(19)国家知识产权局

(12)发明专利

(10)授权公告号 CN 109844130 B
(45)授权公告日 2024.10.11

(21)申请号 201780063220.0

(22)申请日 2017.10.18

(65)同一申请的已公布的文献号
申请公布号 CN 109844130 A

(43)申请公布日 2019.06.04

(30)优先权数据
16194390.7 2016.10.18 EP

(85)PCT国际申请进入国家阶段日
2019.04.12

(86)PCT国际申请的申请数据
PCT/EP2017/076569 2017.10.18

(87)PCT国际申请的公布数据
WO2018/073288 EN 2018.04.26

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(51)Int.Cl.
C12Q 1/37(2006.01)
G01N 33/569(2006.01)

(56)对比文件
US 2006024763 A1,2006.02.02
US 2012164657 A1,2012.06.28
M Wictome等.Development of an in vitro bioassay for Clostridium botulinum type B neurotoxin in foods that is more sensitive than the mouse bioassay.《Appl Environ Microbiol.》.1999,第65卷(第9期),第3787-3792页.

审查员 戴露丹

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(54)发明名称

细胞VAMP切割测定

(57)摘要

本发明涉及适于产生针对VAMP C-末端神经毒素切割产物的抗体的VAMP表位,它们用于产生针对切割的VAMP的抗体的用途以及基于信号增益这些抗体在细胞VAMP切割测定中的用途。

1.一种抗原多肽,其氨基酸序列如AcNH-KLSELDDRADALQ-CONH2、SEQ ID NO:15或SEQ ID NO:49所示。

2.一种产生针对C-末端VAMP切割产物的多克隆抗体的方法,所述C-末端VAMP切割产物是由肉毒神经毒素F(BoNT/F)、BoNT/D、或BoNT/DC对VAMP切割而生成,

所述方法包括从以下中分离和纯化多克隆抗体:

(a).从已经注射抗原多肽的动物获得的血液,其中所述抗原多肽的氨基酸序列如AcNH-KLSELDDRADALQ-CONH2或SEQ ID NO:15所示,

(b).从动物获得的卵,其中所述的卵已经注射抗原多肽,其中所述抗原多肽的氨基酸序列如AcNH-KLSELDDRADALQ-CONH2或SEQ ID NO:

15所示。

3.一种产生针对C-末端VAMP切割产物的多克隆抗体的方法,所述C-末端VAMP切割产物是由BoNT/B、BoNT/F、BoNT/D、BoNT/DC、或破伤风神经毒素(TeNT)对VAMP切割而生成,

所述方法包括从以下中分离和纯化多克隆抗体:

(a).从已经注射抗原多肽的动物获得的血液,其中所述抗原多肽的氨基酸序列如SEQ ID NO:49所示,

(b).从动物获得的卵,其中所述的卵已经注射抗原多肽,其中所述抗原多肽的氨基酸序列如SEQ ID NO:49所示。

4.根据权利要求2的方法可获得的抗体,其中所述抗体是多克隆抗体。

5.根据权利要求3的方法可获得的抗体,其中所述抗体是多克隆抗体。

6.根据权利要求4或5的抗体,其中所述抗体与其表位之间的K_D低于10^{-7}M。

7.权利要求4的抗体在信号增益细胞测定中的用途,所述细胞测定用于通过BoNT/F、BoNT/D、或BoNT/DC进行VAMP切割。

8.权利要求5的抗体在信号增益细胞测定中的用途,所述细胞测定用于通过BoNT/B、BoNT/F、BoNT/D、BoNT/DC、或TeNT进行VAMP切割。

9.一种测定在细胞中BoNT/F、BoNT/D、或BoNT/DC对VAMP的切割的方法,包括:

(a).在适合梭菌神经毒素活性的条件下,使细胞与BoNT/F、BoNT/D、或BoNT/DC接触;

(b).在通过BoNT/F、BoNT/D、或BoNT/DC切割VAMP之后,使所述细胞的细胞质内含物与针对C-末端VAMP切割产物的第一检测抗体在适合于第一检测抗体与C末端VAMP切割产物结合的条件下接触,其中所述第一检测抗体是根据权利要求4的抗体;和

(c).通过适合的手段检测所述第一检测抗体与所述C末端VAMP切割产物的结合。

10.一种测定在细胞中BoNT/B、BoNT/F、BoNT/D、BoNT/DC、或TeNT对VAMP的切割的方法,包括:

(a).在适合梭菌神经毒素活性的条件下,使细胞与BoNT/B、BoNT/F、BoNT/D、BoNT/DC、或TeNT接触;

(b).在通过BoNT/B、BoNT/F、BoNT/D、BoNT/DC、或TeNT切割VAMP之后,使所述细胞的细胞质内含物与针对C-末端VAMP切割产物的第一检测抗体在适合于第一检测抗体与C末端VAMP切割产物结合的条件下接触,其中所述第一检测抗体是根据权利要求5的抗体;和

(c).通过适合的手段检测所述第一检测抗体与所述C末端VAMP切割产物的结合。

11.根据权利要求9或10的方法,还包括(d).通过适合的手段定量与所述第一检测抗体

结合的C末端VAMP切割产物的量。

12.一种试剂盒,其包含易受BoNT/F、BoNT/D、或BoNT/DC中毒的细胞;和一种针对切割的VAMP的第一检测抗体,其中所述第一检测抗体是根据权利要求4的抗体。

13.一种试剂盒,其包含易受BoNT/B、BoNT/F、BoNT/D、BoNT/DC、或TeNT中毒的细胞;和一种针对切割的VAMP的第一检测抗体,其中所述第一检测抗体是根据权利要求5的抗体。

细胞VAMP切割测定

发明领域

[0001] 本发明涉及用于VAMP切割梭菌神经毒素的基于细胞的测定。

[0002] 背景

[0003] 梭菌属中的细菌产生高效且特异性的蛋白质毒素,这些毒素可以毒害它们所传递到的神经元和其他细胞。此类梭菌毒素的实例包括由破伤风梭菌(C.tetani,破伤风神经毒素)和肉毒杆菌(C.botulinum,肉毒杆菌神经毒素血清型A至G)产生的神经毒素,以及由巴氏梭菌(C.baratii)和丁酸梭菌(C.butyricum)产生的神经毒素。

[0004] 梭菌神经毒素通过抑制周围神经系统中,特别是在神经肌肉接头处的胆碱能传递起作用。在自然界中,梭菌神经毒素被合成为单链多肽,其通过蛋白水解切割事件在翻译后修饰以形成通过二硫键连接在一起的两条多肽链。切割发生在特定的切割位点,通常被称为活化位点,其位于提供链间二硫键的半胱氨酸残基之间。正是这种双链形式是毒素的活性形式。这两条链被称为重链(H链)(具有约100kDa的分子量)和轻链(L链)(具有约50kDa的分子量)。H链包含N末端易位组分(H_N结构域)和C末端靶向组分(H_C结构域)。切割位点位于L链和H_N结构域之间。在H_C结构域与其靶神经元结合并通过内体将结合的毒素内化到细胞中之后,H_N结构域将L链转移穿过内体膜并进入胞质溶胶,并且L链提供蛋白酶功能(也称为非细胞毒性蛋白酶)。

[0005] 非细胞毒性蛋白酶通过蛋白水解切割被称为SNARE蛋白(例如SNAP25,VAMP或syntaxin)的细胞内转运蛋白起作用-参见GeraldK(2002)“Cell and Molecular Biology”(第4版)John Wiley&Sons,Inc。缩写SNARE来自术语可溶性NSF附着受体,其中NSF表示N-乙基马来酰亚胺敏感因子。首字母缩写SNAP25来自术语25千道尔顿的突触体相关蛋白。首字母缩写VAMP来自术语囊泡相关膜蛋白。SNARE蛋白是细胞内囊泡融合组成部分,从而是通过囊泡运输而从细胞分泌分子的组成部分。蛋白酶功能是锌依赖性内肽酶活性,对SNARE蛋白显示出高底物特异性。因此,一旦递送到所需的靶细胞,非-细胞毒性蛋白酶能够抑制细胞从靶细胞分泌。梭菌神经毒素的L链蛋白酶是切割SNARE蛋白的非细胞毒性的蛋白酶。BoNT/B,BoNT/D,BoNT/F,BoNT/G,BoNT/X和TeNT的L链蛋白酶切割VAMP(也称为synaptobrevins),BoNT/A和BoNT/E的L链蛋白酶切割SNAP25并且BoNT/C的L链蛋白酶切割SNAP25和突触融合蛋白,这导致神经递质释放的抑制和随后的神经麻痹(Rossetto,O.等人,“Botulinum neurotoxins:genetic,structural and mechanistic insight”.Nature Reviews Microbiology 12.8(2014):535-549)(Zhang等人,“Identification and characterization of a novel botulinum neurotoxin”;Nature Communications,2017,8:14130)。

[0006] 梭菌神经毒素通过其受体结合结构域(H_C)与其特异性受体结合而靶向并进入神经元,这些结构域在文献中有明确定义(Schiavo,G.,Matteoli,M.&Montecucco,C.Neurotoxins affected neuroxocytosis,Physiol Rev),2000,80,717-766)。受体结合决定了BoNT识别神经元的功效和特异性。BoNT/B,D-C和G共有两个同源突触小泡蛋白突触结合蛋白I和II(Syt I/II)作为它们的受体,而BoNT/A,E,D,F和TeNT使用另一个突触小泡

蛋白SV2。除蛋白质受体外,所有BoNT都需要脂质共受体神经节苷脂,其在神经元表面上是丰富的。

[0007] 梭菌神经毒素用于治疗运动和自主神经紊乱的疗法。一些BoNT/A产品(包括**Botox®，Dysport®**和**Xeomin®**)和一种BoNT/B产品**(Neurobloc®/Myobloc®)**已获得监管机构批准用于人类。

[0008] 传统上,BoNT药物产品的效力已经以MLD50(小鼠致死剂量50)单位量化,一个单位对应于小鼠中的半数致死腹膜内剂量。然而,用于肉毒杆菌毒素的MLD50单位不是标准化单位。实际上,市售毒素的不同制造商使用的测定法在稀释缓冲液的选择方面特别不同(Straughan,DW,2006,ATLA34(3),305-313;Hambleton和Pickett,Hambleton,P.和AM Pickett.,1994,Journal of the Royal Society of Medicine 87.11:719)。此外,由于伦理问题和最近的法规,现在优选避免使用基于动物的效力测定。基于细胞的效力测定避免了对动物测试和相关伦理问题的要求。在细胞中毒后,梭菌神经毒素的效力可以通过评估靶细胞内SNARE切割的程度来测量,例如通过Western印迹。或者,可以使用夹心ELISA方法检测和定量SNARE切割。这些方法适用于SNAP25和突触融合蛋白切割(参见例如Pellett,Sabine等人,"Comparison of the primary rat spinal cord cell(RSC)assay and the mouse bioassay for botulinum neurotoxin type A potency determination."Journal of pharmacological and toxicological methods 61.3(2010):304-310;Fernández-Salas,Ester等人,"Botulinum neurotoxin serotype A specific cell-based potency assay to replace the mouse bioassay."PLoS One 7.11(2012):e49516;Kalandakanond S等人,Cleavage of intracellular substrates of botulinum toxins A,C and D in mammalian target tissue"The Journal of Pharmacology and Experimental Therapeutics(2001):749-755;Peng L等人,“Cytotoxicity of botulinum neurotoxins reveals a direct role of syntaxin 1and SNAP25in neuron survival."Nature Communications(2013):4:1472)。然而,迄今为止,已证明来自细胞裂解物的VAMP切割产物极难检测。实际上,尽管可获得识别切割的VAMP的VAMP切割特异性抗体,并且其适于检测细胞外或细胞级分测定中的VAMP切割(Hallis,Bassam,B.A.James,and Clifford C.Shone."Development of novel assays for botulinum type A and B neurotoxins based on their endopeptidase activities."Journal of clinical microbiology 34.8(1996):1934-1938;Kegel,B.等人,"An in vitro assay for detection of tetanus neurotoxin activity:Using antibodies for recognizing the proteolytically generated cleavage product."Toxicology in Vitro21.8(2007):1641-1649;Fujita-Yoshigaki,Junko等人,"Vesicle-associated Membrane Protein 2Is Essential for cAMP-regulated Exocytosis in Rat Parotid Acinar Cells The Inhibition of cAMP-dependent Amylase Release by Botulinum Neurotoxin B."Journal of Biological Chemistry 271.22(1996):13130-13134),这些抗体在细胞研究中未检测到切割的VAMP。

[0009] 迄今为止该领域的普遍共识是切割的VAMP产物必须在细胞中非常快地降解,因此不会促进BoNT作用的持续时间(Foran,Patrick G.,等人,"Evaluation of the Therapeutic Usefulness of Botulinum Neurotoxin B,C1,E,and F Compared with the Long Lasting Type A Basis for Distinct Durations of Inhibition of Exocytosis in Central Neurons."Journal of biological chemistry 278.2(2003):1363-1371。然而,Schiavo等人已经表明,当使用考马斯蓝染色用BoNT/B和TeNT处理时,从大鼠大脑皮层制备的小突触小泡级分中存在两种VAMP切割产物(Schiavo G.,等人(1992),Tetanus and Botulinum-B neurotoxins block neurotransmitter release by proteolytic cleavage of synaptobrevin.Nature 359p832-835)。这表明可以存在来自细胞来源的VAMP产物,尽管突触体制剂可能不包含存在于总细胞裂解物中的所有蛋白酶。Dong等人(2004)描述了在表达YFP-Syb(FL)-CFP的PC12细胞中,来自两种VAMP产物的信号在被BoNT/B切割后可检测到,并且YFP-N末端切割的VAMP产物分散到胞质溶胶中并且将其自身重新分配到细胞核,而CFP-C-末端产物保持定位于囊泡(Dong M.,等人(2004)Using fluorescent sensors to detect botulinum neurotoxin activity in vitro and in living cells.PNAS 101(41)p14701-14706)。该证据表明可能存在两种VAMP产物,但尚未知的细胞过程妨碍了对N端产物的抗体的识别。因此,标准实践是仅通过全长条带的消失来测量VAMP切割(参见例如Pellett,Sabine等人,"A neuronal cell-based botulinum neurotoxin assay for highly sensitive and specific detection of neutralizing serum antibodies."FEBS letters 581.25(2007):4803-4808.;Whitemarsh,Regina CM,等人," Novel application of human neurons derived from induced pluripotent stem cells for highly sensitive botulinum neurotoxin detection Biological Sciences:Applied Biological Sciences."Toxicological Sciences,2012,126(2):426-435)。然而,基于信号丢失的测定法传达了错误的风险,因为总蛋白质负载可能存在差异,这将导致VAMP消失的高估或低估。在BoNT处理期间未改变的管家蛋白质可用于将VAMP消失对对照蛋白质的密度归一化。这里的缺点是抗体之间的信号需要匹配并且在线性标度中以便检测归一化目的的任何差异。虽然定性地讲这可能是BoNT活性的合理表明,但它不适用于更详细的定量,特别是用于确定药物BoNT制剂的效力。

[0010] 因此需要基于信号读数的增益的细胞VAMP切割测定。

[0011] 发明概述

[0012] 在第一方面,本发明提供了包含VAMP表位的抗原多肽,其中所述抗原多肽由10至65个氨基酸残基组成,其中所述VAMP表位包含一氨基酸序列,其与包含直接位于所述VAMP中的梭菌神经毒素切割位点的C-末端的至少8个氨基酸残基的VAMP序列有至少90%同一性。

[0013] 另一方面,本发明涉及包含本发明的抗原多肽的多肽,其中所述多肽不包含具有与天然存在的VAMP氨基酸序列具有100%序列同一性的大于17,优选16,更优选15个连续氨基酸的区域。

[0014] 另一方面,本发明提供了一种抗原蛋白,其包含与载体共价连接的根据本发明的多肽。

[0015] 另一方面,本发明提供了根据本发明的抗原多肽或蛋白质用于产生针对C-末端VAMP切割产物的抗体的用途。在一个实施方案中,本发明的表位用于产生针对C-末端VAMP切割产物的多克隆抗体。在另一个实施方案中,本发明的表位用于产生针对C-末端VAMP切割产物的单克隆抗体。

[0016] 另一方面,本发明提供了结合本发明的抗原多肽或蛋白质的抗体。

[0017] 另一方面,本发明提供了根据本发明的抗体在通过VAMP切割梭菌神经毒素增进VAMP切割的信号细胞测定中的用途。

[0018] 另一方面,本发明提供了一种在细胞中通过切割VAMP的梭菌神经毒素来确定VAMP切割的方法,包括:

[0019] a)在适合梭菌神经毒素活性的条件下,使细胞与梭菌神经毒素接触;

[0020] b)在通过切割VAMP的梭菌神经毒素切割VAMP后,使所述细胞的细胞质内容物与针对C末端VAMP切割产物的第一检测抗体在适于所述第一检测抗体与C末端VAMP切割产物结合的条件下接触,其中所述第一检测抗体是根据本发明的抗体;和

[0021] c)通过适合的方法检测所述第一检测抗体与C末端VAMP切割产物的结合。

[0022] 另一方面,本发明提供了一种测定受试者中针对切割VAMP的梭菌神经毒素的免疫耐受性的方法,包括:

[0023] a)向从受试者获得的测试样品中加入切割VAMP的梭菌神经毒素;

[0024] b)在适合梭菌神经毒素活性的条件下,使细胞与步骤a)的测试样品接触;

[0025] c)在通过切割VAMP的梭菌神经毒素切割VAMP后,使所述细胞的细胞质内含物与针对C末端VAMP切割产物的第一检测抗体在适于所述第一检测抗体与C末端VAMP切割产物结合的条件下接触,其中所述第一检测抗体是根据本发明的抗体;

[0026] d)通过适合的方法检测第一检测抗体与C末端VAMP切割产物的结合;

[0027] e)定量与第一检测抗体结合的C末端VAMP切割产物的量;

[0028] f)用阴性对照样品代替测试样品重复步骤a)至e);和

[0029] g)比较步骤(e)和(f)中与所述第一检测抗体结合的C-末端VAMP切割产物的量,其中相对于在步骤(f)中与所述第一检测抗体结合的C-末端VAMP切割产物的量,在步骤(e)中检测到与所述第一检测抗体结合的较低量的C-末端VAMP切割产物表明存在针对切割VAMP的梭菌神经毒素的中和抗体。

[0030] 另一方面,本发明提供了一种试剂盒,其包含易受切割VAMP的神经毒素中毒的细胞;和针对切割的VAMP的第一检测抗体,其中所述第一检测抗体是根据本发明的抗体。

[0031] 发明详述

[0032] 本发明基于发明人的发现,即基于信号读数的增益,可以产生适用于细胞VAMP切割测定的抗体。

[0033] 特别地,本发明人已经表明,为了在体外检测VAMP切割,检测位于BoNT切割位点的C末端侧的表位是关键。实际上,本发明人已经证明通过Western印迹(WB)使用结合位于BoNT/F和/或BoNT/D和/或BoNT/B切割位点的C末端侧的表位的抗体成功检测了神经元VAMP2切割产物,所述切割位点与BoNT/D和/或BoNT/F和/或BoNT/B切割位点相邻。特别是,这种抗体能够检测细胞裂解物中的全长VAMP和切割产物。该工具通过监测切割的VAMP产物的外观,能够定量评估BoNT在信号增益细胞测定中的效力。

[0034] 在第一方面,本发明提供了包含VAMP表位的抗原多肽,其中所述抗原多肽由10至65个氨基酸残基组成,其中所述VAMP表位包含一氨基酸序列,其与包含直接位于所述VAMP中梭菌神经毒素切割位点的C-末端的至少8个氨基酸残基的VAMP序列有至少90%同一性。

[0035] 本文所用的术语“梭菌神经毒素”是指进入神经元并抑制神经递质释放的任何多肽。该过程包括神经毒素与低或高亲和力受体的结合、神经毒素的内化、神经毒素的内肽酶

部分易位到细胞质中以及神经毒素底物的酶促修饰。更具体地，术语“梭菌神经毒素”包括由梭菌属细菌产生的进入神经元并抑制神经递质释放的任何多肽，以及通过重组技术或化学技术产生的此类多肽。它是双链形式，是神经毒素的活性形式。这两条链被称为重链(H链)，其具有约100kDa的分子量，和轻链(L链)，其具有约50kDa的分子量。梭菌神经毒素包括肉毒杆菌神经毒素(BoNT)和破伤风神经毒素(TeNT)。BoNT血清型A至G可以基于通过特定中和抗血清的失活来区分，血清型的这种分类与氨基酸水平上的百分比序列同一性相关。基于氨基酸百分比序列同一性，将给定血清型的BoNT蛋白进一步分成不同的亚型。

[0036] BoNT/A神经毒素氨基酸序列的实例提供为SEQ ID NO:1(UniProt登录号A5HZZ9)。BoNT/B神经毒素氨基酸序列的实例提供为SEQ ID NO:2(UniProt登录号B1INP5)。BoNT/C神经毒素氨基酸序列的实例提供为SEQ ID NO:3(UniProt登录号P18640)。BoNT/D神经毒素氨基酸序列的实例提供为SEQ ID NO:4(UniProt登录号P19321)。BoNT/E神经毒素氨基酸序列的实例提供为SEQ ID NO:5(NCBI RefSeq登录号WP_003372387)。BoNT/F神经毒素氨基酸序列的实例提供为SEQ ID NO:6(UniProt登录号Q57236)。BoNT/G神经毒素氨基酸序列的实例提供为SEQ ID NO:7(NCBI RefSeq登录号WP_039635782)。BoNT/X神经毒素氨基酸序列的实例提供为SEQ ID NO:41(Genbank登录号BAQ12790.1)。TeNT氨基酸序列的实例提供为SEQ ID NO:8(UniProt登录号P04958)。

[0037] 本文所用的术语“H_C结构域”是指神经毒素重链的功能不同的区域，其分子量约为50kDa，能够使神经毒素与位于靶细胞表面的受体结合。H_C结构域由两个结构不同的亚结构域组成，“H_{CN}亚结构域”(H_C结构域的N末端部分)和“H_{CC}亚结构域”(H_C结构域的C末端部分)，每个亚结构域的分子量均为大约25kDa。

[0038] 本文所用的术语“LH_N结构域”是指缺乏H_C结构域并由内肽酶结构域(“L”或“轻链”)组成的神经毒素和负责内肽酶易位到细胞质中的结构域(重链的H_N结构域)。

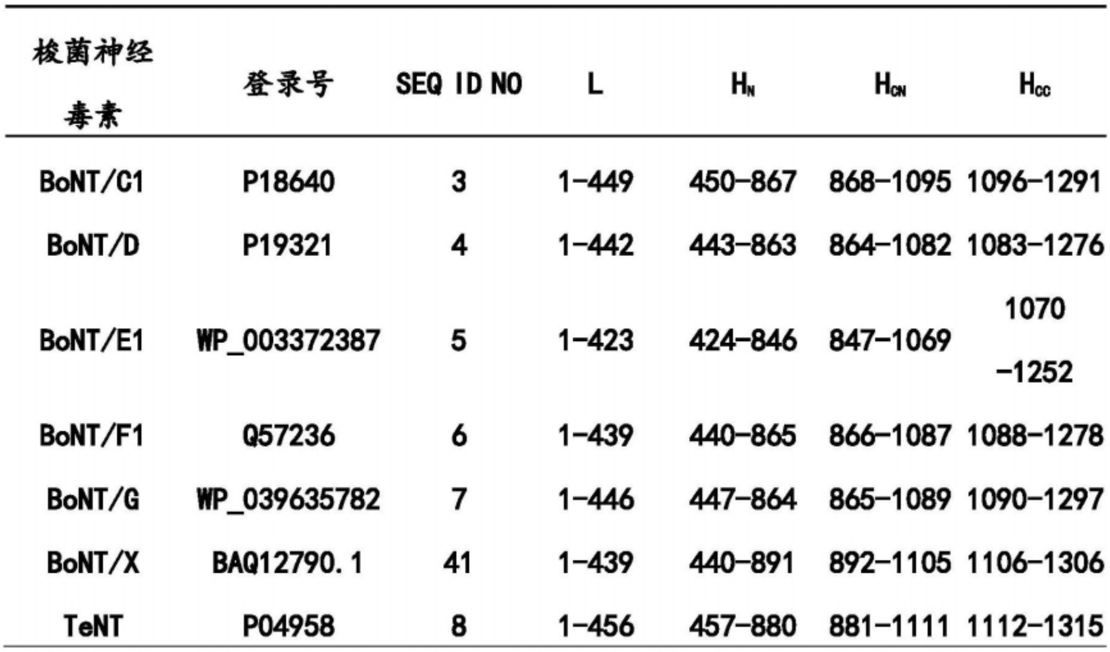
[0039] 示例性的L，H_N，H_{CN}和H_{CC}结构域在表1中示出。

[0040] 表1-示例性L，H_N，H_{CN}和H_{CC}结构域

[0041]

梭菌神经毒素	登录号	SEQ ID NO	L	H_N	H_{CN}	H_{CC}
BoNT/A1	A5HZZ9	1	1-448	449-872	873-1094	1095-1296
BoNT/B1	B1INP5	2	1-441	442-859	860-1081	1082-1291

[0042]



梭菌神经毒素	登录号	SEQ ID NO	L	H_N	H_{CN}	H_{CC}
BoNT/C1	P18640	3	1-449	450-867	868-1095	1096-1291
BoNT/D	P19321	4	1-442	443-863	864-1082	1083-1276
BoNT/E1	WP_003372387	5	1-423	424-846	847-1069	1070 -1252
BoNT/F1	Q57236	6	1-439	440-865	866-1087	1088-1278
BoNT/G	WP_039635782	7	1-446	447-864	865-1089	1090-1297
BoNT/X	BAQ12790.1	41	1-439	440-891	892-1105	1106-1306
TeNT	P04958	8	1-456	457-880	881-1111	1112-1315

[0043] 上述鉴定的参考序列应被视为指导,因为根据亚血清型可能发生轻微变化。举例来说,US 2007/0166332(通过引用整体并入本文)引用了略微不同的梭菌序列。

[0044] 囊泡相关膜蛋白(VAMP)是SNARE蛋白家族,其具有相似的结构并参与囊泡融合和胞吐作用,特别是神经递质释放。VAMP是SNARE蛋白家族的成员,称为突触泡蛋白家族,包括成员如VAMP1,VAMP2(也称为突触泡蛋白),VAMP3(也称为cellubrevin),VAMP4,VAMP5,VAMP7(也称为SYBL1,或破伤风不敏感的VAMP),VAMP8(也称为endobrevin),YKT6,SEC22A等。VAMP1,VAMP2和VAMP3被BoNT/B,BoNT/D,BoNT/F,BoNT/G,BoNT/X和TeNT的轻链切割。BoNT/X还可以切割VAMP4,VAMP5和YKT6。

[0045] 本文所用的术语“VAMP表位”是指抗体结合的VAMP蛋白的一部分。

[0046] 在一个优选的实施方案中,本发明的抗原多肽由10至65,10至60,10至55,10至50,10至45,10至40,10至35,10至30,10至25,10至20,10至19,10至18,10至17,10至16或10至15个氨基酸残基,优选10至15个氨基酸残基组成。例如,本发明的抗原多肽可以由10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64或65个氨基酸残基组成。

[0047] 在一个优选的实施方案中,本发明的抗原多肽包含VAMP表位或由其组成,所述VAMP表位包含一氨基酸序列,其与包含直接位于所述VAMP中的梭菌神经毒素切割位点C末端的至少8,9,10,11,12,13,14,15,16或17个氨基酸残基的VAMP序列有至少90%,91%,92%,93%,94%,95%,96%,97%,98%,99%或100%同一性。

[0048] 天然存在的VAMP,特别是大鼠和人VAMP1,VAMP2,VAMP3,VAMP4,VAMP5和YKT6的氨基酸序列及其相应的梭菌神经毒素VAMP切割位点示于表2和图1中。

[0049] 表2-梭菌神经毒素VAMP切割位点

[0050]

VAMP	SEQ ID NO	BoNT/F5 & BoNT/FA	其他 BoNT/F	BoNT/D & BoNT/DC	BoNT/B & TeNT	BoNT/G	BoNT/X
VAMP1_大 鼠(Q63666)	9	Leu56-Glu57	Gln60-Lys61	Lys61-Leu62	未切割的	Ala83-Ala84	Arg68-Ala69
VAMP1_人(P23763)	10	Leu56-Glu57	Gln60-Lys61	Lys61-Leu62	Gln78-Phe79	Ala83-Ala84	Arg68-Ala69
VAMP2_大 鼠(P63045)	11	Leu54-Glu55	Gln58-Lys59	Lys59-Leu60	Gln76-Phe77	Ala81-Ala82	Arg66-Ala67
VAMP2_	12	Leu54-	Gln58-	Lys59-	Gln76-Phe77	Ala81-	Arg66-Ala67

[0051]

VAMP	SEQ ID NO	BoNT/F5 & BoNT/FA	其他BoNT/F	BoNT/D & BoNT/DC	BoNT/B & TeNT	BoNT/G	BoNT/X
人(P63027)		Glu55	Lys59	Leu60		Ala82	
VAMP3_大鼠(P63025)	13	Leu41-Glu42	Gln45-Lys46	Lys46-Leu47	Gln63-Phe64	Ala68-Ala69	Arg53-Ala54
VAMP3_人(Q15836)	14	Leu37-Glu38	Gln41-Lys42	Lys42-Leu43	Gln59-Phe60	Ala64-Ala65	Arg49-Ala50
VAMP4_大鼠(D4A560)	42	未切割	未切割	未切割	未切割	未切割	Lys87-Ser88
VAMP4_人(O75379)	43	未切割	未切割	未切割	未切割	未切割	Lys87-Ser88
VAMP5_大鼠(Q9Z2J5)	44	未切割	未切割	未切割	未切割	未切割	Arg40-Ser41
VAMP5_	45	未切割	未切割	未切割	未切割	未切割	Arg40-Ser41

[0052]

VAMP	SEQ ID NO	BoNT/F5 & BoNT/FA	其他 BoNT/F	BoNT/D & BoNT/DC	BoNT/B & TeNT	BoNT/G	BoNT/X
人 (095183)							
YKT6_大鼠 (Q5EGY4)	46	未切割	未切割	未切割	未切割	未切割	Lys173-Ser174
YKT6_人 (015498)	47	未切割	未切割	未切割	未切割	未切割	Lys173-Ser174

[0053] 在一个实施方案中,VAMP选自VAMP1,VAMP2,VAMP3,VAMP4,VAMP5和/或YKT6。

[0054] 在一个实施方案中,VAMP选自VAMP1,VAMP2和/或VAMP3。

[0055] 在一个实施方案中,VAMP选自VAMP4,VAMP5和/或YKT6。

[0056] 在优选的实施方案中,VAMP是人VAMP,更优选人VAMP1,VAMP2,VAMP3,VAMP4,VAMP5和/或YKT6。

[0057] 在一个实施方案中,VAMP选自人VAMP1,VAMP2和/或VAMP3。

[0058] 在一个实施方案中,VAMP选自人VAMP4,VAMP5和/或YKT6。

[0059] 在本发明的抗原多肽的一个实施方案中,VAMP表位是BoNT/F切割的VAMP表位,其中至少8个氨基酸残基直接位于VAMP中的BoNT/F切割位点的C末端。

[0060] BoNT/F VAMP表位,更特别是BoNT/F VAMP1,VAMP2和/或VAMP3表位的实例,包括:

[0061] •KLSELDDRADALQ(SEQ ID NO:15)

[0062] •QKLSELDDRADALQ(SEQ ID NO:16)

[0063] •KLSELDDRAD(SEQ ID NO:17)

[0064] •KLSELDDRADALQAGAS(SEQ ID NO:18)

[0065] •DQKLSELDDRADALQ(SEQ ID NO:31)。

[0066] 在一个实施方案中,BoNT/F VAMP表位,特别是BoNT/F VAMP1,VAMP2和/或VAMP3表位,包含与选自SEQ ID NO:15至SEQ ID NO:18和SEQ ID NO:31的序列有至少90%,91%,92%,93%,94%,95%,96%,97%,98%,99%或100%同一性的氨基酸序列或由其组成。在优选的实施方案中,BoNT/F VAMP表位包含与KLSELDDRADALQ(SEQ ID NO:15)有至少90%,

91%,92%,93%,94%,95%,96%,97%,98%,99%或100%同一性的氨基酸序列或由其组成。在更优选的实施方案中,BoNT/F VAMP表位包含KLSELDDRADALQ(SEQ ID NO:15)或由其组成。

[0067] 在本发明的抗原多肽的一个实施方案中,VAMP表位是BoNT/D VAMP表位,其中至少8个氨基酸残基直接位于VAMP中的BoNT/D切割位点的C末端。

[0068] BoNT/D VAMP表位,更特别是BoNT/D VAMP1,VAMP2和/或VAMP3表位的实例,包括:

[0069] •KLSELDDRADALQ(SEQ ID NO:15)

[0070] •LSELDDRADALQ(SEQ ID NO:19)

[0071] •LSELDDRADA(SEQ ID NO:20)

[0072] •LSELDDRADALQAGAS(SEQ ID NO:21)。

[0073] 在一个实施方案中,BoNT/D VAMP表位,特别是BoNT/D VAMP1,VAMP2和/或VAMP3表位,包含与选自SEQ ID NO:15和SEQ ID NO:19至SEQ ID NO:21的序列有至少90%,91%,92%,93%,94%,95%,96%,97%,98%,99%或100%同一性的氨基酸序列或由其组成。在优选的实施方案中,BoNT/D VAMP表位包含与KLSELDDRADALQ(SEQ ID NO:15)的氨基酸序列有至少90%,91%,92%,93%,94%,95%,96%,97%,98%,99%或100%同一性的氨基酸序列或由其组成。在更优选的实施方案中,BoNT/D VAMP表位包含KLSELDDRADALQ(SEQ ID NO:15)或由其组成。

[0074] 在本发明的抗原多肽的一个实施方案中,VAMP表位是BoNT/F5或BoNT/FA切割的VAMP表位,其中至少8个氨基酸残基直接位于VAMP中BoNT/F5或BoNT/FA切割位点的C-末端。

[0075] BoNT/F5或BoNT/FA VAMP表位,更特别是BoNT/F5或BoNT/FA VAMP1,VAMP2和/或VAMP3表位的实例,包括:

[0076] •ERDQKLSELDDRA(SEQ ID NO:32)

[0077] •LERDQKLSELDDRA(SEQ ID NO:33)

[0078] •VLERDQKLSELDDRA(SEQ ID NO:34)。

[0079] 在一个实施方案中,BoNT/F5或BoNT/FA VAMP表位,特别是BoNT/F5或BoNT/FA VAMP1,VAMP2和/或VAMP3表位,包含与选自SEQ ID NO:32至SEQ ID NO:34的序列有至少90%,91%,92%,93%,94%,95%,96%,97%,98%,99%或100%同一性的氨基酸序列或由其组成。在优选的实施方案中,BoNT/F5或BoNT/FA VAMP表位包含与ERDQKLSELDDRA(SEQ ID NO:32)的氨基酸序列有至少90%,91%,92%,93%,94%,95%,96%,97%,98%,99%或100%同一性的氨基酸序列或由其组成。在更优选的实施方案中,BoNT/F5或BoNT/FA VAMP表位包含ERDQKLSELDDRA(SEQ ID NO:32)或由其组成。

[0080] 在本发明的抗原多肽的一个实施方案中,VAMP表位是BoNT/B或TeNT VAMP表位,其中至少8个氨基酸残基直接位于VAMP中的BoNT/B或TeNT切割位点的C末端。

[0081] BoNT/B或TeNT VAMP表位,更特别是BoNT/B或TeNT VAMP1,VAMP2和/或VAMP3表位的实例,包括:

[0082] •FETSAAKLKRKYW(SEQ ID NO:22)

[0083] •FESSAAKLKRKYW(SEQ ID NO:23)

[0084] •QFETSAAKLKRKYW(SEQ ID NO:24)

[0085] •FETSAAKLKR(SEQ ID NO:25)

[0086] •FETSAAKLKRKYWWKN(SEQ ID NO:26)

[0087] •ETSAAKLKRKYWWK(SEQ ID NO:48)

[0088] •FETSAAKLKRKYWWK(SEQ ID NO:49)

[0089] •QFESSAAKLKRKYW(SEQ ID NO:50)

[0090] •FESSAAKLKR(SEQ ID NO:51)

[0091] •FESSAAKLKRKYWWK(SEQ ID NO:52)。

[0092] 在一个实施方案中,BoNT/B或TeNT VAMP表位,特别是BoNT/B或TeNT VAMP1,VAMP2和/或VAMP3表位,包含与选自SEQ ID NO:22至SEQ ID NO:26和SEQ ID NO:48至SEQ ID NO:52的序列有至少90％,91％,92％,93％,94％,95％,96％,97％,98％,99％或100％同一性的氨基酸序列或由其组成。在一个优选的实施方案中,BoNT/B或TeNT VAMP表位包含与FETSAAKLKRKYW(SEQ ID NO:22)或FETSAAKLKRKYWWK(SEQ ID NO:49)有至少90％,91％,92％,93％,94％,95％,96％,97％,98％,99％或100％同一性的氨基酸序列或由其组成。在更优选的实施方案中,BoNT/B或TeNT VAMP表位包含FETSAAKLKRKYW(SEQ ID NO:22)或FETSAAKLKRKYWWK(SEQ ID NO:49)或由其组成。令人惊讶的是,结合后一表位的抗体不仅允许检测BoNT/B VAMP切割,而且还允许检测BoNT/F VAMP切割。

[0093] 在本发明的抗原多肽的一个实施方案中,VAMP表位是BoNT/G VAMP表位,其中至少8个氨基酸残基直接位于VAMP中的BoNT/G切割位点的C-末端。

[0094] BoNT/G VAMP表位,更特别是BoNT/G,VAMP1,VAMP2和/或VAMP3表位的实例,包括:

[0095] •AKLKRKYWWKN(SEQ ID NO:27)

[0096] •AAKLKRKYWWKN(SEQ ID NO:28)

[0097] •AKLKRKYWWKNCKM(SEQ ID NO:29)

[0098] •AKLKRKYWWKNLKM(SEQ ID NO:30)。

[0099] 在一个实施方案中,BoNT/G VAMP表位,特别是BoNT/G VAMP1,VAMP2和/或VAMP3表位,包含与选自SEQ ID NO:27至SEQ ID NO:30的序列有至少90％,91％,92％,93％,94％,95％,96％,97％,98％,99％或100％同一性的氨基酸序列或由其组成。在优选的实施方案中,BoNT/G VAMP表位包含与AKLKRKYWWKN(SEQ ID NO:27)有至少90％,91％,92％,93％,94％,95％,96％,97％,98％,99％或100％同一性的氨基酸序列或由其组成。在更优选的实施方案中,BoNT/G VAMP表位包含AKLKRKYWWKN(SEQ ID NO:27)或由其组成。

[0100] 在本发明的抗原多肽的一个实施方案中,VAMP表位是BoNT/X VAMP表位,其中至少8个氨基酸残基直接位于VAMP中的BoNT/X切割位点的C-末端。

[0101] BoNT/X VAMP表位,更特别是BoNT/X VAMP1,VAMP2和/或VAMP3表位的实例,包括:

[0102] •ADALQAGASQF(SEQ ID NO:53)

[0103] •ADALQAGASQ(SEQ ID NO:54)

[0104] •RADALQAGASQF(SEQ ID NO:55)

[0105] •ADALQAGASQFE(SEQ ID NO:56)

[0106] •ADALQAGASVF(SEQ ID NO:57)

[0107] •ADALQAGASV(SEQ ID NO:58)

[0108] •ADALQAGASVFE(SEQ ID NO:59)

[0109] •RADALQAGASVF(SEQ ID NO:60)

[0110] • RADALQAGAS(SEQ ID NO:61)。

[0111] 在一个实施方案中,BoNT/X VAMP表位,特别是BoNT/X VAMP1,VAMP2和/或VAMP3表位,包含与选自SEQ ID NO:53至SEQ ID NO:61的序列有至少90％,91％,92％,93％,94％,95％,96％,97％,98％,99％或100％同一性的氨基酸序列或由其组成。在优选的实施方案中,BoNT/X VAMP表位包含与ADALQAGASQF(SEQ ID NO:53)有至少90％,91％,92％,93％,94％,95％,96％,97％,98％,99％或100％同一性的氨基酸序列或由其组成。在更优选的实施方案中,BoNT/X VAMP表位包含ADALQAGASQF(SEQ ID NO:53)或由其组成。

[0112] BoNT/X VAMP表位,更特别是BoNT/X VAMP4表位的其他实例,包括:

[0113] • SESLSDNATAF(SEQ ID NO:62)

[0114] • SESLSDNATA(SEQ ID NO:63)

[0115] • KSESLSDNATAF(SEQ ID NO:64)

[0116] • SESLSDNATAFS(SEQ ID NO:65)。

[0117] 在一个实施方案中,BoNT/X VAMP表位,特别是BoNT/X VAMP4表位,包含与选自SEQ ID NO:62至SEQ ID NO:65的序列有至少90％,91％,92％,93％,94％,95％,96％,97％,98％,99％或100％同一性的氨基酸序列或由其组成。在优选的实施方案中,BoNT/X VAMP表位包含与SESLSDNATAF(SEQ ID NO:62)有至少90％,91％,92％,93％,94％,95％,96％,97％,98％,99％或100％同一性的氨基酸序列或由其组成。在更优选的实施方案中,BoNT/X VAMP表位包含SESLSDNATAF(SEQ ID NO:62)或由其组成。

[0118] BoNT/X VAMP表位,更特别是BoNT/X VAMP5表位的其他实例,包括:

[0119] • SDQLLDMSSTF(SEQ ID NO:66)

[0120] • SDQLLDMSST(SEQ ID NO:67)

[0121] • RSDQLLDMSSTF(SEQ ID NO:68)

[0122] • SDQLLDMSSTFN(SEQ ID NO:69)

[0123] • SDQLLDMSSAF(SEQ ID NO:70)

[0124] • SDQLLDMSSA(SEQ ID NO:71)

[0125] • RSDQLLDMSSAF(SEQ ID NO:72)

[0126] • SDQLLDMSSAFS(SEQ ID NO:73)

[0127] • RSDQLLDMSS(SEQ ID NO:74)。

[0128] 在一个实施方案中,BoNT/X VAMP表位,特别是BoNT/X VAMP5表位,包含与选自SEQ ID NO:66至SEQ ID NO:74的序列有至少90％,91％,92％,93％,94％,95％,96％,97％,98％,99％或100％同一性的氨基酸序列或由其组成。在优选的实施方案中,BoNT/X VAMP表位包含与SDQLLDMSSTF(SEQ ID NO:66)有至少90％,91％,92％,93％,94％,95％,96％,97％,98％,99％或100％同一性的氨基酸序列或由其组成。在更优选的实施方案中,BoNT/X VAMP表位包含SDQLLDMSSTF(SEQ ID NO:66)或由其组成。

[0129] BoNT/X VAMP表位,更特别是BoNT/X YKT6表位的其他实例,包括:

[0130] • SEVLGTQSKAF(SEQ ID NO:75)

[0131] • SEVLGTQSKA(SEQ ID NO:76)

[0132] • KSEVLGTQSKAF(SEQ ID NO:77)

[0133] • SEVLGTQSKAFY(SEQ ID NO:78)。

[0134] 在一个实施方案中,BoNT/X VAMP表位,特别是BoNT/X YKT6表位,包含与选自SEQ ID NO:75至SEQ ID NO:78的序列有至少90％,91％,92％,93％,94％,95％,96％,97％,98％,99％或100％同一性的氨基酸序列或由其组成。在优选的实施方案中,BoNT/X VAMP表位包含与SEVLGTQSKAF(SEQ ID NO:75)有至少90％,91％,92％,93％,94％,95％,96％,97％,98％,99％或100％同一性的氨基酸序列或由其组成。在更优选的实施方案中,BoNT/X VAMP表位包含SEVLGTQSKAF(SEQ ID NO:75)或由其组成。

[0135] 本文中,两个或更多个核酸或氨基酸序列之间的“序列同一性百分比”是在由比对序列共有的相同位置处的相同核苷酸或氨基酸的数量的函数。因此,％同一性可以计算为比对中每个位置的相同核苷酸或氨基酸的数量除以比对序列中的核苷酸或氨基酸的总数,乘以100。％序列同一性的计算也可以考虑空位的数量,以及为优化两个或更多个序列的比对需要被引入的每个空位的长度。两个或多个序列之间的序列比较和百分比同一性的确定可以使用本领域技术人员熟悉的特定数学算法来进行,例如全局比对数学算法(例如由Needleman和Wunsch,J.Mol.Biol.48(3),443-453,1972描述的)。

[0136] 另一方面,本发明涉及包含根据本发明的抗原多肽的多肽,其中所述多肽不包含与天然存在的VAMP氨基酸序列具有100％序列同一性的大于17,16,15,14,13,12,11,10,优选16,更优选15个连续氨基酸。本领域技术人员容易理解,这种多肽也是抗原性的。

[0137] 在优选的实施方案中,多肽包含共价接头,优选在其N-末端和/或在C-末端。以下提供根据本发明合适的共价接头的实例。

[0138] 另一方面,本发明提供了一种抗原蛋白,其包含与载体共价连接的本发明多肽。

[0139] 优选地,载体是非免疫原性或弱免疫原性蛋白质。合适的载体的实例包括匙孔血蓝蛋白(KLH),卵清蛋白(OVA),甲状腺球蛋白(THY),牛血清白蛋白(BSA),大豆胰蛋白酶抑制剂(STI)或多附着肽(MAP)。

[0140] 在一个实施方案中,抗原蛋白包含本发明多肽(其可能已经包含如上所述的接头)和载体之间的共价接头。所述接头可以是一种或多种天然或非天然的氨基酸,如本领域所熟知的,由于在它们的N-末端、C-末端和/或侧链存在活性基团,所述氨基酸可与(多肽和/或载体的)其他氨基酸形成共价键。值得注意的是,在N-末端和/或侧链中具有伯胺基团(-NH2)的氨基酸(例如赖氨酸)可以与在C-末端和/或侧链具有羧基(-COOH)的氨基酸(如天冬氨酸或谷氨酸)反应形成共价键;侧链具有巯基(-SH)的氨基酸(如半胱氨酸或硒代半胱氨酸)可与侧链具有巯基(-SH)的氨基酸(如半胱氨酸或硒代半胱氨酸)反应形成共价键。例如,共价接头可以是在本发明多肽的C-末端或N-末端添加的半胱氨酸,所述半胱氨酸与在载体中添加或存在的另一个半胱氨酸形成二硫桥。或者或另外,共价接头可以是形成间隔物的几个氨基酸的形式,例如接头可以是包含具有小侧链R基团的非带电氨基酸(例如甘氨酸,丙氨酸,缬氨酸,亮氨酸或丝氨酸)的肽。本发明的合适间隔物的实例包括G-间隔物,例如GGG,GGGG和GGGGS,或A-间隔物,例如AAA,AAAA和AAAAV。在一个实施方案中,接头由约1至约30个氨基酸残基组成,优选约2至约25个氨基酸残基,更优选约3至约20个氨基酸残基,例如,4,5,6,7,8,9,10,11,12,13,14或15个氨基酸残基。

[0141] 另一方面,本发明提供了根据本发明的抗原多肽或蛋白质用于产生针对C-末端VAMP切割产物的抗体的用途。在一个实施方案中,本发明的表位用于产生针对C-末端VAMP切割产物的多克隆抗体。在另一个实施方案中,本发明的表位用于产生针对C-末端VAMP切

割产物的单克隆抗体。

[0142] 产生抗体的方法是本领域熟知的,参见例如Greenfield,Edward A.,编,Antibodies:a laboratory manual.Cold Spring Harbor Laboratory Press,2014;Leenaars,Marlies,和Coenraad FM Hendriksen."Critical steps in the production of polyclonal and monoclonal antibodies:evaluation and recommendations."Ilar Journal 46.3(2005):269-279。

[0143] 结合如本文所述VAMP表位的多克隆抗体可以通过用本发明的抗原多肽或蛋白质注射动物,例如,哺乳动物,如兔子,山羊,小鼠,仓鼠或猴子,或卵,如鸡蛋来产生。本文公开的VAMP表位的多克隆抗体可以从动物(例如从血液)或卵中分离,并通过众所周知的技术进一步纯化,例如蛋白质亲和层析以获得IgG级分,或通过针对用于产生抗体的VAMP表位的亲和纯化。一些合同研究组织提供定制的抗体生成服务,例如Eurogentec公司提供“快速28天计划”,他们在第0天进行免疫,然后在第7天,第10天和第18天进行3次加强免疫注射,第21天中间取血和第28天最后取血。这是本领域众所周知的多克隆抗体生产的一般技术的一个实例。

[0144] 可以使用杂交瘤方法产生结合如本文所述的VAMP表位的单克隆抗体。参见,例如,第7章,Greenfield,Edward A.,编,Antibodies:a laboratory manual.Cold Spring Harbor Laboratory Press,2014。简言之,将宿主动物,例如哺乳动物如兔,山羊,小鼠,仓鼠或猴子暴露于一次或多次注射本发明的抗原多肽或蛋白质以引发产生或能够产生特异性结合切割的VAMP的抗体的淋巴细胞。可以通过标准技术随时间监测免疫动物中的抗体滴度,例如用ELISA(酶联免疫吸附测定法)。或者,可以使用合适的细胞培养系在体外免疫淋巴细胞。在免疫后的适当时间,例如当抗体滴度最高时,从动物中分离产生抗体的细胞。通常,如果需要人源细胞,则使用外周血淋巴细胞,或者如果需要非人哺乳动物来源,则使用脾细胞或淋巴结细胞。使用合适的融合剂(例如聚乙二醇)将分离的抗体生成细胞与永生细胞系融合,以形成杂交瘤细胞。永生细胞系通常是转化的哺乳动物细胞,特别是啮齿动物,牛和人来源的骨髓瘤细胞。通常,将鼠骨髓瘤细胞系与从适当免疫的小鼠收获的脾细胞融合以产生杂交瘤。优选的永生细胞系是对含有次黄嘌呤、氨基蝶呤和胸苷(HAT)的培养基敏感的小鼠骨髓瘤细胞系。根据标准技术,许多骨髓瘤细胞系中的任何一种都可以用作融合伴侣,例如P3-NS1/1-Ag4-1,P3-x63-Ag8.653或Sp2/0-Ag14骨髓瘤系。然后使用HAT培养基选择由融合产生的杂交瘤细胞,其杀死未融合和非生产性融合的骨髓瘤细胞(未融合的脾细胞在培养几天后死亡,因为它们未被转化)。然后可以测定其中生长杂交瘤细胞的培养基中是否存在结合如本文所述的VAMP表位的单克隆抗体。例如,可以在免疫沉淀测定中使用切割的VAMP阳性培养基,体外结合测定法,例如放射免疫测定法(RIA)或酶联免疫吸附测定法(ELISA),或在基于细胞的活性测定中筛选杂交瘤上清液。单克隆抗体的结合亲和力也可以通过例如Scatchard分析来确定。参见,例如,Peter J.Munson和David Rodbard,Ligand:A Versatile Computerized Approach For Characterization of Ligand-Binding Systems,107(1)Anal.Biochem.220-239(1980)。在鉴定出所需的杂交瘤细胞后,使用有限稀释程序分离源自单个细胞的克隆,直至获得表达所需单克隆抗体的克隆细胞系。或者,可以通过用本发明的抗原多肽、蛋白质或肽筛选重组组合免疫球蛋白文库,例如抗体噬菌体展示文库,来产生结合如本文所述的VAMP表位的单克隆抗体。用于产生和筛选噬菌体展示

文库的试剂盒是可商购的,例如重组噬菌体抗体系统(Amersham GE Healthcare,Piscataway,NJ);和**SurfZAP™**噬菌体展示试剂盒(Stratagene,La JoIIa,CA)。另外,用于产生和筛选抗体展示文库的方法和试剂的实例可以在例如Ladner等人美国专利5,223,409;Borrebaeck等人美国专利5,712,089;Griffiths等人美国专利5,885,793;Griffiths等人美国专利5,962,255;McCafferty等人美国专利5,969,108;Griffiths等人美国专利6,010,884;Jespers等人美国专利6,017,732;Borrebaeck等人美国专利6,027,930;Johnson等人美国专利6,140,471;McCafferty等人美国专利6,172,197中找到,其每个都通过引用整体并入本文。

[0145] 另一方面,本发明提供了结合本发明的抗原多肽或蛋白质的抗体。

[0146] 在一个实施方案中,抗体是多克隆抗体。

[0147] 在一个实施方案中,抗体是单克隆抗体。

[0148] 抗体与抗原多肽或蛋白质之间的结合亲和力可以通过测定平衡解离常数(K_D)来评估,该平衡解离常数测量新抗体-抗原复合物形成的速率等于抗体-抗原复合物在平衡时解离的速率。平衡解离常数以M表示,并且由平衡时的Kd/Ka比定义,其中Ka是抗体的结合速率常数,Kd是抗体的解离速率常数。K_D=[Ab]x[Ag]/[Ab+Ag],其中[Ab]是抗体的摩尔浓度,[Ag]是抗原的摩尔浓度,并且[Ab+Ag]是抗体-抗原复合物的摩尔浓度,其中当系统处于平衡时,所有浓度是这些组分的浓度。平衡解离常数越小,抗体与其抗原的结合越紧密,或抗体与抗原之间的结合亲和力越高。

[0149] 在一个实施方案中,本发明的抗体与抗原多肽或蛋白质表位之间的K_D低于10^{-6}M。在优选的实施方案中,本发明的抗体与抗原多肽或蛋白质之间的K_D低于10^{-7}M。在更优选的实施方案中,本发明的抗体与抗原多肽或蛋白质之间的K_D低于10^{-8}M。在更优选的实施方案中,本发明的抗体与抗原多肽或蛋白质之间的K_D低于10^{-9}M。在更优选的实施方案中,本发明的抗体与抗原多肽或蛋白质之间的K_D低于10^{-10}M。在更优选的实施方案中,本发明的抗体与抗原多肽或蛋白质之间的K_D低于10^{-11}M。在更优选的实施方案中,本发明的抗体与抗原多肽或蛋白质之间的K_D低于10^{-12}M。

[0150] 另一方面,本发明提供了根据本发明的抗体在通过VAMP切割梭菌神经毒素进行VAMP切割的信号增益细胞测定中的用途。

[0151] 在一个实施方案中,该用途是体外或离体使用。

[0152] 另一方面,本发明提供了一种在细胞中通过切割VAMP的梭菌神经毒素来确定VAMP切割的方法,包括:

[0153] a)在适合梭菌神经毒素活性的条件下,使细胞与梭菌神经毒素接触;

[0154] b)在通过切割VAMP的梭菌神经毒素切割VAMP后,使所述细胞的细胞质内容物与针对C末端VAMP切割产物的第一检测抗体在适于第一检测抗体与C末端VAMP切割产物结合的条件下接触,其中所述第一检测抗体是根据本发明的抗体;和

[0155] c)通过适合的方法检测所述第一检测抗体与C末端VAMP切割产物的结合。

[0156] 在一个实施方案中,根据本发明的方法还包括d)通过适合的方法定量与所述第一检测抗体结合的C末端VAMP切割产物的量。

[0157] 在本发明方法的一个实施方案中,步骤b)包括在适于所述第二检测抗体与全长VAMP结合的条件下,使所述细胞的细胞质内容物与针对全长VAMP的第二检测抗体接触;步

骤c)包括通过适合的方法检测第二检测抗体与全长VAMP的结合,步骤d)包括通过适合的方法定量与所述第二检测抗体结合的全长VAMP的量。

[0158] 在一个实施方案中,该方法是体外或离体方法。

[0159] 本领域技术人员清楚的是,与第一抗体结合的C末端VAMP切割产物的量的增加和/或与第二检测抗体结合的全长VAMP的量的减少表明切割VAMP的梭菌神经毒素导致的VAMP切割增加。

[0160] 在一个实施方案中,第二检测抗体与第一检测抗体相同,并结合C末端VAMP切割产物和全长VAMP。

[0161] 在一个备选实施方案中,第二检测抗体不同于所述第一检测抗体,并且与全长VAMP结合但不与C-末端VAMP切割产物结合。合适地,第二检测抗体与梭菌神经毒素切割位点N末端的VAMP表位结合。合适的抗体的实例包括市售抗体,例如ab3347(Abcam)或ab181869(Abcam)。

[0162] 在一个具体实施方案中,通过确定在细胞中切割VAMP的梭菌神经毒素的VAMP切割的方法,包括:

[0163] a)在适合梭菌神经毒素活性的条件下,使细胞与梭菌神经毒素接触;

[0164] b)使所述细胞的细胞质内容物进行:

[0165] ·在通过切割VAMP的梭菌神经毒素切割VAMP后,在适于第一检测抗体与C末端VAMP切割产物结合的条件下,接触针对C末端VAMP切割产物的第一检测抗体,其中所述第一检测抗体是根据本发明的抗体,其与C末端VAMP切割产物和全长VAMP结合;和

[0166] ·接触第二检测抗体,其与全长VAMP结合但不与C末端VAMP切割产物结合;

[0167] c)通过适合的方法检测

[0168] ·第一抗体与C末端VAMP切割产物和全长VAMP的结合;和

[0169] ·第二检测抗体与全长VAMP的结合;和

[0170] d)通过适当的方式量化:

[0171] ·C末端VAMP切割产物和与第一检测抗体结合的全长VAMP的组合量;和

[0172] ·与第二检测抗体结合的全长VAMP的量。

[0173] 本领域技术人员清楚的是,与第二检测抗体结合的全长VAMP的量减少,以及与第一检测抗体结合的全长和C末端VAMP切割产物的组合量没有变化表明切割VAMP的梭菌神经毒素的VAMP切割。

[0174] 在另一个具体的实施方案中,确定在细胞中通过切割VAMP的梭菌神经毒素的VAMP切割的方法包括:

[0175] a)在适合梭菌神经毒素活性的条件下,使细胞与梭菌神经毒素接触;

[0176] b)通过切割VAMP的梭菌神经毒素切割VAMP后,使所述细胞的细胞质内容物与针对C末端VAMP切割产物的第一检测抗体在适于第一检测抗体与C末端VAMP结合的条件下接触,其中所述第一检测抗体是根据本发明的抗体,其结合C末端VAMP切割产物和全长VAMP;

[0177] c)通过适合的方法检测

[0178] ·第一抗体与C末端VAMP切割产物的结合;和

[0179] ·第一检测抗体与全长VAMP的结合;

[0180] 其中,第一检测抗体与C末端VAMP切割产物结合产生的信号可以与第一检测抗体

与全长VAMP结合产生的信号区分开;和

[0181] d)通过适当的方式量化:

[0182] •与第一检测抗体结合的C末端VAMP切割产物的量;和

[0183] •与第一检测抗体结合的全长VAMP的量。

[0184] 本领域技术人员清楚的是,与第一抗体结合的C末端VAMP切割产物的量的增加和与第一检测抗体结合的全长VAMP的量的减少表明通过切割VAMP的梭菌神经毒素的VAMP切割增加。

[0185] 另一方面,本发明提供了一种测定受试者中对切割VAMP的梭菌神经毒素的免疫耐受性的方法,包括:

[0186] a)向从受试者获得的测试样品中加入切割VAMP的梭菌神经毒素;

[0187] b)在适合梭菌神经毒素活性的条件下,使细胞与步骤a)的测试样品接触;

[0188] c)通过切割VAMP的梭菌神经毒素切割VAMP后,使所述细胞的细胞质内容物与针对C末端VAMP切割产物的第一检测抗体在适于第一检测抗体与C末端VAMP切割产物结合的条件下接触,其中所述第一检测抗体是根据本发明的抗体;

[0189] d)通过适合的方法检测第一检测抗体与C末端VAMP切割产物的结合;

[0190] e)量化与第一检测抗体结合的C末端VAMP切割产物的量;

[0191] f)用阴性对照样品代替测试样品重复步骤a)至e);和

[0192] g)比较步骤(e)和(f)中与所述第一检测抗体结合的C-末端VAMP切割产物的量,其中相对于在步骤(f)中与所述第一检测抗体结合的C-末端VAMP切割产物的量,在步骤(e)中检测到与所述第一检测抗体结合的较低量的C-末端VAMP切割产物,表明存在针对切割VAMP的梭菌神经毒素的中和抗体。

[0193] 在一个实施方案中,步骤f)还包括用阳性对照样品重复步骤a)至e)。

[0194] 如本文所用,术语“针对切割VAMP的梭菌神经毒素的中和抗体”是指在生理条件下将以减少或防止切割VAMP的梭菌神经毒素在受试者中发挥其治疗作用的方式结合切割VAMP的梭菌神经毒素的任何抗体。

[0195] 在一个实施方案中,受试者是哺乳动物。在优选的实施方案中,受试者是人。

[0196] 在一个实施方案中,样品选自从受试者获得的血液、血浆、血清和淋巴液。

[0197] 在暴露于切割VAMP的梭菌神经毒素之前,在用切割VAMP的梭菌神经毒素的单次处理之后或在用切割VAMP的梭菌神经毒素的多次处理之后,可以从受试者获得测试样品。在一个具体实施方案中,测试样品来自对用切割VAMP的梭菌神经毒素处理具有抗性的受试者。

[0198] 如本文所用,术语“对照样品”是指其中已知存在或不存在测试样品的任何样品,并且包括阴性和阳性对照样品。关于针对切割VAMP的梭菌神经毒素的中和抗体,可以从未暴露于切割VAMP的梭菌神经毒素的个体获得阴性对照样品,并且可以包括但不限于来自提供测试样品但在接受切割VAMP的梭菌神经毒素治疗前获取的相同个体的样品;取自从未暴露于切割VAMP的梭菌神经毒素的不同个体的样品;从多个从未暴露于切割VAMP的梭菌神经毒素的不同个体中获取的合并样品。

[0199] 关于针对切割VAMP的梭菌神经毒素的中和抗体,可以从对切割VAMP的梭菌神经毒素表现出免疫抗性的个体获得阳性对照样品,并且包括但不限于在基于患者的测试测定中

的测试阳性的个体;在体内生物测定中的测试阳性的个体;以及显示超免疫的个体,例如接种针对切割VAMP的梭菌神经毒素的受试者。

[0200] 在一个实施方案中,该方法是体外或离体方法。

[0201] 在用于确定免疫抗性的方法的一个实施方案中,步骤c)包括在适于所述第二检测抗体与全长VAMP结合的条件下,使所述细胞的细胞质内容物与针对全长VAMP的第二检测抗体接触;步骤d)包括通过适合的方法检测第二检测抗体与全长VAMP的结合,步骤e)包括定量与所述第二检测抗体结合的全长VAMP的量。

[0202] 在一个实施方案中,第二检测抗体与第一检测抗体相同,并结合C末端VAMP切割产物和全长VAMP。

[0203] 在一个备选实施方案中,第二检测抗体不同于所述第一检测抗体,并且与全长VAMP结合但不与C-末端VAMP切割产物结合。合适地,第二检测抗体与梭菌神经毒素切割位点N末端的VAMP表位结合。合适的抗体的实例包括市售抗体,例如ab3347(Abcam)或ab181869(Abcam)。

[0204] 在一个具体实施方案中,确定受试者中切割VAMP的梭菌神经毒素的免疫耐受性的方法包括:

[0205] a)向从受试者获得的测试样品中加入切割VAMP的梭菌神经毒素;

[0206] b)在适合梭菌神经毒素活性的条件下,使细胞与步骤a)的测试样品接触;

[0207] c)使所述细胞的细胞质内容物进行

[0208] •通过切割VAMP的梭菌神经毒素的VAMP切割后,在适于第一检测抗体与C末端VAMP切割产物结合的条件下,接触针对C末端VAMP切割产物的第一检测抗体,其中所述第一检测抗体是根据本发明的抗体,其与C末端VAMP切割产物和全长VAMP结合;和

[0209] •接触第二检测抗体,其与全长VAMP结合但不与C末端VAMP切割产物结合;

[0210] d)通过适合的方法检测

[0211] •第一抗体与C末端VAMP切割产物和全长VAMP的结合;和

[0212] •第二检测抗体与全长VAMP的结合;

[0213] e)量化

[0214] •C末端VAMP切割产物和与第一检测抗体结合的全长VAMP的组合量;和

[0215] •与第二检测抗体结合的全长VAMP的量;

[0216] f)用阴性对照样品代替测试样品重复步骤a)至e);和

[0217] g)比较步骤(e)和(f)中与所述第一检测抗体结合的C-末端VAMP切割产物的量,其中相对于在步骤(f)中的相应量,在步骤(e)中检测到与所述第一检测抗体结合的C-末端VAMP切割产物和全长VAMP的较低量和/或与第二检测抗体结合的全长VAMP的较高量表明存在针对切割VAMP的梭菌神经毒素的中和抗体。

[0218] 在另一个具体实施方案中,确定受试者中对切割VAMP的梭菌神经毒素的免疫耐受性的方法包括:

[0219] a)向从受试者获得的测试样品中加入切割VAMP的梭菌神经毒素;

[0220] b)在适合梭菌神经毒素活性的条件下,使细胞与步骤a)的测试样品接触;

[0221] c)通过切割VAMP的梭菌神经毒素的VAMP切割后,在适于第一检测抗体与C末端VAMP切割产物结合的条件下,所述细胞的细胞质内容物接触针对C末端VAMP切割产物的第

一检测抗体,其中所述第一检测抗体是根据本发明的抗体,其结合C末端VAMP切割产物和全长VAMP;

[0222] d)通过适合的手段检测

[0223] •第一抗体与C末端VAMP切割产物和全长VAMP的结合;和

[0224] •第一检测抗体与全长VAMP的结合;

[0225] 其中第一检测抗体与C末端VAMP切割产物结合产生的信号可以与第一检测抗体与全长VAMP结合产生的信号区分开;

[0226] e)量化

[0227] •与第一检测抗体结合的C末端VAMP切割产物的量;和

[0228] •与第一检测抗体结合的全长VAMP的量;

[0229] f)用阴性对照样品代替试样重复步骤a)到e);

[0230] g)比较步骤(e)和(f)中与所述第一检测抗体结合的C-末端VAMP切割产物的量,其中相对于在步骤(f)中的相应量,在步骤(e)中与所述第一检测抗体结合的C-末端VAMP切割产物的较低量和/或与第一检测抗体结合的全长VAMP的较高量表明存在针对切割VAMP的梭菌神经毒素的中和抗体。

[0231] 本文中,“切割VAMP的梭菌神经毒素”是指梭菌神经毒素,其与靶细胞上的受体结合,将梭菌轻链(L)转移到胞质溶胶中,然后蛋白水解切割VAMP,从而通过由细胞囊泡运输破坏分子的分泌。

[0232] 优选地,在本发明的方法或用途中,切割VAMP的梭菌神经毒素包括BoNT/B,BoNT/D,BoNT/F,BoNT/G,BoNT/X或TeNT轻链。合适地,BoNT/B,BoNT/D,BoNT/F,BoNT/G,BoNT/X或TeNT轻链包含选自以下的序列:

[0233] -SEQ ID NO:2的氨基酸残基1至441,或与其具有至少70%,优选至少75%,80%,85%,90%,95%或99%序列同一性的多肽序列,

[0234] -SEQ ID NO:4的氨基酸残基1至442,或与其具有至少70%,优选至少75%,80%,85%,90%,95%或99%序列同一性的多肽序列,

[0235] -SEQ ID NO:6的氨基酸残基1至439,或与其具有至少70%,优选至少75%,80%,85%,90%,95%或99%序列同一性的多肽序列,

[0236] -SEQ ID NO:7的氨基酸残基1至446,或与其具有至少70%,优选至少75%,80%,85%,90%,95%或99%序列同一性的多肽序列,

[0237] -SEQ ID NO:41的氨基酸残基1至439,或与其具有至少70%,优选至少75%,80%,85%,90%,95%或99%序列同一性的多肽序列,和

[0238] -SEQ ID NO:8的氨基酸残基1至456,或与其具有至少70%,优选至少75%,80%,85%,90%,95%或99%序列同一性的多肽序列。

[0239] 应理解,如本文所述的BoNT/B,BoNT/D,BoNT/F,BoNT/G,BoNT/X或TeNT轻链具有切割VAMP的能力。

[0240] 在本发明方法或用途的一个实施方案中,切割VAMP的梭菌神经毒素选自BoNT/B,BoNT/D,BoNT/F,BoNT/G,BoNT/X和TeNT。适当地,BoNT/B,BoNT/D,BoNT/F,BoNT/G,BoNT/X或TeNT包含选自以下的序列:

[0241] -SEQ ID NO:2或与其具有至少70%,优选至少75%,80%,85%,90%,95%或99%

序列同一性的多肽序列，

[0242] -SEQ ID NO:4，或与其具有至少70％，优选至少75％，80％，85％，90％，95％或99％序列同一性的多肽序列，

[0243] -SEQ ID NO:6，或与其具有至少70％，优选至少75％，80％，85％，90％，95％或99％序列同一性的多肽序列，

[0244] -SEQ ID NO:7，或与其具有至少70％，优选至少75％，80％，85％，90％，95％或99％序列同一性的多肽序列，

[0245] -SEQ ID NO:41，或与其具有至少70％，优选至少75％，80％，85％，90％，95％或99％序列同一性的多肽序列，和

[0246] -SEQ ID NO:8，或与其具有至少70％，优选至少75％，80％，85％，90％，95％或99％序列同一性的多肽序列。

[0247] 应理解，如本文所述的BoNT/B，BoNT/D，BoNT/F，BoNT/G，BoNT/X或TeNT梭菌神经毒素具有结合靶细胞上的受体，将梭菌轻链转移到细胞质和切割VAMP的能力。

[0248] 在一个实施方案中，切割VAMP的梭菌神经毒素是镶嵌(mosaic)神经毒素。在本文中使用的术语“镶嵌神经毒素”是指天然存在的梭菌神经毒素，其包含来自另一种梭菌神经毒素(例如不同血清型的梭菌神经毒素)的至少一个功能结构域，所述梭菌神经毒素通常不包含至少一个功能结构域。天然存在的切割VAMP的镶嵌神经毒素的实例是BoNT/DC和BoNT/FA。BoNT/DC包含血清型D的L链和H_N结构域和血清型C的H_C结构域，Nakamura K等人，“Characterization of the D/C mosaic neurotoxin produced by Clostridium botulinum associated with bovine botulism in Japan.”Vet.Microbiol.(2010):140:147-154，而BoNT/FA由BoNT/F5轻链，与亚型F1密切相关的H_N结构域和BoNT/A1H_C结构域组成(Pellett，Sabine等人，"Purification and Characterization of Botulinum Neurotoxin FA from a Genetically Modified Clostridium botulinum Strain." mSphere 1.1(2016):e00100-15)。

[0249] 在一个实施方案中，切割VAMP的梭菌神经毒素是选自BoNT/DC和BoNT/FA的镶嵌神经毒素。

[0250] 在一个实施方案中，切割VAMP的梭菌神经毒素是嵌合(chimeric)神经毒素。本文所用的术语“嵌合神经毒素”是指这样的神经毒素，其包含一个或多个源自第一神经毒素的结构域和一个或多个源自第二神经毒素的结构域。例如，嵌合神经毒素可包含源自第一神经毒素的LH_N结构域和源自第二神经毒素的H_C结构域。嵌合神经毒素的另一个实例是这样的神经毒素，其包含源自第一神经毒素的LH_NH_{CN}结构域和源自第二神经毒素的H_{CC}结构域。嵌合神经毒素的实例在GB1607901.4(尚未公开)中提供，其通过引用并入本文。

[0251] 在一个实施方案中，切割VAMP的梭菌神经毒素是嵌合神经毒素，其包含：

[0252] -来自BoNT/B，BoNT/D，BoNT/F，BoNT/G，BoNT/X或TeNT的轻链(L)，

[0253] -来自BoNT/A，BoNT/B，BoNT/C，BoNT/D，BoNT/E，BoNT/F，BoNT/G，BoNT/X或TeNT的H_N结构域，

[0254] -来自BoNT/A，BoNT/B，BoNT/C，BoNT/D，BoNT/E，BoNT/F，BoNT/G，BoNT/X或TeNT的H_{CN}结构域，

[0255] -来自BoNT/A，BoNT/B，BoNT/C，BoNT/D，BoNT/E，BoNT/F，BoNT/G，BoNT/X或TeNT的

H_{CC}结构域，

[0256]　其中至少两个结构域来自不同的梭菌神经毒素。

[0257]　在一个实施方案中，切割VAMP的梭菌神经毒素是嵌合神经毒素，其包含：

[0258]　-来自选自BoNT/B，BoNT/D，BoNT/F，BoNT/G，BoNT/X或TeNT的第一种梭菌神经毒素的LH_N结构域，

[0259]　-来自选自BoNT/A，BoNT/B，BoNT/C，BoNT/D，BoNT/E，BoNT/F，BoNT/G，BoNT/X或TeNT的第二种梭菌神经毒素的$H_{CN}H_{CC}$结构域，

[0260]　其中第一和第二梭菌神经毒素是不同的。

[0261]　在一个实施方案中，切割VAMP的梭菌神经毒素是嵌合神经毒素，其包含：

[0262]　-来自选自BoNT/B，BoNT/D，BoNT/F，BoNT/G，BoNT/X或TeNT的第一种梭菌神经毒素的LH_NH_{CN}结构域，

[0263]　-来自选自BoNT/A，BoNT/B，BoNT/C，BoNT/D，BoNT/E，BoNT/F，BoNT/G，BoNT/X或TeNT的第二种梭菌神经毒素的H_{CC}结构域，

[0264]　其中第一和第二梭菌神经毒素是不同的。

[0265]　切割VAMP的梭菌神经毒素可以是修饰的神经毒素或其衍生物，包括但不限于下面描述的那些。修饰的神经毒素或衍生物可含有一种或多种氨基酸，其与天然(未修饰的)形式的神经毒素相比已被修饰，或可含有一个或多个插入的氨基酸，其不存在于天然(未修饰的)形式的毒素中。举例来说，相对于天然(未修饰的)梭菌神经毒素序列，修饰的梭菌神经毒素可在一个或多个结构域中具有修饰的氨基酸序列。此类修饰可以修饰神经毒素的功能方面，例如生物活性或持久性。

[0266]　如本文所述的修饰的切割VAMP的梭菌神经毒素保留了与靶细胞上的受体结合，将轻链转移到细胞质中并切割VAMP的能力。

[0267]　修饰的切割VAMP的梭菌神经毒素可以在重链的氨基酸序列中具有一个或多个修饰(例如修饰的H_C结构域)，其中所述修饰的重链以比天然(未修饰的)神经毒素更高或更低的亲和力结合靶神经细胞。H_C结构域中的此类修饰可包括修饰神经节苷脂结合位点或H_{CC}结构域的蛋白质受体结合位点中的残基，其改变与神经节苷脂受体和/或靶神经细胞的蛋白质受体的结合。此类修饰的神经毒素的实例描述于WO 2006/027207和WO 2006/114308中，这两篇文献均通过引用整体并入本文。例如，来自BoNT/B神经毒素的H_{CC}结构域包含至少一个氨基酸残基取代、添加或缺失，其具有与天然BoNT/B H_{CC}序列相比增加BoNT/B H_{CC}结构域对人Syt II的结合亲和力的作用。BoNT/B H_{CC}亚结构域中合适的氨基酸残基取代、添加或缺失已在WO2013/180799和尚未公布的PCT/US2016/024211中公开(均通过引用并入本文)。H_{CC}亚结构域中合适的氨基酸残基取代、添加或缺失包括选自下组的取代突变：V1118M；Y1183M；E1191M；E1191I；E1191Q；E1191T；S1199Y；S1199F；S1199L；S1201V；E1191C，E1191V，E1191L，E1191Y，S1199W，S1199E，S1199H，W1178Y，W1178Q，W1178A，W1178S，Y1183C，Y1183P及其组合。

[0268]　在一个实施方案中，切割VAMP的梭菌神经毒素是重新靶向的神经毒素。本文所用的术语“重新靶向的神经毒素”(也称为“靶向分泌抑制剂”，“TSI”，“TVEMP”或“TEM”)是指包含与非梭菌受体结合的靶向部分(TM)的梭菌神经毒素。TM可以替代梭菌神经毒素重链的部分或全部H_C或H_{CC}结构域。重新靶向的神经毒素的实例公开于WO96/33273，WO98/07864，

WO00/10598,WO01/21213,WO01/53336;WO02/07759,WO2005/023309,WO2006/026780,WO2006/099590,WO2006/056093,WO2006/059105,WO2006/059113,WO2007/138339,WO2007/106115,WO2007/106799,WO2009/150469,WO2009/150470,WO2010/055358。,WO2010/020811,WO2010/138379,WO2010/138395,WO2010/138382,WO2011/020052,WO2011/020056,WO2011/020114,WO2011/020117,WO2011/20119,WO2012/156743,WO2012/134900,WO2012/134897,WO2012/134904,WO2012/134902,WO2012/135343,WO2012/135448,WO2012/135304,WO2012/134902,WO2014/033441,WO2014/128497,WO2014/053651,WO2015/004464,所有这些文献均通过引用并入本文。

[0269] 适用于本发明方法或用途的细胞的实例包括原核细胞,例如大肠杆菌细胞,酵母细胞,昆虫细胞,动物细胞,哺乳动物细胞,人细胞,小鼠细胞,灵长类动物细胞和/或神经元细胞。优选地,细胞是神经元细胞,特别是对BoNT具有高敏感性的细胞。

[0270] 对BoNT具有高敏感性的细胞是易受BoNT中毒影响的细胞。在一些实施方案中,对BoNT具有高敏感性的细胞是对例如,通过约500pM或更小,约400pM或更小,约300pM或更小,约200pM或更小,约100pM或更小,约90pM或更小,约80pM或更小,约70pM或更小,约60pM或更小,约50pM或更小,约40pM或更小,约30pM或更小,约20pM或更小,约10pM或更小,约9pM或更小,约8pM或更小,约7pM或更小,约6pM或更小,约5pM或更小,约4pM或更小,约3pM或更小,约2pM或更小,约1pM或更小,约0.9pM或更小,约0.8pM或更小,约0.7pM或更小,约0.6pM或更小,约0.5pM或更小,约0.4pM或更小,约0.3pM或更小,约0.2pM,约0.1pM或更小,约90fM或更小,约80fM或更小,约70fM或更小,约60fM或更小,约50fM或更小,约40fM或更小,约30fM或更小,约20fM或更小,或约10fM或更小的BoNT中毒敏感的细胞。

[0271] 优选地,细胞对切割VAMP的BoNT具有高灵敏度(如上所定义)。

[0272] 在一个实施方案中,细胞是对BoNT具有高度敏感性的原代神经元细胞,例如皮质神经元、海马神经元和/或脊髓神经元。例如,细胞是大鼠皮质神经元。

[0273] 在一个实施方案中,细胞来自对BoNT具有高敏感性的神经元细胞系,例如BE(2)-M17,Kelly,LA1-55n,N1E-115,N4TG3,N18,Neuro-2a,NG108-15,PC12,SH-SY5Y,SiMa和/或SK-N-BE(2)-C。

[0274] 在一个实施方案中,细胞是源自干细胞的神经元细胞,特别是来自诱导的多能干细胞(iPS细胞)的神经元细胞,例如**i-Cell**®神经元,**i-Cell**® DopaNeurons iCell谷氨酸能神经元,iCell MotoNeurons(细胞动力学公司)大脑皮层神经元,神经干细胞(Axol Biosciences),Peri.4U神经元,CNS.4U神经元,Dopa.4UNeurons(Axiogenesis),MNP细胞(Lonza),皮层神经元,运动神经元(iStem)和/或iPSC衍生的神经细胞(MTI-GlobalStem)。

[0275] 在一个实施方案中,可以通过重组技术修饰细胞以表达高水平的VAMP,例如VAMP1,VAMP2VAMP3,VAMP4,VAMP5和/或YKT6,更优选VAMP1,VAMP2和/或VAMP3。

[0276] 在其中切割VAMP的神经毒素是BoNT/B,BoNT/DC或BoNT/G的一个实施方案中,细胞表达高水平的突触结合蛋白I和/或突触结合蛋白II(Syt I/Syt II)。在其中切割VAMP的神经毒素是BoNT/B,BoNT/DC或BoNT/G的一个实施方案中,通过重组技术修饰细胞以表达高水平的突触结合蛋白I和/或突触结合蛋白II(Syt I/Syt II)。

[0277] 在其中切割VAMP的神经毒素是BoNT/FA,BoNT/F,BoNT/D或TeNT的一个实施方案中,细胞表达高水平的突触小泡蛋白(SV2)。在其中切割VAMP的神经毒素是BoNT/FA,BoNT/

F,BoNT/D或TeNT的一个实施方案中,通过重组技术修饰细胞以表达高水平的突触小泡蛋白(SV2)。

[0278] 如本文所用,“适合梭菌神经毒素活性的条件”是指梭菌神经毒素可与细胞膜上存在的梭菌神经毒素受体结合,将梭菌神经毒素轻链转移到细胞质和切割VAMP的条件(例如温度、pH、辅因子等)。

[0279] 在本发明方法的一个实施方案中,适合梭菌神经毒素活性的条件可包括在约37℃温育约1小时至约48小时的时间。在本发明方法的一个实施方案中,适合梭菌神经毒素活性的条件可包括在约37℃温育约2小时至约36小时的时间。在本发明方法的一个实施方案中,适合梭菌神经毒素活性的条件可包括在约37℃温育约4小时至约24小时的时间。

[0280] 例如,适合梭菌神经毒素活性的条件可包括在37℃下温育24小时。

[0281] 如本文所用,“适合于第一检测抗体与切割的VAMP结合的条件”和“适合于第二检测抗体与全长VAMP结合的条件”是指其中第一和/或第二检测抗体可以与切割的VAMP和/或全长VAMP结合的条件(例如温度、pH、辅因子等)。

[0282] 在本发明方法的一个实施方案中,适于抗体结合的条件可包括在约4℃温育约8小时至约48小时的时间。在本发明方法的一个实施方案中,适于抗体结合的条件可包括在约4℃温育约10小时至约24小时的时间。在本发明方法的一个实施方案中,适于抗体结合的条件可包括在约4℃温育约12小时至约16小时的时间。

[0283] 在本发明方法的一个实施方案中,适合抗体结合的条件可包括在约25℃温育约30分钟至约8小时的时间。在本发明方法的一个实施方案中,适合抗体结合的条件可包括在约25℃温育约1小时至约4小时的时间。在本发明方法的一个实施方案中,适于抗体结合的条件可包括在约25℃温育约1.5小时至约3小时的时间。

[0284] 适用于检测和定量检测抗体与切割或全长VAMP结合的方法是本领域熟知的。例如,可以通过蛋白质印迹检测和定量检测抗体与切割的或全长的VAMP的结合。当每种蛋白质通过SDS-PAGE以特定分子量运行时,将以比全长VAMP更低的分子量检测切割的VAMP。通过光密度测定法对条带的分析允许使用凝胶上相同泳道内的全长条带和切割条带的百分比切割读数。或者,可以使用酶联免疫吸附测定(ELISA),例如夹心ELISA检测和定量VAMP切割。

[0285] 在本发明方法的一个实施方案中,第一检测抗体是多克隆抗体,并且在酶联免疫吸附测定中检测并定量第一检测抗体与C端VAMP切割产物的结合。

[0286] 在本发明方法的一个实施方案中,第一检测抗体是多克隆抗体,并且在蛋白质印迹测定中检测并定量第一检测抗体与C末端VAMP切割产物的结合。

[0287] 在本发明方法的一个实施方案中,第一检测抗体是单克隆抗体,并且在酶联免疫吸附测定中检测并定量第一检测抗体与C端VAMP切割产物的结合。

[0288] 在本发明方法的一个实施方案中,第一检测抗体是单克隆抗体,并且在蛋白质印迹测定中检测并定量第一检测抗体与C末端VAMP切割产物的结合。

[0289] 在本发明方法的一个实施方案中,在细胞质内容物与检测抗体接触之前裂解细胞。

[0290] 在本发明方法的备选实施方案中,细胞在其细胞质内容物与检测抗体接触之前被透化。

[0291] 另一方面,本发明提供了一种试剂盒,其包含易受切割VAMP的神经毒素中毒的细胞;和针对切割的VAMP的第一检测抗体,其中所述第一检测抗体是本发明的抗体。

[0292] 在一个实施方案中,所述试剂盒还包含第二检测抗体,其与全长VAMP结合但不与C-末端VAMP切割产物结合。合适地,第二检测抗体与梭菌神经毒素切割位点N末端的VAMP表位结合。合适的抗体的实例包括市售抗体,例如ab3347(Abcam)或ab181869(Abcam)。

[0293] 本公开不受本文公开的示例性方法和材料的限制,并且与本文描述的那些类似或等同的任何方法和材料可以用于实践或测试本公开的实施方案。数字范围包括定义范围的数字。除非另有说明,否则任何核酸序列以5’至3’方向从左至右书写;氨基酸序列分别以氨基至羧基取向从左至右书写。

[0294] 在提供一系列值的情况下,应当理解,除非上下文另有明确规定,否则还具体公开了该范围的上限和下限之间的每个中间值,至下限单位的十分之一。在所述范围内的任何所述值或中间值与所述范围内的任何其他所述或中间值之间的每个较小范围都包含在本公开内。这些较小范围的上限和下限可以独立地包括在该范围内或在该范围内排除,并且在该较小范围内包括任一个、没有或两个极限的每个范围也包括在本公开内容中,受限于在所述范围中任何特别排除的极限。在所述范围包括一个或两个极限的情况下,排除那些被包括的极限之一或两者的范围也包括在本公开中。

[0295] 必须注意的是,除非上下文另有明确说明,如本文和所附权利要求中所使用的,单数形式“一”、“一个”和“该”包括复数参照。因此,例如,提及“一个梭菌神经毒素”包括多种这样的候选剂,并且提及“该梭菌神经毒素”包括提及一种或多种梭菌神经毒素及其本领域技术人员已知的等同物等。

[0296] 现在将参照以下附图和实施例仅通过举例描述本发明。

[0297] 附图

[0298] 图1-具有梭菌神经毒素切割位点的VAMP序列。(A)具有BoNT/F5和BoNT/FA,BoNT/F,BoNT/D和BoNT/DC,BoNT/B,BoNT/G,TeNT和BoNT/X切割位点的人和大鼠VAMP1,VAMP2和VAMP3序列。(B)具有BoNT/X切割位点的人和大鼠VAMP4,VAMP5和YKT6序列。

[0299] 图2-具有Ab表位(即免疫原性表位区域)和BoNT/F,BoNT/D和BoN/B切割位点的VAMP序列。显示人和大鼠VAMP1,VAMP2和VAMP3的序列用于比较。大鼠和人VAMP2序列在所选择的表位区域中是相同的。切割位点用箭头表示:BoNT/F和BoNT/D的VAMP2切割点分别位于相邻氨基酸Q58-K59和K59-L60上,而BoNT/B的切割点位于氨基酸Q76-F77(基于人VAMP2序列氨基酸位置)上。

[0300] 图3-用MBP-LF和LH_ND对重组VAMP2-GFP进行无细胞切割。将重组VAMP2-GFP与0.01μg/μl LH_ND或MBP-LF在37℃温育1小时。加入等体积的样品缓冲液,通过SDS-PAGE运行0.5μg(考马斯)和0.3μg(印迹)蛋白质,并用考马斯染色或用各种抗VAMP2抗体印迹。草图表示抗体表位的位置。重组蛋白的表示和表位的线长度未按比例绘制。1-BSA,2-VAMP2-GFP,3-切割的VAMP2-GFP(aa59/60-末端),4-切割的VAMP2-GFP(aa1-58/59)。

[0301] 图4-BoNT/F和BoNT/D处理后的体外VAMP切割。在96孔板中生长的大鼠皮质神经元直至DIV18-21用BoNT/F(A)或BoNT/D(B)处理24小时。通过SDS-PAGE电泳裂解物并用定制的抗VAMP2抗体印迹:抗Pep1,抗Pep2或抗Pep3,或用商业抗体ab1818691-全长VAMP2,2-切割的VAMP2印迹。抗pep 2数据显示全长VAMP2的剂量依赖性消失和低分子量切割片段的出现。

两个带信号用于量化BoNT/F(C)和BoNT/D(D)对VAMP2切割的剂量依赖性百分比。

[0302] 图5-(A)用天然BoNT/F1(),天然BoNT/A1(●)或重组BoNT/FA(Δ)处理大鼠皮质神经元24小时。裂解细胞,在SDS-PAGE上电泳并进行VAMP-2或SNAP-25切割的印迹。通过光密度分析从全长与切割蛋白的比例确定SNARE切割百分比。使用四参数逻辑方程拟合数据,并测定50%最大SNARE切割(pEC50)所需的BoNT浓度(B)。数据是平均值±s.e.m.(n=3(BoNT/F1和BoNT/A1)或4(BoNT/FA)独立实验,一式三份)。

[0303] 图6-BoNT/B和BoNT/F处理后的体外VAMP切割。生长至DIV18-21的大鼠皮层神经元用BoNT/F或BoNT/B处理24小时。通过SDS-PAGE电泳裂解物并用新定制的抗VAMP2抗体(抗Pep4),BoNT/B切割特异性抗VAMP2抗体或抗Pep1,抗Pep2或抗Pep3抗体印迹。

实施例

[0304] 实施例1:通过BoNT/D和BoNT/F检测VAMP蛋白水解切割

[0305] A-方法

[0306] 1.抗体生成

[0307] Eurogentec使用他们的Speedy 28天计划(https://secure.eurogentec.com/product/research-anti-protein-28-day-speedy-polyclonal-packages.html?country=gbr)生成抗体。用以下肽以每个肽两只兔子来进行免疫:

[0308] -VAMP PEP1:H2N-SNR RLQ QTQ AQV DEC-CONH2(SEQ ID NO:39);

[0309] -VAMP PEP2:AcNH-KLS ELD DRA DAL Q-CONH2(SEQ ID NO:15);或

[0310] -VAMP PEP3:H2N-CLQ AGA SQ-CONH2(SEQ ID NO:40)。

[0311] 动物进行第一次免疫和三次后续加强免疫。进行免疫前取血,中间取血和最终取血。

[0312] 2.重组蛋白质切割

[0313] 如前所述产生含有BoNT/D的轻链和易位结构域或与麦芽糖结合蛋白(MPB)融合的等同BoNT/F结构域的活性构建体(Masuyer等人,“Structure and activity of a functional derivative of Clostridium botulinum neurotoxin B.J Struct Biol”,174,p52-57,2011;Sutton等人,“Preparation of specifically activatable endopeptidase derivatives of Clostridium botulinum toxins type A,B,and C and their applications.Protein Expression and Purification 40:31-41,2005)。简而言之,LH_ND(SEQ ID NO:35)或称为MBP-LF(SEQ ID NO:36)的融合蛋白(后者是MBP与BoNT/F1轻链和C-末端6-组氨酸基序的融合体,并且MPB和6-组氨酸基序是通常已知的亲和标签)在测定缓冲液(50mM HEPES pH7.2,200μM ZnCl2,1μg/μlBSA,10mM DTT)中稀释至0.01μg/μl。将VAMP2-GFP(SEQ ID NO:37)(人VAMP2的氨基酸2-94和可检测标记绿色荧光蛋白(GFP)的融合蛋白)在测定缓冲液(50mM HEPES pH7.2,200μM ZnCl2,1μg/μlBSA,10mM DTT)中稀释至8μM。将等体积的LH_ND或MBP-LF和VAMP2-GFP(SEQ ID NO:37)(8μM)合并,并在37℃下温育1小时。通过加入2x还原样品缓冲液(NuPage LDS样品缓冲液,100mM DTT)终止反应。

[0314] 3.大鼠皮层神经元细胞培养

[0315] 从E17-E18CD大鼠胚胎制备大鼠皮质神经元。将解剖的皮质组织收集到冰冷的Hank’s平衡盐溶液(HBSS)w/o Ca2+或Mg2+中,然后按照制造商的说明书(Worthington

Biochemical,NJ,US)在37℃下在木瓜蛋白酶溶液中解离40分钟。将皮质细胞以20,000个细胞/孔的密度接种在聚-L-鸟氨酸(PLO)包被的96孔板上,在125μl含有2%B27补充物,0.5mM GlutaMAX,1%胎牛血清(FBS)和100U/ml青霉素/链霉素的Neurobasal培养基中。将细胞保持在37℃,含有5%CO2的潮湿气氛中。在DIV(体外天数)4时,加入另外125μl含有2%B27,0.5mM GlutaMAX的Neurobasal培养基。通过每周两次更换半培养基维持细胞。在DIV11时,向培养基中加入1.5μM胞嘧啶β-D-阿拉伯呋喃糖苷(AraC)以防止非神经元细胞的增殖。

[0316] 4.BoNT治疗

[0317] 用浓度范围的天然BoNT/F1(Metabiologics,US)(1nM-0.1pM)或BoNT/D(Metabiologics,US)(10nM-1pM)在37℃下一式三份的孔中处理DIV18-21的大鼠皮层神经元24小时。除去培养基,用PBS洗涤细胞一次。将细胞在40μl LRS样品缓冲液(NuPage LDS缓冲液,1mM DTT,1:500Benzonase)中在室温下裂解10分钟。

[0318] 5.SDS-PAGE和蛋白质印迹

[0319] 将神经元裂解物在90℃下煮沸5分钟。每个泳道加载15μl裂解物至12%Bis-Tris凝胶,并在200μV的MES缓冲液中运行50min。使用低MW程序通过Transblot Turbo(Biorad)将蛋白质转移到硝酸纤维素膜上。将膜在室温下用5%低脂乳/PBS-Tween封闭1小时,然后与定制的抗Pep1,抗Pep2或抗Pep3抗VAMP2一级抗体或与商业抗VAMP2抗体(Abcam ab3347和ab181869)一起在4℃过夜温育。将膜在PBS-Tween中洗涤3次,并与抗兔-HRP二级抗体在室温下温育1小时。将膜在PBS-Tween中洗涤3×5分钟,然后用SuperSignal West Femto化学发光底物显色,并使用Syngene PXi系统显现。

[0320] B-结果

[0321] 重组蛋白检测的评估

[0322] 图2中示出了来自VAMP2的所选三个肽表位相对于BoNT切割位点的区域。显示了人和大鼠VAMP1,VAMP2和VAMP3的序列用于比较。大鼠和人VAMP2序列在所选择的表位区域中是相同的。BoNT/B和BoNT/D的切割位点位于相邻的氨基酸上。

[0323] 最初,使用重组VAMP2-GFP在无细胞试验中测试抗体。含有毒素的酶促轻链结构域的BoNT/F和BoNT/D替代物(MBP-LF和$LH_{N}D$)用于切割VAMP蛋白(图3)。另外,使用另外两种市售的VAMP2抗体ab3347(表位aa1-18)和ab181869(aa1-100内的表位)作为比较。图3显示抗Pep1抗体检测到全长VAMP2和N-末端切割部分(aa1-58/59),信号大大减少。如所预期的,该抗体没有检测到C末端切割产物,因为其表位不在该部分上。抗Pep2和抗Pep3抗体检测到VAMP2-GFP的全长蛋白质和C末端切割产物。Ab3347仅检测到全长VAMP2而未检测到N末端切割片段,而ab181869检测到两者。

[0324] 这些第一结果表明所述抗体能够检测重组VAMP2的全长和预期的切割产物。例外是ab3347,其仅检测到全长VAMP2而不是N末端切割片段。

[0325] 内源性蛋白质检测的评估

[0326] 接下来的问题是这些抗体是否能够在神经细胞测定中检测到任何的切割产物,在所述神经细胞测定中将存在内源蛋白酶。用BoNT/F或BoNT/D处理大鼠原代皮质神经元并裂解用于WB分析(图4)。抗Pep1抗体仅识别全长蛋白质,并且没有可检测的切割产物。抗Pep 2抗体检测到全长和C末端切割的产物。抗Pep3抗体在细胞裂解物中显示出对单体VAMP非常差的亲和力的弱信号,并且检测到最可能是二聚体和其他蛋白质的更高分子量种类(数据

未示出)。全长单体信号非常低,但是存在BoNT/F和BoNT/D切割的C-末端产物的条带。换句话说,抗Pep3不检测全长VAMP,但弱检测到BoNT/F和BoNT/D切割的C末端片段。这与早期的无细胞结果形成对比,后者显示来自全长和切割的重组VAMP的强信号。由于在无细胞试验中不存在切割的蛋白检测,因此未在体外测试商业抗体Ab3347。尽管在无细胞试验中与N-末端切割的重组片段具有阳性结合,但商业抗体ab181869在皮质裂解物中检测到全长VAMP2,但在皮质裂解物中未检测到切割的片段。Pep 2数据用于量化BoNT/F(图4C)和BoNT/D(图4D)对VAMP2的剂量依赖性切割。

[0327] 本发明人最初表明,在无细胞系统中,可以检测两种重组VAMP切割产物。然而,当转移至细胞裂解物时,发明人还表明N端产物是不可检测的,但除了降解之外,还可能存在其他尚未知的机制。相反,本发明人已经表明,仍然与囊泡膜结合的C-末端VAMP片段不会以防止抗体结合和Western印迹检测的方式降解或改变。Pep2表位与BoNT/D和BoNT/F切割位点相邻并且针对该肽产生的抗体检测全长VAMP和切割产物。相反,针对更远离BoNT F/D切割位点的较短表位产生的抗Pep3抗体也检测到切割产物,尽管是弱的。

[0328] 实施例2:在大鼠皮质神经元中检测BoNT/FA和BoNT/F1的VAMP蛋白水解切割

[0329] A-方法

[0330] 1.大鼠皮层神经元细胞培养

[0331] 如实施例1中详述的制备大鼠皮质神经元。

[0332] 2.BoNT处理

[0333] 在一式三份孔中,在37℃下用浓度范围(1pM-1fM)的重组BoNT/FA(SEQ ID NO:38)或浓度范围(1nM-1pM)的天然BoNT/F1(Metabiologics,US)或浓度范围(1nM-1fM)的天然BoNT/A1(List Biological Laboratories Inc.,US)处理DIV18-21的大鼠皮层神经元24小时。除去培养基,用PBS洗涤细胞一次。将细胞在40μl LRS样品缓冲液(NuPage LDS缓冲液,1mM DTT,1:500Benzonase)中在室温下裂解10分钟。

[0334] 3.大鼠皮层神经元的SDS Page和Western印迹

[0335] 在室温下将大鼠皮质神经元在40μl裂解缓冲液(NuPage LDS样品缓冲液,1mM DTT和1:500Benzonase)中裂解10分钟。将样品在90℃下煮沸5分钟,每个泳道加入15μl裂解物至12%Bis-Tris凝胶,并在MOPS缓冲液中在200V下运行80min(SNAP-25)或MES缓冲液在200V下运行50min(VAMP2)。使用混合MW(SNAP25)或低MW(VAMP2)程序,通过Transblot Turbo(Biorad)将蛋白质转移到硝酸纤维素膜上。将膜在室温下用5%低脂乳/PBS-Tween封闭1小时,然后与抗SNAP25抗体(Sigma S9684 1:4000)或抗Pep2(1:500),一种如实施例1中所述的定制的抗体-VAMP2(Eurogentec)抗体一起温育;将每种一级抗体在4℃温育过夜。将膜在PBS-Tween中洗涤3次,并与抗兔-HRP二级抗体在室温下温育1小时。将膜在PBS-Tween中洗涤3×5min,然后用SuperSignal West Dura或West Femto化学发光底物显色,并使用Syngene Pxi系统显现。使用Genetools软件分析条带光密度测定法,并使用SNAP-25和VAMP2的全长蛋白质与切割产物的比率来测定%蛋白质切割。

[0336] B-结果

[0337] 用BoNT/F1,BoNT/A1或BoNT/FA处理24小时后,裂解大鼠皮质神经元,在SDS-PAGE上电泳并对VAMP-2(BoNT/F1和BoNT/FA)或SNAP-25(BoNT/A1)进行Western印迹。通过光密度测定法分析从全长与切割蛋白的比例来确定SNARE切割百分比。

[0338] 结果如图5所示。重组BoNT/FA切割的VAMP-2，效力pEC50＝12.75±0.14，n＝4。天然BoNT/F1切割的VAMP-2，效力pEC50＝10.77±0.12，n＝3。天然BoNT/A1切割的SNAP-25，效力pEC50＝12.38±0.14，n＝3。

[0339] 实施例3：在大鼠皮质神经元中检测BoNT/B的VAMP蛋白水解切割

[0340] A-方法

[0341] 1.抗体生成

[0342] Abcam使用用肽Pep4：FETSAAKLKRKYWWK(SEQ ID NO:49)免疫的兔产生单克隆抗体。

[0343] BoNT/B切割特异性抗VAMP2抗体(Kegel等人，Toxicology in Vitro；2007，21：p1641-1649)用于比较研究。

[0344] 2.大鼠皮层神经元细胞培养

[0345] 从E17-E18CD大鼠胚胎制备大鼠皮质神经元。将解剖的皮质组织收集到冰冷的Hank's平衡盐溶液(HBSS)w/o Ca2+或Mg2+中，然后按照制造商的说明书(Worthington Biochemical，NJ，US)在37℃下在木瓜蛋白酶溶液中解离40分钟。将皮质细胞以20,000个细胞/孔的密度接种在聚-L-鸟氨酸(PLO)包被的96孔板上，在125μl含有2％B27补充物，0.5mM GlutaMAX，1％胎牛血清(FBS)和100U/ml青霉素/链霉素的Neurobasal培养基中。将细胞保持在37℃，含有5％CO2的潮湿气氛中。在DIV(体外天数)4时，加入另外125μl含有2％B27，0.5mM GlutaMAX的Neurobasal培养基。通过每周两次更换半培养基维持细胞。在DIV11时，向培养基中加入1.5μM胞嘧啶β-D-阿拉伯呋喃糖苷(AraC)以防止非神经元细胞的增殖。

[0346] 3.BoNT治疗

[0347] 将大鼠皮质神经元在T25烧瓶中培养，并用1nM和10pM的BoNT/B(由List Biological Laboratories，Inc.提供)(SEQ ID NO:2)在37℃处理24小时。除去培养基，用PBS洗涤细胞一次。将细胞在1.5ml NuPage样品缓冲液(NuPage LDS缓冲液，1mM DTT，1：500Benzonase)中在室温下裂解10分钟。

[0348] 4.SDS-PAGE和Western印迹

[0349] 将神经元裂解物在90℃下煮沸5分钟。每个泳道加入15μl裂解物至12％Bis-Tris凝胶，并在200μV的MES缓冲液中运行50分钟。使用低MW程序通过Transblot Turbo(Biorad)将蛋白质转移到硝酸纤维素膜上。将膜在室温下用5％低脂乳/PBS-Tween封闭1小时，然后与定制的抗Pep1，抗Pep2，抗Pep3或抗Pep4抗体或与BoNT-B切割特异性抗体一起温育，在4℃过夜。将膜在PBS-Tween中洗涤3次，并与抗兔-HRP二级抗体在室温下温育1小时。将膜在PBS-Tween中洗涤3×5分钟，然后用SuperSignal West Femto化学发光底物显色，并使用Syngene PXi系统显现。

[0350] B-结果

[0351] 基于在上述实施例1和2中获得的结果，其暗示表位的位置是体外检测切割的VAMP的关键，产生针对位于C-末端侧上BoNT/B切割位点附近的表位的新的单克隆抗体。

[0352] 在BoNT/B和BoNT/F处理后，在相同的大鼠皮层测定中测试该抗体，并与抗Pep1，抗Pep2，抗Pep3和BoNT/B切割特异性抗体进行比较(图6)。所有比较抗体的表位区域位于BoNT/B切割位点的N末端侧。图6显示针对Pep4的新抗体表位的位置使得能够检测全长VAMP2，以及用于BoNT/B和BoNT/F处理的切割产物。相反，抗Pep2和抗Pep3抗体仅检测到

BoNT/F切割产物,但未检测到BoNT/B切割产物。抗Pep1抗体未检测到任何预期的裂解产物。BoNT/B切割特异性抗体也未在这些细胞裂解物中检测到任何BoNT/B切割产物。

[0353] 总之,本数据显示切割的VAMP检测的重要考虑因素是抗体表位的位置。只有针对切割后位于膜结合的VAMP片段上的表位产生的抗体才能够检测到该片段。通过将单克隆抗体表位定位于VAMP的C末端,假设该区域应存在于由切割VAMP的神经毒素血清型B,D和F产生的VAMP片段中。事实证明是这种情况,使得能够产生单一抗体(抗Pep4Mab),其为BoNT/B和BoNT/F处理的神经元提供阳性结果。此外,由于TeNT与BoNT/B具有相同的切割位点,并且BoNT/D切割位点与BoNT/F切割位点紧密相邻,因此预期该抗体也适用于TeNT和BoNT/D切割。

[0354] Pep4表位区域的另一个优点是针对该区域的抗体可以以相似的灵敏度检测全长和切割的VAMP。同时检测同一样品中的两种蛋白质形式的能力提供了用于归一化的强大工具,而无需额外的管家蛋白质的印迹。这提供了非常有用且直接的信号增益蛋白质印迹分析,用于定量细胞模型中的BoNT效力。

[0355] 本数据还显示了无细胞重组蛋白测定和全细胞模型之间VAMP检测的差异。正是这种无法检测细胞切割的VAMP构成了细胞中VAMP降解发生得非常快的假说的基础(Foran等人,“Evaluation of the therapeutic usefulness of botulinum neurotoxin B,C1,E and F compared with the long-lasting type A”.J.Biol Chem 278(2)pp1363-1371 2003)。与本发明的抗体相反,大多数商业上可获得的VAMP抗体针对蛋白质N-末端区域内的表位产生,因此N-末端VAMP片段是那些早期研究的焦点。尽管本文显示较小的C-末端VAMP片段在细胞中不降解,但也未检测到较大的N-末端片段。然而有趣的是,无细胞结果显示,即使它存在于缺乏任何蛋白酶的无细胞系统中,并非所有商业抗体都能够检测到预期的N-末端片段。从目前的数据可以得出结论,VAMP降解假说最肯定仅与N末端片段有关,并且C末端VAMP片段不降解并保持与囊泡膜结合。

[0356] 序列信息

[0357] • SEQ ID NO:1-BoNT/A1-UniProtKB登录号P10845(肉毒杆菌)

[0358] MPFVNKQFNYKDPVNGVDIAYIKIPNAGQMQPVKAFKIHNKIWVIPERDTFTNPEEGDLNPPPEAKQVPVSYYDSTYLSTDNEKDNYLKGVTKLFERIYSTDLGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDGSYRSEELNLVIIGPSADIIQFECKSFGHEVLNLTRNGYGSTQYIRFSPDFTFGFEESLEVDTNPLLGAGKFATDPAVTLAHELIHAGHRLYGIAINPNRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYYNKFKDIASTLNKAKSIVGTTASLQYMKNVFKEKYLLSEDTSGKFSVDKLKFDKLYKMLTEIYTEDNFVKFFKVLNRKTYLNFDKAVFKINIVPKVNYTIYDGFNLRNTNLAANFNGQNTEINNMNFTKLKNFTGLFEFYKLLCVRGIITSKTKSLDKGYNKALNDLCIKVNNWDLFFSPSEDNFTNDLNKGEEITSDTNIEAAEENISLDLIQQYYLTFNFDNEPENISIENLSSDIIGQLELMPNIERFPNGKKYELDKYTMFHYLRAQEFEHGKSRIALTNSVNEALLNPSRVYTFFSSDYVKKVNKATEAAMFLGWVEQLVYDFTDETSEVSTTDKIADITIIIPYIGPALNIGNMLYKDDFVGALIFSGAVILLEFIPEIAIPVLGTFALVSYIANKVLTVQTIDNALSKRNEKWDEVYKYIVTNWLAKVNTQIDLIRKKMKEALENQAEATKAIINYQYNQYTEEEKNNINFNIDDLSSKLNESINKAMININKFLNQCSVSYLMNSMIPYGVKRLEDFDASLKDALLKYIYDNRGTLIGQVDRLKDKVNNTLSTDIPFQLSKYVDNQRLLSTFTEYIKNIINTSILNLRYESNHLIDLSRYASKINIGSKVNFDPIDKNQIQLFNLESSKIEVILKNAIVYNSMYENFSTSFWIRIPKYFNSISLNNEYTIINCMENNSGWKVSLNYGEIIWTLQDTQEIKQRVVFKYSQMINISDYINRWIFVTITNNRLNNSKIYINGRLIDQKPISNLGNIHASNNIMFKLDGCRDTHRYIWIK

YFNLFDKELNEKEIKDLYDNQSNSGILKDFWGDYLQYDKPYYMLNLYDPNKYVDVNNVGIRGYMYLKGPRGSVMTTN
IYLNSSLYRGTKFIIKKYASGNKDNIVRNNDRVYINVVVKNKEYRLATNASQAGVEKILSALEIPDVGNLSQVVVMK
SKNDQGITNKCKMNLQDNNGNDIGFIGFHQFNNIAKLVASNWYNRQIERSSRTLGCSWEFIPVDDGWGERPL

[0359] • SEQ ID NO:2-BoNT/B1-UniProtKB登录号P10844(肉毒杆菌)

[0360] MPVTINNFNYNDPIDNNNIIMMEPPFARGTGRYYKAFKITDRIWIIPERYTFGYKPEDFNKSSGIFNR
DVCEYYDPDYLNTNDKKNIFLQTMIKLFNRIKSKPLGEKLLEMIINGIPYLGDRRVPLEEFNTNIASVTVNKLISN
PGEVERKKGIFANLIIFGPGPVLNENETIDIGIQNHFASREGFGGIMQMKFCPEYVSVFNNVQENKGASIFNRRGY
FSDPALILMHELIHVLHGLYGIKVDDLPIVPNEKKFFMQSTDAIQAEELYTFGGQDPSIITPSTDKSIYDKVLQNF
RGIVDRLNKVLVCISDPNININIYKNKFKDKYKFVEDSEGKYSIDVESFDKLYKSLMFGFTETNIAENYKIKTRAS
YFSDSLPPVKIKNLLDNEIYTIEEGFNISDKDMEKEYRGQNKAINKQAYEEISKEHLAVYKIQMCKSVKAPGICID
VDNEDLFFIADKNSFSDDLSKNERIEYNTQSNYIENDFPINELILDTDLISKIELPSENTESLTDFNVDVPVYEKQ
PAIKKIFTDENTIFQYLYSQTFPLDIRDISLTSSFDDALLFSNKVYSFFSMDYIKTANKVVEAGLFAGWVKQIVND
FVIEANKSNTMDKIADISLIVPYIGLALNVGNETAKGNFENAFEIAGASILLEFIPELLIPVVGAFLLESYIDNKN
KIIKTIDNALTKRNEKWSDMYGLIVAQWLSTVNTQFYTIKEGMYKALNYQAQALEEIIKYRYNIYSEKEKSNINID
FNDINSKLNEGINQAIDNINNFINGCSVSYLMKKMIPLAVEKLLDFDNTLKKNLLNYIDENKLYLIGSAEYEKSKV
NKYLKTIMPFDLSIYTNDTILIEMFNKYNSEILNNIILNLRYKDNNLIDLSGYGAKVEVYDGVELNDKNQFKLTSS
ANSKIRVTQNQNIIFNSVFLDFSVSFWIRIPKYKNDGIQNYIHNEYTIINCMKNNSGWKISIRGNRIIWTLIDING
KTKSVFFEYNIREDISEYINRWFFVTITNNLNNAKIYINGKLESNTDIKDIREVIANGEIIFKLDGDIDRTQFIWM
KYFSIFNTELSQSNIEERYKIQSYSEYLKDFWGNPLMYNKEY YMFNAGNKNSYIKLKKDSPVGEILTRSKYNQNS
KYINYRDLYIGEKFIIRRKSNSQSINDDIVRKEDYIYLDFFNLNQEWRVYTYKYFKKEEEKLFLAPISDSDEFYNTI
QIKEYDEQPTYSCQLLFKKDEESTDEIGLIGIHRFYESGIVFEEYKDYFCISKWYLKEVKRKPYNLKLGCNWQFIPK
DEGWTE

[0361] • SEQ ID NO:3-BoNT/C1-UniProtKB登录号P18640(肉毒杆菌)

[0362] MPITINNFNYSDPVDNKNILYLDTHLNTLANEPEKAFRITGNIWVIPDRFSRNSNPNLNKPPRVTSPK
SGYYDPNYLSTDSDKDPFLKEIIKLFKRINSREIGEELIYRLSTDIPFPGNNNTPINTFDFDVDFNSVDVKTRQGN
NWVKTGSINPSVIITGPRENIIDPETSTFKLTNNTFAAQEGFGALSIISISPRFMLTYSNATNDVGEGRFSKSEFC
MDPILILMHELNHAMHNLYGIAIPNDQTISSVTSNIFYSQYNVKLEYAEIYAFGGPTIDLIPKSARKYFEEKALDY
YRSIAKRLNSITTANPSSFNKYIGEYKQKLIRKYRFVVESSGEVTVNRNKFVELYNELTQIFTEFNYAKIYNVQNR
KIYLSNVYTPVTANILDDNVYDIQNGFNIPKSNLNVLFMGQNLSRNPALRKVNPENMLYLFTKFCHKAIDGRSLYN
KTLDCRELLVKNTDLPFIGDISDVKTDIFLRKDINEETEVIYYPDNVSVDQVILSKNTSEHGQLDLLYPSIDSESE
ILPGENQVFYDNRTQNVDYLNSYYYLESQKLSDNVEDFTFTRSIEEALDNSAKVYTYFPTLANKVNAGVQGGLFLM
WANDVVEDFTTNILRKDTLDKISDVSAIIPYIGPALNISNSVRRGNFTEAFAVTGVTILLEAFPEFTIPALGAFVI
YSKVQERNEIIKTIDNCLEQRIKRWKDSYEWMMGTWLSRIITQFNNISYQMYDSLNYQAGAIKAKIDLEYKKYSGS
DKENIKSQVENLKNSLDVKISEAMNNINKFIRECSVTYLFKNMLPKVIDELNEFDRNTKAKLINLIDSHNIILVGE
VDKLKAKVNNSFQNTIPFNIFSYTNNSLLKDIINEYFNNINDSKILSLQNRKNTLVDTSGYNAEVSEEGDVQLNPI
FPFDFKLGSSGEDRGKVIVTQNENIVYNSMYESFSISFWIRINKWVSNLPGYTIIDSVKNNSGWSIGIISNFLVFT
LKQNEDSEQSINFSYDISNNAPGYNKWFFVTVTNNMMGNMKIYINGKLIDTIKVKELTGINFSKTITFEINKIPDT
GLITSDSDNINMWIRDFYIFAKELDGKDINILFNSLQYTNVVKDYWGNDLRYNKEYYMVNIDYLNRYMYANSRQIV
FNTRRNNNDFNEGYKIIIKRIRGNTNDTRVRGGDILYFDMTINNKAYNLFMKNETMYADNHSTEDIYAIGLREQTK

DINDNIIFQIQPMNNTYYYASQ IFKSNFNGENISGICSIGTYRFRLGGDWYRHNYLVPTVKQGNYASLLESTSTHWGFVPVSE

[0363] • SEQ ID NO:4-BoNT/D-UniProtKB登录号P19321(肉毒杆菌)

[0364] MTWPVKDFNYSDPVNDNDILYLRIPQNKLITTPVKAFMITQNIWVIPERFSSDTNPSLSKPPRPTSKYQSYYDPSYLSTDEQKDTFLKGIIKLFKRINERDIGKKLINYLVVGSPFMGDSSTPEDTFDFTRHTTNIAVEKFENGSWKVTNIITPSVLIFGPLPNILDYTASLTLQGQQSNPSFEGFGTLSILKVAPEFLLTFSDVTSNQSSAVLGKSIFCMDPVIALMHELTHSLHQLYGINIPSDKRIRPQVSEGFFSQDGPNVQFEELYTFGGLDVEIIPQIERSQLREKALGHYKDIAKRLNNINKTIPSSWISNIDKYKKIFSEKYNFDKDNTGNFVVNIDKFNSLYSDLTNVMSEVVYSSQYNVKNRTHYFSRHYLPVFANILDDNIYTIRDGFNLTNKGFNIENSGQNIERNPALQKLSSESVVDLFTKVCLRLTKNSRDDSTCIKVKNNRLPYVADKDSISQEIFENKIITDETNVQNYSDKFSLDESILDGQVPINPEIVDPLLPNVNMEPLNLPGEEIVFYDDITKYVDYLNSYYYLESQKLSNNVENITLTTSVEEALGYSNKIYTFLPSLAEKVNKGVQAGLFLNWANEVVEDFTTNIMKKDTLDKISDVSVIIPYIGPALNIGNSALRGNFNQAFATAGVAFLLEGFPEFTIPALGVFTFYSSIQEREKIIKTIENCLEQRVKRWKDSYQWMVSNWLSRITTQFNHINYQMYDSLSYQADAIKAKIDLEYKKYSGSDKENIKSQVENLKNSLDVKISEAMNNINKFIRECSVTYLFKNMLPKVIDELNKFDLRTKTELINLIDSHNIILVGEVDRLKAKVNESFENTMPFNIFSYTNNSLLKDIINEYFNSINDSKILSLQNKKNALVDTSGYNAEVRVGDNVQLNTIYTNDFKLSSSGDKIIVNLNNNILYSAIYENSSVSFWIKISKDLTNSHNEYTIINSIEQNSGWKLCIRNGNIEWILQDVNRKYKSLIFDYSESLSHTGYTNKWFFVTITNNIMGYMKLYINGELKQSQKIEDLDEVKLDKTIVFGIDENIDENQMLWIRDFNIFSKELSNEDINIVYEGQILRNVIKDYWGNPLKFDTEYYIINDNYIDRYIAPESNVLVLVQYPDRSKLYTGNPITIKSVSDKNPYSRILNGDNIILHMLYNSRKYMIIRDTDTIYATQGGECSQNCVYALKLQSNLGNYGIGIFSIKNIVSKNKYCSQIFSSFRENTMLLADIYKPWRFSFKNAYTPVAVTNYETKLLSTSSFWKFISRDPGWVE

[0365] • SEQ ID NO:5-BoNT/E-登录号WP_003372387(肉毒杆菌)

[0366] MPKINSFNYNDPVNDRTILYIKPGGCQEFYKSFNIMKNIWIIPERNVIGTTPQDFHPPTSLKNGDSSYYDPNYLQSDEEKDRFLKIVTKIFNRINNNLSGGILLEELSKANPYLGNDNTPDNQFHIGDASAVEIKFSNGSQDILLPNVIIMGAEPDLFETNSSNISLRNNYMPSNHGFGSIAIVTFSPEYSFRFNDNSMNEFIQDPALTLMHELIHSLHGLYGAKGITTKYTITQKQNPLITNIRGTNIEEFLTFGGTDLNIITSAQSNDIYTNLLADYKKIASKLSKVQVSNPLLNPYKDVFEAKYGLDKDASGIYSVNINKFNDIFKKLYSFTEFDLATKFQVKCRQTYIGQYKYFKLSNLLNDSIYNISEGYNINNLKVNFRGQNANLNPRIITPITGRGLVKKIIRFCKNIVSVKGIRKSICIEINNGELFFVASENSYNDDNINTPKEIDDTVTSNNNYENDLDQVILNFNSESAPGLSDEKLNLTIQNDAYIPKYDSNGTSDIEQHDVNELNVFFYLDAQKVPEGENNVNLTSSIDTALLEQPKIYTFFSSEFINNVNKPVQAALFVSWIQQVLVDFTTEANQKSTVDKIADISIVVPYIGLALNIGNEAQKGNFKDALELLGAGILLEFEPELLIPTILVFTIKSFLGSSDNKNKVIKAINNALKERDEKWKEVYSFIVSNWMTKINTQFNKRKEQMYQALQNQVNAIKTIIESKYNSYTLEEKNELTNKYDIKQIENELNQKVSIAMNNIDRFLTESSISYLMKLINEVKINKLREYDENVKTYLLNYIIQHGSILGESQQELNSMVTDTLNNSIPFKLSSYTDDKILISYFNKFFKRIKSSSVLNMRYKNDKYVDTSGYDSNININGDVYKYPTNKNQFGIYNDKLSEVNISQNDYIIYDNKYKNFSISFWVRIPNYDNKIVNVNNEYTIINCMRDNNSGWKVSLNHNEIIWTLQDNAGINQKLAFNYGNANGISDYINKWIFVTITNDRLGDSKLYINGNLIDQKSILNLGNIHVSDNILFKIVNCSYTRYIGIRYFNIFDKELDETEIQTLYSNEPNTNILKDFWGNYLLYDKEYYLLNVLKPNNFIDRRKDSTLSINNIRSTILLANRLYSGIKVKIQRVNNSSTNDNLVRKNDQVYINFVASKTHLFPLYADTATTNKEKTIKISSSGNRFNQVVVMNSVGNNCTMNFKNNNGNNIGLLGFKADTVVASTWYYTHMRDHTNSNGCFWNFISEEHGWQEK

[0367] • SEQ ID NO:6-BoNT/F-UniProtKB登录号YP_001390123(肉毒杆菌)

[0368] MPVVINSFNYNDPVNDDTILYMQIPYEEKSKKYYKAFEIMRNVWIIPERNTIGTDPSDFDPPASLENGSSAYYDPNYLTTDAEKDRYLKTTIKLFKRINSNPAGEVLLQEISYAKPYLGNEHTPINEFHPVTRTTSVNIKSSTNVKSSIILNLLVLGAGPDIFENSSYPVRKLMDSGGVYDPSNDGFGSINIVTFSPEYEYTFNDISGGYNSSTESFIADPAISLAHELIHALHGLYGARGVTYKETIKVKQAPLMIAEKPIRLEEFLTFGGQDLNIITSAMKEKIYNNLLANYEKIATRLSRVNSAPPEYDINEYKDYFQWKYGLDKNADGSYTVNENKFNEIYKKLYSFTEIDLANKFKVKCRNTYFIKYGFLKVPNLLDDDIYTVSEGFNIGNLAVNNRGQNIKLNPKIIDSIPDKGLVEKIVKFCKSVIPRKGTKAPPRLCIRVNNRELFFVASESSYNENDINTPKEIDDTTNLNNNYRNNLDEVILDYNSETIPQISNQTLNTLVQDDSYVPRYDSNGTSEIEEHNVVDLNVFFYLHAQKVPEGETNISLTSSIDTALSEESQVYTFFSSEFINTINKPVHAALFISWINQVIRDFTTEATQKSTFDKIADISLVVPYVGLALNIGNEVQKENFKEAFELLGAGILLEFVPELLIPTILVFTIKSFIGSSENKNKIIKAINNSLMERETKWKEIYSWIVSNWLTRINTQFNKRKEQMYQALQNQVDAIKTVIEYKYNNYTSDERNRLESEYNINNIREELNKKVSLAMENIERFITESSIFYLMKLINEAKVSKLREYDEGVKEYLLDYISEHRSILGNSVQELNDLVTSTLNNSIPFELSSYTNDKILILYFNKLYKKIKDNSILDMRYENNKFIDISGYGSNISINGDVYIYSTNRNQFGIYSSKPSEVNIAQNNDIIYNGRYQNFSISFWVRIPKYFNKVNLNNEYTIIDCIRNNNSGWKISLNYNKIIWTLQDTAGNNQKLVFNYTQMISISDYINKWIFVTITNNRLGNSRIYINGNLIDEKSISNLGDIHVSDNILFKIVGCNDTRYVGIRYFKVFDTELGKTEIETLYSDEPDPSILKDFWGNYLLYNKRYYLLNLLRTDKSITQNSNFLNINQQRGVYQKPNIFSNTRLYTGVEVIIRKNGSTDISNTDNFVRKNDLAYINVVDRDVEYRLYADISIAKPEKIIKLIRTSNSNNSLGQIIVMDSIGNNCTMNFQNNNGGNIGLLGFHSNNLVASSWYYNNIRKNTSSNGCFWSFISKEHGWQEN

[0369] • SEQ ID NO:7-BoNT/G-UniProtKB登录号WP_039635782(肉毒杆菌)

[0370] MPVNIKNFNYNDPINNDDIIMMEPFNDPGPGTYYKAFRIIDRIWIVPERFTYGFQPDQFNASTGVFSKDVYEYYDPTYLKTDAEKDKFLKTMIKLFNRINSKPSGQRLLDMIVDAIPYLGNASTPPDKFAANVANVSINKKIIQPGAEDQIKGLMTNLIIFGPGPVLSDNFTDSMIMNGHSPISEGFGARMMIRFCPSCLNVFNNVQENKDTSIFSRRAYFADPALTLMHELIHVLHGLYGIKISNLPITPNTKEFFMQHSDPVQAEELYTFGGHDPSVISPSTDMNIYNKALQNFQDIANRLNIVSSAQGSGIDISLYKQIYKNKYDFVEDPNGKYSVDKDKFDKLYKALMFGFTETNLAGEYGIKTRYSYFSEYLPPIKTEKLLDNTIYTQNEGFNIASKNLKTEFNGQNKAVNKEAYEEISLEHLVIYRIAMCKPVMYKNTGKSEQCIIVNNEDLFFIANKDSFSKDLAKAETIAYNTQNNTIENNFSIDQLILDNDLSSGIDLPNENTEPFTNFDDIDIPVYIKQSALKKIFVDGDSLFEYLHAQTFPSNIENLQLTNSLNDALRNNNKVYTFFSTNLVEKANTVVGASLFVNWVKGVIDDFTSESTQKSTIDKVSDVSIIIPYIGPALNVGNETAKENFKNAFEIGGAAILMEFIPELIVPIVGFFTLESYVGNKGHIIMTISNALKKRDQKWTDMYGLIVSQWLSTVNTQFYTIKERMYNALNNQSQAIEKIIEDQYNRYSEEDKMNINIDFNDIDFKLNQSINLAINNIDDFINQCSISYLMNRMIPLAVKKLKDFDDNLKRDLLEYIDTNELYLLDEVNILKSKVNRHLKDSIPFDLSLYTKDTILIQVFNNYISNISSNAILSLSYRGGRLIDSSGYGATMNVGSDVIFNDIGNGQFKLNNSENSNITAHQSKFVVYDSMFDNFSINFWVRTPKYNNNDIQTYLQNEYTIISCIKNDSGWKVSIKGNRIIWTLIDVNAKSKSIFFEYSIKDNISDYINKWFSITITNDRLGNANIYINGSLKKSEKILNLDRINSSNDIDFKLINCTDTTKFVWIKDFNIFGRELNATEVSSLYWIQSSTNTLKDFWGNPLRYDTQYYLFNQGMQNIYIKYFSKASMGETAPRTNFNNAAINYQNLYLGLRFIIKKASNSRNINNDNIVREGDYIYLNIDNISDESYRVYVLVNSKEIQTQLFLAPINDDPTFYDVLQIKKYYEKTTYNCQILCEKDTKTFGLFGIGKFVKDYGYVWDTYDNYFCISQWYLRRISENINKLRLGCNWQFIPVDEGWTE

[0371] • SEQ ID NO:8-TeNT-UniProtKB登录号P04958(破伤风梭菌)

[0372] MPITINNFRYSDPVNNDTIIMMEPPYCKGLDIYYKAFKITDRIWIVPERYEFGTKPEDFNPPSSLIEGASEYYDPNYLRTDSDKDRFLQTMVKLFNRIKNNVAGEALLDKIINAIPYLGNSYSLLDKFDTNSNSVSFNLLEQDPSGATTKSAMLTNLIIFGPGPVLNKNEVRGIVLRVDNKNYFPCRDGFGSIMQMAFCPEYVPTFDNVIENITSLTIGK

SKYFQDPALLLMHELIHVLHGLYGMQVSSHEIIPSKQEIYMQHTYPISAEELFTFGGQDANLISIDIKNDLYEKTL
NDYKAIANKLSQVTSCNDPNIDIDSYKQIYQQKYQFDKDSNGQYIVNEDKFQILYNSIMYGFTEIELGKKFNIKTR
LSYFSMNHDPVKIPNLLDDTIYNDTEGFNIESKDLKSEYKGQNMRVNTNAFRNVDGSGLVSKLIGLCKKIIPPTNI
RENLYNRTASLTDLGGELCIKIKNEDLTFIAEKNSFSEEPFQDEIVSYNTKNKPLNFNYSLDKIIVDYNLQSKITL
PNDRTTPVTKGIPYAPEYKSNAASTIEIHNIDDNTIYQYLYAQKSPTTLQRITMTNSVDDALINSTKIYSYFPSVI
SKVNQGAQGILFLQWVRDIIDDFTNESSQKTTIDKISDVSTIVPYIGPALNIVKQGYEGNFIGALETTGVVLLLEY
IPEITLPVIAALSIAESSTQKEKIIKTIDNFLEKRYEKWIEVYKLVKAKWLGTVNTQFQKRSYQMYRSLEYQVDAI
KKIIDYEYKIYSGPDKEQIADEINNLKNKLEEKANKAMININIFMRESSRSFLVNQMINEAKKQLLEFDTQSKNIL
MQYIKANSKFIGITELKKLESKINKVFSTPIPFSYSKNLDCWVDNEEDIDVILKKSTILNLDINNDIISDISGFNS
SVITYPDAQLVPGINGKAIHLVNNESSEVIVHKAMDIEYNDMFNNFTVSFWLRVPKVSASHLEQYGTNEYSIISSM
KKHSLSIGSGWSVSLKGNNLIWTLKDSAGEVRQITFRDLPDKFNAYLANKWVFITITNDRLSSANLYINGVLMGSA
EITGLGAIREDNNITLKLDRCNNNNQYVSIDKFRIFCKALNPKEIEKLYTSYLSITFLRDFWGNPLRYDTEYYLIP
VASSSKDVQLKNITDYMYLTNAPSYTNGKLNIYYRRLYNGLKFIIKRYTPNNEIDSFVKSGDFIKLYVSYNNNEHI
VGYPKDGNAFNN LDRILRVGYNAPGIPLYKKMEAVKLRDLKTYSVQLKLYDDKNASLGLVGTHNGQIGNDPNRDI
LIASNWYFNHLKDKILGCDWYFVPTDEGWTND

[0373] • SEQ ID NO:9-VAMP1_大鼠(Q63666)

[0374] MSAPAQPPAEGTEGAAPGGGPPGPPPNTTSNRRLQQTQAQVEEVVDIMRVNVDKVLERDQKLSELDDRA
DALQAGASVFESSAAKLKRKYWWKNCKMMIMLGAICAIIVVVIVIYIFT

[0375] • SEQ ID NO:10-VAMP1_人(P23763)

[0376] MSAPAQPPAEGTEGTAPGGGPPGPPPNMTSNRRLQQTQAQVEEVVDIIRVNVDKVLERDQKLSELDDRA
DALQAGASQFESSAAKLKRKYWWKNCKMMIMLGAICAIIVVVIVIYFFT

[0377] • SEQ ID NO:11-VAMP2_大鼠(P63045)

[0378] MSATAATVPPAAPAGEGGPPAPPPNLTSNRRLQQTQAQVDEVVDIMRVNVDKVLERDQKLSELDDRADA
LQAGASQFETSAAKLKRKYWWKNLKMMIILGVICAIILIIIIVYFST

[0379] • SEQ ID NO:12-VAMP2_人(P63027)

[0380] MSATAATAPPAAPAGEGGPPAPPPNLTSNRRLQQTQAQVDEVVDIMRVNVDKVLERDQKLSELDDRADA
LQAGASQFETSAAKLKRKYWWKNLKMMIILGVICAIILIIIIVYFST

[0381] • SEQ ID NO:13-VAMP3_大鼠(P63025)

[0382] MSTGVPSGSSAATGSNRRLQQTQNQVDEVVDIMRVNVDKVLERDQKLSELDDRADALQAGASQFETSAA
KLKRKYWWKNCKMWAIGISVLVIIVIIIIVWCVS

[0383] • SEQ ID NO:14-VAMP3_人(Q15836)

[0384] MSTGPTAATGSNRRLQQTQNQVDEVVDIMRVNVDKVLERDQKLSELDDRADALQAGASQFETSAAKLKR
KYWWKNCKMWAIGITVLVIFIIIIIVWVVSS

[0385] • SEQ ID NO:15-VAMP表位

[0386] KLSELDDRADALQ

[0387] • SEQ ID NO:16-VAMP表位

[0388] QKLSELDDRADALQ

[0389] • SEQ ID NO:17-VAMP表位

[0390] KLSELDDRAD

[0391] • SEQ ID NO:18-VAMP表位

[0392] KLSELDDRADALQAGAS

[0393] • SEQ ID NO:19-VAMP表位

[0394] LSELDDRADALQ

[0395] • SEQ ID NO:20-VAMP表位

[0396] LSELDDRADA

[0397] • SEQ ID NO:21-VAMP表位

[0398] LSELDDRADALQAGAS

[0399] • SEQ ID NO:22-VAMP表位

[0400] FETSAAKLKRKYW

[0401] • SEQ ID NO:23-VAMP表位

[0402] FESSAAKLKRKYW

[0403] • SEQ ID NO:24-VAMP表位

[0404] QFETSAAKLKRKYW

[0405] • SEQ ID NO:25-VAMP表位

[0406] FETSAAKLKR

[0407] • SEQ ID NO:26-VAMP表位FETSAAKLKRKYWWKN

[0408] • SEQ ID NO:27-VAMP表位

[0409] AKLKRKYWWKN

[0410] • SEQ ID NO:28-VAMP表位

[0411] AAKLKRKYWWKN

[0412] • SEQ ID NO:29-VAMP表位

[0413] AKLKRKYWWKNCKM

[0414] • SEQ ID NO:30-VAMP表位

[0415] AKLKRKYWWKNLKM

[0416] • SEQ ID NO:31-VAMP表位

[0417] DQKLSELDDRADALQ

[0418] • SEQ ID NO:32-VAMP表位

[0419] ERDQKLSELDDRA

[0420] • SEQ ID NO:33-VAMP表位

[0421] LERDQKLSELDDRA

[0422] • SEQ ID NO:34-VAMP表位

[0423] VLERDQKLSELDDRA

[0424] • SEQ ID NO:35-LH$_N$D

[0425] MGSMTWPVKDFNYSDPVNDNDILYLRIPQNKLITTPVKAFMITQNIWVIPERFSSDTNPSLSKPPRPTSKYQSYYDPSYLSTDEQKDTFLKGIIKLFKRINERDIGKKLINYLVVGSPFMGDSSTPEDTFDFTRHTTNIAVEKFENGSWKVTNIITPSVLIFGPLPNILDYTASLTLQGQQSNPSFEGFGTLSILKVAPEFLLTFSDVTSNQSSAVLGKSIFCMDPVIALMHELTHSLHQLYGINIPSDKRIRPQVSEGFFSQDGPNVQFEELYTFGGLDVEIIPQIERSQLREKALGHYKDIAKRLNNINKTIPSSWISNIDKYKKIFSEKYNFDKDNTGNFVVNIDKFNSLYSDLTNVMSEVVYSSQYNVKNRTH

YFSRHYLPVFANILDDNIYTIRDGFNLTNKGFNIENSGQNIERNPALQKLSSESVVDLFTKVCVDKSEEKLYDDDDK
DRWGSSLQCIKVKNNRLPYVADKDSISQEIFENKIITDETNVQNYSDKFSLDESILDGQVPINPEIVDPLLPNVNME
PLNLPGEEIVFYDDITKYVDYLNSYYYLESQKLSNNVENITLTTSVEEALGYSNKIYTFLPSLAEKVNKGVQAGLFL
NWANEVVEDFTTNIMKKDTLDKISDVSVIIPYIGPALNIGNSALRGNFNQAFATAGVAFLLEGFPEFTIPALGVFTF
YSSIQEREKIIKTIENCLEQRVKRWKDSYQWMVSNWLSRITTQFNHINYQMYDSLSYQADAIKAKIDLEYKKYSGSD
KENIKSQVENLKNSLDVKISEAMNNINKFIRECSVTYLFKNMLPKVIDELNKFDLRTKTELINLIDSHNIILVGEVD
RLKAKVNESFENTMPFNIFSYTNNSLLKDIINEYFNLEAHHHHHHHHHH

[0426] • SEQ ID NO:36-MBP-LF

[0427] MKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAHDRFG
GYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDKELKAKGKS
ALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAEAAFNKGET
AMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPL
GAVALKSYEEELAKDPRIAATMENAQKGEIMPNIPQMSAFWYAVRTAVINAASGRQTVDEALKDAQTNSSSNNNNNN
NNNNLGIEGRISEFGSMPVAINSFNYNDPVNDDTILYMQIPYEEKSKKYYKAFEIMRNVWIIPERNTIGTNPSDFDP
PASLKNGSSAYYDPNYLTTDAEKDRYLKTTIKLFKRINSNPAGKVLLQEISYAKPYLGNDHTPIDEFSPVTRTTSVN
IKLSTNVESSMLLNLLVLGAGPDIFESCCYPVRKLIDPDVVYDPSNYGFGSINIVTFSPEYEYTFNDISGGHNSSTE
SFIADPAISLAHELIHALHGLYGARGVTYEETIEVKQAPLMIAEKPIRLEEFLTFGGQDLNIITSAMKEKIYNNLLA
NYEKIATRLSEVNSAPPEYDINEYKDYFQWKYGLDKNADGSYTVNENKFNEIYKKLYSFTESDLANKFKVKCRNTYF
IKYEFLKVPNLLDDDIYTVSEGFNIGNLAVNNRGQSIKLNPKIIDSIPDKGLVEKIVKFAVDKLAAALEHHHHHH

[0428] • SEQ ID NO:37(重组VAMP2-GFP)

[0429] GPLGSSATAATAPPAAPAGEGGPPAPPPNLTSNRRLQQTQAQVDEVVDIMRVNVDKVLERDQKLSELDD
RADALQAGASQFETSAAKLKRKYWWKNLKLENVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLK
FICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNR
IELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNH
YLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK

[0430] • SEQ ID NO:38-重组BoNT/FA

[0431] MPVVINSFNYDDPVNDNTIIYIRPPYYETSNTYFKAFQIMDNVWIIPERYRLGIDPSLFNPPVSLKAGS
DGYFDPNYLSTNTEKNKYLQIMIKLFKRINSKPAGQILLEEIKNAIPYLGNSYTQEEQFTTNNRTVSFNVKLANGNI
VQQMANLIIWGPGPDLTTNKTGGIIYSPYQSMEATPYKDGFGSIMTVEFSPEYATAFNDISIASHSPSLFIKDPALI
LMHELIHVLHGLYGTYITEYKITPNVVQSYMKVTKPITSAEFLTFGGRDRNIVPQSIQSQLYNKVLSDYKRIASRLN
KVNTATALINIDEFKNLYEWKYQFAKDSNGVYSVDLNKFEQLYKKIYSFTEFNLAYEFKIKTRLGYLAENFGPFYLP
NLLDDSIYTEVDGFNIGALSINYQGQNIGSDINSIKKLQGQGVVSRVVRLCKSVIPRKGTKAPPRLCITVNNRDLFF
IASQESYGENTINTYKEIDDTTTLDPSFEDILDKVILNFNEQVIPQMPNRNVSTDIQKDNYIPKYDYNRTDIIDSYE
VGRNYNTFFYLNAQKFSPNESNITLTSSFDTGLLEGSKVYTFFSSDFINNINKPVQALLFIEWVKQVIRDFTTEATK
TSTVDKLKDISLVVPYIGLALNIGDEIYKQHFAEAVELVGAGLLLEFSPEFLIPTLLIFTIKGYLTGSIRDKDKIIK
TLDNALNVRDQKWKELYRWVVSKWLTTINTQFNKRKEQMYKALKNQATAIKKIIENKYNNYTTDEKSKIDSSYNINE
IERTLNEKINLAMKNIEQFITESSIAYLINIINNETIQKLKSYDDLVRRYLLGYIRNHSSILGNSVEELNSKVNNHL
DNGIPFELSSYTNDSLLIRYFNKNYGELKYNCILNIKYEMDRDKLVDSSGYRSRINIGTGVKFSEIDKNQVQLSNLE
SSKIEVILNNGVIYNSMYENFSTSFWIRIPKYFRNINNEYKIISCMQNNSGWEVSLNFSNMNSKIIWTLQDTEGIKK

TVVFQYTQNINISDYINRWIFVTITNNRLSNSKIYINGRLINEESISDLGNIHASNNIMFKLDGCRDPHRYIWIKYFNLFDKELNKKEIKDLYDNQSNSGILKDFWGDYLQYDKPYYMLNLYDPNKYLDVNNVGIRGYMYLKGPRGRIVTTNIYLNSTLYMGTKFIIKKYASGNKDNIVRNNDRVYINVVVKNKEYRLATNASQAGVEKILSAVEIPDVGNLSQVVVMKSENDQGIRNKCKMNLQDNNGNDIGFIGFHQFNNIAKLVASNWYNRQIGKASRTFGCSWEFIPVDDGWGESSLHHHHHHHHH

[0432] • SEQ ID NO:39-Pep1

[0433] SNRRLQQTQAQVDEC

[0434] • SEQ ID NO:40-Pep3

[0435] CLQAGASQ

[0436] • SEQ ID NO:41-BoNT/X Genbank登录号BAQ12790(肉毒杆菌)

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[0438] • SEQ ID NO:42-VAMP4_大鼠(D4A560)

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[0440] • SEQ ID NO:43-VAMP4_人(O75379)

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[0442] • SEQ ID NO:44-VAMP5_大鼠(Q9Z2J5)

[0443] MAGKELERCQRQADQVTEIMLNNFDKVLERDGKLSELQQRSDQLLDMSSAFSKTTKTLAQQKRWENIRCRVYLGLAVAGGLLLILVVLLVIFLPSGEDSSKP

[0444] • SEQ ID NO:45-VAMP5_人(O95183)

[0445] MAGIELERCQQQANEVTEIMRNNFGKVLERGVKLAELQQRSDQLLDMSSTFNKTTQNLAQKKCWENIRY

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[0447] MKLYSLSVFYKGEPKAVLLKAAYDVSSFSFFQRSSVQEFMTFTSQLIVERSAKGSRASVKEQEYLCHVYVRSDSLAGVVIADSEYPSRVAFTLLEKVLDEFSKQVDRIDWPVGSPATIHYTALDGHLSRYQNPREADPMSKVQAELDETKIILHNTMESLLERGEKLDDLVSKSEVLGTQSKAFYKTARKQNSCCAIM

[0448] • SEQ ID NO:47-YKT6_人(O15498)

[0449] MKLYSLSVLYKGEAKVVLLKAAYDVSSFSFFQRSSVQEFMTFTSQLIVERSSKGTRASVKEQDYLCHVYVRNDSLAGVVIADNEYPSRVAFTLLEKVLDEFSKQVDRIDWPVGSPATIHYPALDGHLSRYQNPREADPMTKVQAELDETKIILHNTMESLLERGEKLDDLVSKSEVLGTQSKAFYKTARKQNSCCAIM

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[0453] FETSAAKLKRKYWWK

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[0455] QFESSAAKLKRKYW

[0456] • SEQ ID NO:51-VAMP表位

[0457] FESSAAKLKR

[0458] • SEQ ID NO:52-VAMP表位

[0459] FESSAAKLKRKYWWK

[0460] • SEQ ID NO:53-VAMP表位

[0461] ADALQAGASQF

[0462] • SEQ ID NO:54-VAMP表位

[0463] ADALQAGASQ

[0464] • SEQ ID NO:55-VAMP表位

[0465] RADALQAGASQF

[0466] • SEQ ID NO:56-VAMP表位

[0467] ADALQAGASQFE

[0468] • SEQ ID NO:57-VAMP表位

[0469] ADALQAGASVF

[0470] • SEQ ID NO:58-VAMP表位

[0471] ADALQAGASV

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[0484] • SEQ ID NO:65-VAMP表位

[0485] SESLSDNATAFS

[0486] • SEQ ID NO:66-VAMP表位

[0487] SDQLLDMSSTF

[0488] • SEQ ID NO:67-VAMP表位

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[0490] • SEQ ID NO:68-VAMP表位

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[0492] • SEQ ID NO:69-VAMP表位

[0493] SDQLLDMSSTFN

[0494] • SEQ ID NO:70-VAMP表位

[0495] SDQLLDMSSAF

[0496] • SEQ ID NO:71-VAMP表位

[0497] SDQLLDMSSA

[0498] • SEQ ID NO:72-VAMP表位

[0499] RSDQLLDMSSAF

[0500] • SEQ ID NO:73-VAMP表位

[0501] SDQLLDMSSAFS

[0502] • SEQ ID NO:74-VAMP表位

[0503] RSDQLLDMSS

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Ser Pro Ser Glu Asp Asn Phe Thr Asn Asp Leu Asn Lys Gly Glu Glu
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Leu Asp Lys Tyr Thr Met Phe His Tyr Leu Arg Ala Gln Glu Phe Glu
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Pro Arg Glu Asn Ile Ile Asp Pro Glu Thr Ser Thr Phe Lys Leu Thr
165 170 175
Asn Asn Thr Phe Ala Ala Gln Glu Gly Phe Gly Ala Leu Ser Ile Ile
180 185 190
Ser Ile Ser Pro Arg Phe Met Leu Thr Tyr Ser Asn Ala Thr Asn Asp
195 200 205
Val Gly Glu Gly Arg Phe Ser Lys Ser Glu Phe Cys Met Asp Pro Ile
210 215 220
Leu Ile Leu Met His Glu Leu Asn His Ala Met His Asn Leu Tyr Gly
225 230 235 240
Ile Ala Ile Pro Asn Asp Gln Thr Ile Ser Ser Val Thr Ser Asn Ile
245 250 255
Phe Tyr Ser Gln Tyr Asn Val Lys Leu Glu Tyr Ala Glu Ile Tyr Ala
260 265 270
Phe Gly Gly Pro Thr Ile Asp Leu Ile Pro Lys Ser Ala Arg Lys Tyr
275 280 285
Phe Glu Glu Lys Ala Leu Asp Tyr Tyr Arg Ser Ile Ala Lys Arg Leu
290 295 300
Asn Ser Ile Thr Thr Ala Asn Pro Ser Ser Phe Asn Lys Tyr Ile Gly
305 310 315 320

Glu Tyr Lys Gln Lys Leu Ile Arg Lys Tyr Arg Phe Val Val Glu Ser

325 330 335

Ser Gly Glu Val Thr Val Asn Arg Asn Lys Phe Val Glu Leu Tyr Asn

340 345 350

Glu Leu Thr Gln Ile Phe Thr Glu Phe Asn Tyr Ala Lys Ile Tyr Asn

355 360 365

Val Gln Asn Arg Lys Ile Tyr Leu Ser Asn Val Tyr Thr Pro Val Thr

370 375 380

Ala Asn Ile Leu Asp Asp Asn Val Tyr Asp Ile Gln Asn Gly Phe Asn

385 390 395 400

Ile Pro Lys Ser Asn Leu Asn Val Leu Phe Met Gly Gln Asn Leu Ser

405 410 415

Arg Asn Pro Ala Leu Arg Lys Val Asn Pro Glu Asn Met Leu Tyr Leu

420 425 430

Phe Thr Lys Phe Cys His Lys Ala Ile Asp Gly Arg Ser Leu Tyr Asn

435 440 445

Lys Thr Leu Asp Cys Arg Glu Leu Leu Val Lys Asn Thr Asp Leu Pro

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Phe Ile Gly Asp Ile Ser Asp Val Lys Thr Asp Ile Phe Leu Arg Lys

465 470 475 480

Asp Ile Asn Glu Glu Thr Glu Val Ile Tyr Tyr Pro Asp Asn Val Ser

485 490 495

[0010] Val Asp Gln Val Ile Leu Ser Lys Asn Thr Ser Glu His Gly Gln Leu

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Asp Leu Leu Tyr Pro Ser Ile Asp Ser Glu Ser Glu Ile Leu Pro Gly

515 520 525

Glu Asn Gln Val Phe Tyr Asp Asn Arg Thr Gln Asn Val Asp Tyr Leu

530 535 540

Asn Ser Tyr Tyr Tyr Leu Glu Ser Gln Lys Leu Ser Asp Asn Val Glu

545 550 555 560

Asp Phe Thr Phe Thr Arg Ser Ile Glu Glu Ala Leu Asp Asn Ser Ala

565 570 575

Lys Val Tyr Thr Tyr Phe Pro Thr Leu Ala Asn Lys Val Asn Ala Gly

580 585 590

Val Gln Gly Gly Leu Phe Leu Met Trp Ala Asn Asp Val Val Glu Asp

595 600 605

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

610 615 620

Val Ser Ala Ile Ile Pro Tyr Ile Gly Pro Ala Leu Asn Ile Ser Asn

625 630 635 640

Ser Val Arg Arg Gly Asn Phe Thr Glu Ala Phe Ala Val Thr Gly Val

645 650 655

Thr Ile Leu Leu Glu Ala Phe Pro Glu Phe Thr Ile Pro Ala Leu Gly

660 665 670

Ala Phe Val Ile Tyr Ser Lys Val Gln Glu Arg Asn Glu Ile Ile Lys
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Thr Ile Asp Asn Cys Leu Glu Gln Arg Ile Lys Arg Trp Lys Asp Ser
690 695 700
Tyr Glu Trp Met Met Gly Thr Trp Leu Ser Arg Ile Ile Thr Gln Phe
705 710 715 720
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725 730 735
Ala Ile Lys Ala Lys Ile Asp Leu Glu Tyr Lys Lys Tyr Ser Gly Ser
740 745 750
Asp Lys Glu Asn Ile Lys Ser Gln Val Glu Asn Leu Lys Asn Ser Leu
755 760 765
Asp Val Lys Ile Ser Glu Ala Met Asn Asn Ile Asn Lys Phe Ile Arg
770 775 780
Glu Cys Ser Val Thr Tyr Leu Phe Lys Asn Met Leu Pro Lys Val Ile
785 790 795 800
Asp Glu Leu Asn Glu Phe Asp Arg Asn Thr Lys Ala Lys Leu Ile Asn
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Leu Ile Asp Ser His Asn Ile Ile Leu Val Gly Glu Val Asp Lys Leu
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Lys Ala Lys Val Asn Asn Ser Phe Gln Asn Thr Ile Pro Phe Asn Ile
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[0011]
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Phe Asn Asn Ile Asn Asp Ser Lys Ile Leu Ser Leu Gln Asn Arg Lys
865 870 875 880
Asn Thr Leu Val Asp Thr Ser Gly Tyr Asn Ala Glu Val Ser Glu Glu
885 890 895
Gly Asp Val Gln Leu Asn Pro Ile Phe Pro Phe Asp Phe Lys Leu Gly
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Ser Ser Gly Glu Asp Arg Gly Lys Val Ile Val Thr Gln Asn Glu Asn
915 920 925
Ile Val Tyr Asn Ser Met Tyr Glu Ser Phe Ser Ile Ser Phe Trp Ile
930 935 940
Arg Ile Asn Lys Trp Val Ser Asn Leu Pro Gly Tyr Thr Ile Ile Asp
945 950 955 960
Ser Val Lys Asn Asn Ser Gly Trp Ser Ile Gly Ile Ile Ser Asn Phe
965 970 975
Leu Val Phe Thr Leu Lys Gln Asn Glu Asp Ser Glu Gln Ser Ile Asn
980 985 990
Phe Ser Tyr Asp Ile Ser Asn Asn Ala Pro Gly Tyr Asn Lys Trp Phe
995 1000 1005
Phe Val Thr Val Thr Asn Asn Met Met Gly Asn Met Lys Ile Tyr
1010 1015 1020

Ile Asn Gly Lys Leu Ile Asp Thr Ile Lys Val Lys Glu Leu Thr
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Gly Ile Asn Phe Ser Lys Thr Ile Thr Phe Glu Ile Asn Lys Ile
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Pro Asp Thr Gly Leu Ile Thr Ser Asp Ser Asp Asn Ile Asn Met
1055 1060 1065
Trp Ile Arg Asp Phe Tyr Ile Phe Ala Lys Glu Leu Asp Gly Lys
1070 1075 1080
Asp Ile Asn Ile Leu Phe Asn Ser Leu Gln Tyr Thr Asn Val Val
1085 1090 1095
Lys Asp Tyr Trp Gly Asn Asp Leu Arg Tyr Asn Lys Glu Tyr Tyr
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Met Val Asn Ile Asp Tyr Leu Asn Arg Tyr Met Tyr Ala Asn Ser
1115 1120 1125
Arg Gln Ile Val Phe Asn Thr Arg Arg Asn Asn Asn Asp Phe Asn
1130 1135 1140
Glu Gly Tyr Lys Ile Ile Ile Lys Arg Ile Arg Gly Asn Thr Asn
1145 1150 1155
Asp Thr Arg Val Arg Gly Gly Asp Ile Leu Tyr Phe Asp Met Thr
1160 1165 1170
Ile Asn Asn Lys Ala Tyr Asn Leu Phe Met Lys Asn Glu Thr Met
1175 1180 1185
[0012] Tyr Ala Asp Asn His Ser Thr Glu Asp Ile Tyr Ala Ile Gly Leu
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Arg Glu Gln Thr Lys Asp Ile Asn Asp Asn Ile Ile Phe Gln Ile
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Gln Pro Met Asn Asn Thr Tyr Tyr Tyr Ala Ser Gln Ile Phe Lys
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Pro Val Lys Ala Phe Met Ile Thr Gln Asn Ile Trp Val Ile Pro Glu
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Thr Ser Lys Tyr Gln Ser Tyr Tyr Asp Pro Ser Tyr Leu Ser Thr Asp
65                  70                  75                  80
Glu Gln Lys Asp Thr Phe Leu Lys Gly Ile Ile Lys Leu Phe Lys Arg
                85                  90                  95
Ile Asn Glu Arg Asp Ile Gly Lys Lys Leu Ile Asn Tyr Leu Val Val
            100                 105                 110
Gly Ser Pro Phe Met Gly Asp Ser Ser Thr Pro Glu Asp Thr Phe Asp
        115                 120                 125
Phe Thr Arg His Thr Thr Asn Ile Ala Val Glu Lys Phe Glu Asn Gly
    130                 135                 140
Ser Trp Lys Val Thr Asn Ile Ile Thr Pro Ser Val Leu Ile Phe Gly
145                 150                 155                 160
Pro Leu Pro Asn Ile Leu Asp Tyr Thr Ala Ser Leu Thr Leu Gln Gly
                165                 170                 175
Gln Gln Ser Asn Pro Ser Phe Glu Gly Phe Gly Thr Leu Ser Ile Leu
            180                 185                 190
[0013]
Lys Val Ala Pro Glu Phe Leu Leu Thr Phe Ser Asp Val Thr Ser Asn
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Gln Ser Ser Ala Val Leu Gly Lys Ser Ile Phe Cys Met Asp Pro Val
    210                 215                 220
Ile Ala Leu Met His Glu Leu Thr His Ser Leu His Gln Leu Tyr Gly
225                 230                 235                 240
Ile Asn Ile Pro Ser Asp Lys Arg Ile Arg Pro Gln Val Ser Glu Gly
                245                 250                 255
Phe Phe Ser Gln Asp Gly Pro Asn Val Gln Phe Glu Glu Leu Tyr Thr
            260                 265                 270
Phe Gly Gly Leu Asp Val Glu Ile Ile Pro Gln Ile Glu Arg Ser Gln
        275                 280                 285
Leu Arg Glu Lys Ala Leu Gly His Tyr Lys Asp Ile Ala Lys Arg Leu
    290                 295                 300
Asn Asn Ile Asn Lys Thr Ile Pro Ser Ser Trp Ile Ser Asn Ile Asp
305                 310                 315                 320
Lys Tyr Lys Lys Ile Phe Ser Glu Lys Tyr Asn Phe Asp Lys Asp Asn
                325                 330                 335
Thr Gly Asn Phe Val Val Asn Ile Asp Lys Phe Asn Ser Leu Tyr Ser
            340                 345                 350
Asp Leu Thr Asn Val Met Ser Glu Val Val Tyr Ser Ser Gln Tyr Asn
        355                 360                 365
Val Lys Asn Arg Thr His Tyr Phe Ser Arg His Tyr Leu Pro Val Phe
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Ala Asn Ile Leu Asp Asp Asn Ile Tyr Thr Ile Arg Asp Gly Phe Asn
385 390 395 400
Leu Thr Asn Lys Gly Phe Asn Ile Glu Asn Ser Gly Gln Asn Ile Glu
405 410 415
Arg Asn Pro Ala Leu Gln Lys Leu Ser Ser Glu Ser Val Val Asp Leu
420 425 430
Phe Thr Lys Val Cys Leu Arg Leu Thr Lys Asn Ser Arg Asp Asp Ser
435 440 445
Thr Cys Ile Lys Val Lys Asn Asn Arg Leu Pro Tyr Val Ala Asp Lys
450 455 460
Asp Ser Ile Ser Gln Glu Ile Phe Glu Asn Lys Ile Ile Thr Asp Glu
465 470 475 480
Thr Asn Val Gln Asn Tyr Ser Asp Lys Phe Ser Leu Asp Glu Ser Ile
485 490 495
Leu Asp Gly Gln Val Pro Ile Asn Pro Glu Ile Val Asp Pro Leu Leu
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Pro Asn Val Asn Met Glu Pro Leu Asn Leu Pro Gly Glu Glu Ile Val
515 520 525
Phe Tyr Asp Asp Ile Thr Lys Tyr Val Asp Tyr Leu Asn Ser Tyr Tyr
530 535 540
[0014] Tyr Leu Glu Ser Gln Lys Leu Ser Asn Asn Val Glu Asn Ile Thr Leu
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Thr Thr Ser Val Glu Glu Ala Leu Gly Tyr Ser Asn Lys Ile Tyr Thr
565 570 575
Phe Leu Pro Ser Leu Ala Glu Lys Val Asn Lys Gly Val Gln Ala Gly
580 585 590
Leu Phe Leu Asn Trp Ala Asn Glu Val Val Glu Asp Phe Thr Thr Asn
595 600 605
Ile Met Lys Lys Asp Thr Leu Asp Lys Ile Ser Asp Val Ser Val Ile
610 615 620
Ile Pro Tyr Ile Gly Pro Ala Leu Asn Ile Gly Asn Ser Ala Leu Arg
625 630 635 640
Gly Asn Phe Asn Gln Ala Phe Ala Thr Ala Gly Val Ala Phe Leu Leu
645 650 655
Glu Gly Phe Pro Glu Phe Thr Ile Pro Ala Leu Gly Val Phe Thr Phe
660 665 670
Tyr Ser Ser Ile Gln Glu Arg Glu Lys Ile Ile Lys Thr Ile Glu Asn
675 680 685
Cys Leu Glu Gln Arg Val Lys Arg Trp Lys Asp Ser Tyr Gln Trp Met
690 695 700
Val Ser Asn Trp Leu Ser Arg Ile Thr Thr Gln Phe Asn His Ile Asn
705 710 715 720
Tyr Gln Met Tyr Asp Ser Leu Ser Tyr Gln Ala Asp Ala Ile Lys Ala

725 730 735
Lys Ile Asp Leu Glu Tyr Lys Lys Tyr Ser Gly Ser Asp Lys Glu Asn
740 745 750
Ile Lys Ser Gln Val Glu Asn Leu Lys Asn Ser Leu Asp Val Lys Ile
755 760 765
Ser Glu Ala Met Asn Asn Ile Asn Lys Phe Ile Arg Glu Cys Ser Val
770 775 780
Thr Tyr Leu Phe Lys Asn Met Leu Pro Lys Val Ile Asp Glu Leu Asn
785 790 795 800
Lys Phe Asp Leu Arg Thr Lys Thr Glu Leu Ile Asn Leu Ile Asp Ser
805 810 815
His Asn Ile Ile Leu Val Gly Glu Val Asp Arg Leu Lys Ala Lys Val
820 825 830
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850 855 860
Asn Asp Ser Lys Ile Leu Ser Leu Gln Asn Lys Lys Asn Ala Leu Val
865 870 875 880
Asp Thr Ser Gly Tyr Asn Ala Glu Val Arg Val Gly Asp Asn Val Gln
885 890 895
[0015] Leu Asn Thr Ile Tyr Thr Asn Asp Phe Lys Leu Ser Ser Ser Gly Asp
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Lys Ile Ile Val Asn Leu Asn Asn Asn Ile Leu Tyr Ser Ala Ile Tyr
915 920 925
Glu Asn Ser Ser Val Ser Phe Trp Ile Lys Ile Ser Lys Asp Leu Thr
930 935 940
Asn Ser His Asn Glu Tyr Thr Ile Ile Asn Ser Ile Glu Gln Asn Ser
945 950 955 960
Gly Trp Lys Leu Cys Ile Arg Asn Gly Asn Ile Glu Trp Ile Leu Gln
965 970 975
Asp Val Asn Arg Lys Tyr Lys Ser Leu Ile Phe Asp Tyr Ser Glu Ser
980 985 990
Leu Ser His Thr Gly Tyr Thr Asn Lys Trp Phe Phe Val Thr Ile Thr
995 1000 1005
Asn Asn Ile Met Gly Tyr Met Lys Leu Tyr Ile Asn Gly Glu Leu
1010 1015 1020

Lys Gln Ser Gln Lys Ile Glu Asp Leu Asp Glu Val Lys Leu Asp
1025 1030 1035
Lys Thr Ile Val Phe Gly Ile Asp Glu Asn Ile Asp Glu Asn Gln
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Asn Glu Asp Ile Asn Ile Val Tyr Glu Gly Gln Ile Leu Arg Asn
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Val Ile Lys Asp Tyr Trp Gly Asn Pro Leu Lys Phe Asp Thr Glu
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Tyr Tyr Ile Ile Asn Asp Asn Tyr Ile Asp Arg Tyr Ile Ala Pro
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Glu Ser Asn Val Leu Val Leu Val Gln Tyr Pro Asp Arg Ser Lys
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Leu Tyr Thr Gly Asn Pro Ile Thr Ile Lys Ser Val Ser Asp Lys
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Asn Pro Tyr Ser Arg Ile Leu Asn Gly Asp Asn Ile Ile Leu His
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Met Leu Tyr Asn Ser Arg Lys Tyr Met Ile Ile Arg Asp Thr Asp
1160 1165 1170
Thr Ile Tyr Ala Thr Gln Gly Gly Glu Cys Ser Gln Asn Cys Val
1175 1180 1185
Tyr Ala Leu Lys Leu Gln Ser Asn Leu Gly Asn Tyr Gly Ile Gly
1190 1195 1200
Ile Phe Ser Ile Lys Asn Ile Val Ser Lys Asn Lys Tyr Cys Ser
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Gln Ile Phe Ser Ser Phe Arg Glu Asn Thr Met Leu Leu Ala Asp
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[0016] Ile Tyr Lys Pro Trp Arg Phe Ser Phe Lys Asn Ala Tyr Thr Pro
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Gly Thr Thr Pro Gln Asp Phe His Pro Pro Thr Ser Leu Lys Asn Gly
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Asp Ser Ser Tyr Tyr Asp Pro Asn Tyr Leu Gln Ser Asp Glu Glu Lys
65 70 75 80
Asp Arg Phe Leu Lys Ile Val Thr Lys Ile Phe Asn Arg Ile Asn Asn

85 90 95
Asn Leu Ser Gly Gly Ile Leu Leu Glu Glu Leu Ser Lys Ala Asn Pro
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Tyr Leu Gly Asn Asp Asn Thr Pro Asp Asn Gln Phe His Ile Gly Asp
115 120 125
Ala Ser Ala Val Glu Ile Lys Phe Ser Asn Gly Ser Gln Asp Ile Leu
130 135 140
Leu Pro Asn Val Ile Ile Met Gly Ala Glu Pro Asp Leu Phe Glu Thr
145 150 155 160
Asn Ser Ser Asn Ile Ser Leu Arg Asn Asn Tyr Met Pro Ser Asn His
165 170 175
Gly Phe Gly Ser Ile Ala Ile Val Thr Phe Ser Pro Glu Tyr Ser Phe
180 185 190
Arg Phe Asn Asp Asn Ser Met Asn Glu Phe Ile Gln Asp Pro Ala Leu
195 200 205
Thr Leu Met His Glu Leu Ile His Ser Leu His Gly Leu Tyr Gly Ala
210 215 220
Lys Gly Ile Thr Thr Lys Tyr Thr Ile Thr Gln Lys Gln Asn Pro Leu
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245 250 255
[0017] Gly Thr Asp Leu Asn Ile Ile Thr Ser Ala Gln Ser Asn Asp Ile Tyr
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Thr Asn Leu Leu Ala Asp Tyr Lys Lys Ile Ala Ser Lys Leu Ser Lys
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290 295 300
Ala Lys Tyr Gly Leu Asp Lys Asp Ala Ser Gly Ile Tyr Ser Val Asn
305 310 315 320
Ile Asn Lys Phe Asn Asp Ile Phe Lys Lys Leu Tyr Ser Phe Thr Glu
325 330 335
Phe Asp Leu Ala Thr Lys Phe Gln Val Lys Cys Arg Gln Thr Tyr Ile
340 345 350
Gly Gln Tyr Lys Tyr Phe Lys Leu Ser Asn Leu Leu Asn Asp Ser Ile
355 360 365
Tyr Asn Ile Ser Glu Gly Tyr Asn Ile Asn Asn Leu Lys Val Asn Phe
370 375 380
Arg Gly Gln Asn Ala Asn Leu Asn Pro Arg Ile Ile Thr Pro Ile Thr
385 390 395 400
Gly Arg Gly Leu Val Lys Lys Ile Ile Arg Phe Cys Lys Asn Ile Val
405 410 415
Ser Val Lys Gly Ile Arg Lys Ser Ile Cys Ile Glu Ile Asn Asn Gly
420 425 430
Glu Leu Phe Phe Val Ala Ser Glu Asn Ser Tyr Asn Asp Asp Asn Ile

435 440 445
Asn Thr Pro Lys Glu Ile Asp Asp Thr Val Thr Ser Asn Asn Asn Tyr
450 455 460
Glu Asn Asp Leu Asp Gln Val Ile Leu Asn Phe Asn Ser Glu Ser Ala
465 470 475 480
Pro Gly Leu Ser Asp Glu Lys Leu Asn Leu Thr Ile Gln Asn Asp Ala
485 490 495
Tyr Ile Pro Lys Tyr Asp Ser Asn Gly Thr Ser Asp Ile Glu Gln His
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Asp Val Asn Glu Leu Asn Val Phe Phe Tyr Leu Asp Ala Gln Lys Val
515 520 525
Pro Glu Gly Glu Asn Asn Val Asn Leu Thr Ser Ser Ile Asp Thr Ala
530 535 540
Leu Leu Glu Gln Pro Lys Ile Tyr Thr Phe Phe Ser Ser Glu Phe Ile
545 550 555 560
Asn Asn Val Asn Lys Pro Val Gln Ala Ala Leu Phe Val Ser Trp Ile
565 570 575
Gln Gln Val Leu Val Asp Phe Thr Thr Glu Ala Asn Gln Lys Ser Thr
580 585 590
Val Asp Lys Ile Ala Asp Ile Ser Ile Val Val Pro Tyr Ile Gly Leu
595 600 605
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Leu Ile Pro Thr Ile Leu Val Phe Thr Ile Lys Ser Phe Leu Gly Ser
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Ser Asp Asn Lys Asn Lys Val Ile Lys Ala Ile Asn Asn Ala Leu Lys
660 665 670
Glu Arg Asp Glu Lys Trp Lys Glu Val Tyr Ser Phe Ile Val Ser Asn
675 680 685
Trp Met Thr Lys Ile Asn Thr Gln Phe Asn Lys Arg Lys Glu Gln Met
690 695 700
Tyr Gln Ala Leu Gln Asn Gln Val Asn Ala Ile Lys Thr Ile Ile Glu
705 710 715 720
Ser Lys Tyr Asn Ser Tyr Thr Leu Glu Glu Lys Asn Glu Leu Thr Asn
725 730 735
Lys Tyr Asp Ile Lys Gln Ile Glu Asn Glu Leu Asn Gln Lys Val Ser
740 745 750
Ile Ala Met Asn Asn Ile Asp Arg Phe Leu Thr Glu Ser Ser Ile Ser
755 760 765
Tyr Leu Met Lys Leu Ile Asn Glu Val Lys Ile Asn Lys Leu Arg Glu
770 775 780
Tyr Asp Glu Asn Val Lys Thr Tyr Leu Leu Asn Tyr Ile Ile Gln His

785 790 795 800
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Asp Thr Leu Asn Asn Ser Ile Pro Phe Lys Leu Ser Ser Tyr Thr Asp
820 825 830
Asp Lys Ile Leu Ile Ser Tyr Phe Asn Lys Phe Phe Lys Arg Ile Lys
835 840 845
Ser Ser Ser Val Leu Asn Met Arg Tyr Lys Asn Asp Lys Tyr Val Asp
850 855 860
Thr Ser Gly Tyr Asp Ser Asn Ile Asn Ile Asn Gly Asp Val Tyr Lys
865 870 875 880
Tyr Pro Thr Asn Lys Asn Gln Phe Gly Ile Tyr Asn Asp Lys Leu Ser
885 890 895
Glu Val Asn Ile Ser Gln Asn Asp Tyr Ile Ile Tyr Asp Asn Lys Tyr
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Lys Asn Phe Ser Ile Ser Phe Trp Val Arg Ile Pro Asn Tyr Asp Asn
915 920 925
Lys Ile Val Asn Val Asn Asn Glu Tyr Thr Ile Ile Asn Cys Met Arg
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Lys Asn Asp Gln Val Tyr Ile Asn Phe Val Ala Ser Lys Thr His

1145 1150 1155

Leu Phe Pro Leu Tyr Ala Asp Thr Ala Thr Thr Asn Lys Glu Lys

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Thr Ile Lys Ile Ser Ser Ser Gly Asn Arg Phe Asn Gln Val Val

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Val Met Asn Ser Val Gly Asn Asn Cys Thr Met Asn Phe Lys Asn

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35 40 45

Arg Asn Thr Ile Gly Thr Asp Pro Ser Asp Phe Asp Pro Pro Ala Ser

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

65 70 75 80

Asp Ala Glu Lys Asp Arg Tyr Leu Lys Thr Thr Ile Lys Leu Phe Lys

85 90 95

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

100 105 110

Tyr Ala Lys Pro Tyr Leu Gly Asn Glu His Thr Pro Ile Asn Glu Phe

115 120 125

His Pro Val Thr Arg Thr Thr Ser Val Asn Ile Lys Ser Ser Thr Asn

130 135 140

Val Lys Ser Ser Ile Ile Leu Asn Leu Leu Val Leu Gly Ala Gly Pro

145 150 155 160

Asp Ile Phe Glu Asn Ser Ser Tyr Pro Val Arg Lys Leu Met Asp Ser

165 170 175
Gly Gly Val Tyr Asp Pro Ser Asn Asp Gly Phe Gly Ser Ile Asn Ile
180 185 190
Val Thr Phe Ser Pro Glu Tyr Glu Tyr Thr Phe Asn Asp Ile Ser Gly
195 200 205
Gly Tyr Asn Ser Ser Thr Glu Ser Phe Ile Ala Asp Pro Ala Ile Ser
210 215 220
Leu Ala His Glu Leu Ile His Ala Leu His Gly Leu Tyr Gly Ala Arg
225 230 235 240
Gly Val Thr Tyr Lys Glu Thr Ile Lys Val Lys Gln Ala Pro Leu Met
245 250 255
Ile Ala Glu Lys Pro Ile Arg Leu Glu Glu Phe Leu Thr Phe Gly Gly
260 265 270
Gln Asp Leu Asn Ile Ile Thr Ser Ala Met Lys Glu Lys Ile Tyr Asn
275 280 285
Asn Leu Leu Ala Asn Tyr Glu Lys Ile Ala Thr Arg Leu Ser Arg Val
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Gln Trp Lys Tyr Gly Leu Asp Lys Asn Ala Asp Gly Ser Tyr Thr Val
325 330 335
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Glu Ile Asp Leu Ala Asn Lys Phe Lys Val Lys Cys Arg Asn Thr Tyr
355 360 365
Phe Ile Lys Tyr Gly Phe Leu Lys Val Pro Asn Leu Leu Asp Asp Asp
370 375 380
Ile Tyr Thr Val Ser Glu Gly Phe Asn Ile Gly Asn Leu Ala Val Asn
385 390 395 400
Asn Arg Gly Gln Asn Ile Lys Leu Asn Pro Lys Ile Ile Asp Ser Ile
405 410 415
Pro Asp Lys Gly Leu Val Glu Lys Ile Val Lys Phe Cys Lys Ser Val
420 425 430
Ile Pro Arg Lys Gly Thr Lys Ala Pro Pro Arg Leu Cys Ile Arg Val
435 440 445
Asn Asn Arg Glu Leu Phe Phe Val Ala Ser Glu Ser Ser Tyr Asn Glu
450 455 460
Asn Asp Ile Asn Thr Pro Lys Glu Ile Asp Asp Thr Thr Asn Leu Asn
465 470 475 480
Asn Asn Tyr Arg Asn Asn Leu Asp Glu Val Ile Leu Asp Tyr Asn Ser
485 490 495
Glu Thr Ile Pro Gln Ile Ser Asn Gln Thr Leu Asn Thr Leu Val Gln
500 505 510
Asp Asp Ser Tyr Val Pro Arg Tyr Asp Ser Asn Gly Thr Ser Glu Ile

515 520 525
Glu Glu His Asn Val Val Asp Leu Asn Val Phe Phe Tyr Leu His Ala
530 535 540
Gln Lys Val Pro Glu Gly Glu Thr Asn Ile Ser Leu Thr Ser Ser Ile
545 550 555 560
Asp Thr Ala Leu Ser Glu Glu Ser Gln Val Tyr Thr Phe Phe Ser Ser
565 570 575
Glu Phe Ile Asn Thr Ile Asn Lys Pro Val His Ala Ala Leu Phe Ile
580 585 590

Ser Trp Ile Asn Gln Val Ile Arg Asp Phe Thr Thr Glu Ala Thr Gln
595 600 605
Lys Ser Thr Phe Asp Lys Ile Ala Asp Ile Ser Leu Val Val Pro Tyr
610 615 620
Val Gly Leu Ala Leu Asn Ile Gly Asn Glu Val Gln Lys Glu Asn Phe
625 630 635 640
Lys Glu Ala Phe Glu Leu Leu Gly Ala Gly Ile Leu Leu Glu Phe Val
645 650 655
Pro Glu Leu Leu Ile Pro Thr Ile Leu Val Phe Thr Ile Lys Ser Phe
660 665 670
Ile Gly Ser Ser Glu Asn Lys Asn Lys Ile Ile Lys Ala Ile Asn Asn
675 680 685
[0022]
Ser Leu Met Glu Arg Glu Thr Lys Trp Lys Glu Ile Tyr Ser Trp Ile
690 695 700
Val Ser Asn Trp Leu Thr Arg Ile Asn Thr Gln Phe Asn Lys Arg Lys
705 710 715 720
Glu Gln Met Tyr Gln Ala Leu Gln Asn Gln Val Asp Ala Ile Lys Thr
725 730 735
Val Ile Glu Tyr Lys Tyr Asn Asn Tyr Thr Ser Asp Glu Arg Asn Arg
740 745 750
Leu Glu Ser Glu Tyr Asn Ile Asn Asn Ile Arg Glu Glu Leu Asn Lys
755 760 765
Lys Val Ser Leu Ala Met Glu Asn Ile Glu Arg Phe Ile Thr Glu Ser
770 775 780
Ser Ile Phe Tyr Leu Met Lys Leu Ile Asn Glu Ala Lys Val Ser Lys
785 790 795 800
Leu Arg Glu Tyr Asp Glu Gly Val Lys Glu Tyr Leu Leu Asp Tyr Ile
805 810 815
Ser Glu His Arg Ser Ile Leu Gly Asn Ser Val Gln Glu Leu Asn Asp
820 825 830
Leu Val Thr Ser Thr Leu Asn Asn Ser Ile Pro Phe Glu Leu Ser Ser
835 840 845
Tyr Thr Asn Asp Lys Ile Leu Ile Leu Tyr Phe Asn Lys Leu Tyr Lys
850 855 860

Lys Ile Lys Asp Asn Ser Ile Leu Asp Met Arg Tyr Glu Asn Asn Lys
865 870 875 880

Phe Ile Asp Ile Ser Gly Tyr Gly Ser Asn Ile Ser Ile Asn Gly Asp
885 890 895

Val Tyr Ile Tyr Ser Thr Asn Arg Asn Gln Phe Gly Ile Tyr Ser Ser
900 905 910

Lys Pro Ser Glu Val Asn Ile Ala Gln Asn Asn Asp Ile Ile Tyr Asn
915 920 925

Gly Arg Tyr Gln Asn Phe Ser Ile Ser Phe Trp Val Arg Ile Pro Lys
930 935 940

Tyr Phe Asn Lys Val Asn Leu Asn Asn Glu Tyr Thr Ile Ile Asp Cys
945 950 955 960

Ile Arg Asn Asn Asn Ser Gly Trp Lys Ile Ser Leu Asn Tyr Asn Lys
965 970 975

Ile Ile Trp Thr Leu Gln Asp Thr Ala Gly Asn Asn Gln Lys Leu Val
980 985 990

Phe Asn Tyr Thr Gln Met Ile Ser Ile Ser Asp Tyr Ile Asn Lys Trp
995 1000 1005

Ile Phe Val Thr Ile Thr Asn Asn Arg Leu Gly Asn Ser Arg Ile
1010 1015 1020

Tyr Ile Asn Gly Asn Leu Ile Asp Glu Lys Ser Ile Ser Asn Leu
1025 1030 1035

[0023] Gly Asp Ile His Val Ser Asp Asn Ile Leu Phe Lys Ile Val Gly
1040 1045 1050

Cys Asn Asp Thr Arg Tyr Val Gly Ile Arg Tyr Phe Lys Val Phe
1055 1060 1065

Asp Thr Glu Leu Gly Lys Thr Glu Ile Glu Thr Leu Tyr Ser Asp
1070 1075 1080

Glu Pro Asp Pro Ser Ile Leu Lys Asp Phe Trp Gly Asn Tyr Leu
1085 1090 1095

Leu Tyr Asn Lys Arg Tyr Tyr Leu Leu Asn Leu Leu Arg Thr Asp
1100 1105 1110

Lys Ser Ile Thr Gln Asn Ser Asn Phe Leu Asn Ile Asn Gln Gln
1115 1120 1125

Arg Gly Val Tyr Gln Lys Pro Asn Ile Phe Ser Asn Thr Arg Leu
1130 1135 1140

Tyr Thr Gly Val Glu Val Ile Ile Arg Lys Asn Gly Ser Thr Asp
1145 1150 1155

Ile Ser Asn Thr Asp Asn Phe Val Arg Lys Asn Asp Leu Ala Tyr
1160 1165 1170

Ile Asn Val Val Asp Arg Asp Val Glu Tyr Arg Leu Tyr Ala Asp
1175 1180 1185

Ile Ser Ile Ala Lys Pro Glu Lys Ile Ile Lys Leu Ile Arg Thr
1190 1195 1200

Ser Asn Ser Asn Asn Ser Leu Gly Gln Ile Ile Val Met Asp Ser
1205 1210 1215
Ile Gly Asn Asn Cys Thr Met Asn Phe Gln Asn Asn Asn Gly Gly
1220 1225 1230
Asn Ile Gly Leu Leu Gly Phe His Ser Asn Asn Leu Val Ala Ser
1235 1240 1245
Ser Trp Tyr Tyr Asn Asn Ile Arg Lys Asn Thr Ser Ser Asn Gly
1250 1255 1260
Cys Phe Trp Ser Phe Ile Ser Lys Glu His Gly Trp Gln Glu Asn
1265 1270 1275
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<213> 肉毒杆菌
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Asp Asp Ile Ile Met Met Glu Pro Phe Asn Asp Pro Gly Pro Gly Thr
20 25 30
Tyr Tyr Lys Ala Phe Arg Ile Ile Asp Arg Ile Trp Ile Val Pro Glu
35 40 45
[0024] Arg Phe Thr Tyr Gly Phe Gln Pro Asp Gln Phe Asn Ala Ser Thr Gly
50 55 60
Val Phe Ser Lys Asp Val Tyr Glu Tyr Tyr Asp Pro Thr Tyr Leu Lys
65 70 75 80
Thr Asp Ala Glu Lys Asp Lys Phe Leu Lys Thr Met Ile Lys Leu Phe
85 90 95
Asn Arg Ile Asn Ser Lys Pro Ser Gly Gln Arg Leu Leu Asp Met Ile
100 105 110
Val Asp Ala Ile Pro Tyr Leu Gly Asn Ala Ser Thr Pro Pro Asp Lys
115 120 125
Phe Ala Ala Asn Val Ala Asn Val Ser Ile Asn Lys Lys Ile Ile Gln
130 135 140
Pro Gly Ala Glu Asp Gln Ile Lys Gly Leu Met Thr Asn Leu Ile Ile
145 150 155 160
Phe Gly Pro Gly Pro Val Leu Ser Asp Asn Phe Thr Asp Ser Met Ile
165 170 175
Met Asn Gly His Ser Pro Ile Ser Glu Gly Phe Gly Ala Arg Met Met
180 185 190
Ile Arg Phe Cys Pro Ser Cys Leu Asn Val Phe Asn Asn Val Gln Glu
195 200 205
Asn Lys Asp Thr Ser Ile Phe Ser Arg Arg Ala Tyr Phe Ala Asp Pro
210 215 220
Ala Leu Thr Leu Met His Glu Leu Ile His Val Leu His Gly Leu Tyr

225 230 235 240

Gly Ile Lys Ile Ser Asn Leu Pro Ile Thr Pro Asn Thr Lys Glu Phe
245 250 255

Phe Met Gln His Ser Asp Pro Val Gln Ala Glu Glu Leu Tyr Thr Phe
260 265 270

Gly Gly His Asp Pro Ser Val Ile Ser Pro Ser Thr Asp Met Asn Ile
275 280 285

Tyr Asn Lys Ala Leu Gln Asn Phe Gln Asp Ile Ala Asn Arg Leu Asn
290 295 300

Ile Val Ser Ser Ala Gln Gly Ser Gly Ile Asp Ile Ser Leu Tyr Lys
305 310 315 320

Gln Ile Tyr Lys Asn Lys Tyr Asp Phe Val Glu Asp Pro Asn Gly Lys
325 330 335

Tyr Ser Val Asp Lys Asp Lys Phe Asp Lys Leu Tyr Lys Ala Leu Met
340 345 350

Phe Gly Phe Thr Glu Thr Asn Leu Ala Gly Glu Tyr Gly Ile Lys Thr
355 360 365

Arg Tyr Ser Tyr Phe Ser Glu Tyr Leu Pro Pro Ile Lys Thr Glu Lys
370 375 380

Leu Leu Asp Asn Thr Ile Tyr Thr Gln Asn Glu Gly Phe Asn Ile Ala
385 390 395 400

[0025] Ser Lys Asn Leu Lys Thr Glu Phe Asn Gly Gln Asn Lys Ala Val Asn
405 410 415

Lys Glu Ala Tyr Glu Glu Ile Ser Leu Glu His Leu Val Ile Tyr Arg
420 425 430

Ile Ala Met Cys Lys Pro Val Met Tyr Lys Asn Thr Gly Lys Ser Glu
435 440 445

Gln Cys Ile Ile Val Asn Asn Glu Asp Leu Phe Phe Ile Ala Asn Lys
450 455 460

Asp Ser Phe Ser Lys Asp Leu Ala Lys Ala Glu Thr Ile Ala Tyr Asn
465 470 475 480

Thr Gln Asn Asn Thr Ile Glu Asn Asn Phe Ser Ile Asp Gln Leu Ile
485 490 495

Leu Asp Asn Asp Leu Ser Ser Gly Ile Asp Leu Pro Asn Glu Asn Thr
500 505 510

Glu Pro Phe Thr Asn Phe Asp Asp Ile Asp Ile Pro Val Tyr Ile Lys
515 520 525

Gln Ser Ala Leu Lys Lys Ile Phe Val Asp Gly Asp Ser Leu Phe Glu
530 535 540

Tyr Leu His Ala Gln Thr Phe Pro Ser Asn Ile Glu Asn Leu Gln Leu
545 550 555 560

Thr Asn Ser Leu Asn Asp Ala Leu Arg Asn Asn Asn Lys Val Tyr Thr
565 570 575

Phe Phe Ser Thr Asn Leu Val Glu Lys Ala Asn Thr Val Val Gly Ala

580 585 590
Ser Leu Phe Val Asn Trp Val Lys Gly Val Ile Asp Asp Phe Thr Ser
595 600 605
Glu Ser Thr Gln Lys Ser Thr Ile Asp Lys Val Ser Asp Val Ser Ile
610 615 620
Ile Ile Pro Tyr Ile Gly Pro Ala Leu Asn Val Gly Asn Glu Thr Ala
625 630 635 640
Lys Glu Asn Phe Lys Asn Ala Phe Glu Ile Gly Gly Ala Ala Ile Leu
645 650 655
Met Glu Phe Ile Pro Glu Leu Ile Val Pro Ile Val Gly Phe Phe Thr
660 665 670
Leu Glu Ser Tyr Val Gly Asn Lys Gly His Ile Ile Met Thr Ile Ser
675 680 685
Asn Ala Leu Lys Lys Arg Asp Gln Lys Trp Thr Asp Met Tyr Gly Leu
690 695 700
Ile Val Ser Gln Trp Leu Ser Thr Val Asn Thr Gln Phe Tyr Thr Ile
705 710 715 720
Lys Glu Arg Met Tyr Asn Ala Leu Asn Asn Gln Ser Gln Ala Ile Glu
725 730 735
Lys Ile Ile Glu Asp Gln Tyr Asn Arg Tyr Ser Glu Glu Asp Lys Met
740 745 750
[0026] Asn Ile Asn Ile Asp Phe Asn Asp Ile Asp Phe Lys Leu Asn Gln Ser
755 760 765
Ile Asn Leu Ala Ile Asn Asn Ile Asp Asp Phe Ile Asn Gln Cys Ser
770 775 780
Ile Ser Tyr Leu Met Asn Arg Met Ile Pro Leu Ala Val Lys Lys Leu
785 790 795 800
Lys Asp Phe Asp Asp Asn Leu Lys Arg Asp Leu Leu Glu Tyr Ile Asp
805 810 815
Thr Asn Glu Leu Tyr Leu Leu Asp Glu Val Asn Ile Leu Lys Ser Lys
820 825 830
Val Asn Arg His Leu Lys Asp Ser Ile Pro Phe Asp Leu Ser Leu Tyr
835 840 845
Thr Lys Asp Thr Ile Leu Ile Gln Val Phe Asn Asn Tyr Ile Ser Asn
850 855 860
Ile Ser Ser Asn Ala Ile Leu Ser Leu Ser Tyr Arg Gly Gly Arg Leu
865 870 875 880
Ile Asp Ser Ser Gly Tyr Gly Ala Thr Met Asn Val Gly Ser Asp Val
885 890 895
Ile Phe Asn Asp Ile Gly Asn Gly Gln Phe Lys Leu Asn Asn Ser Glu
900 905 910
Asn Ser Asn Ile Thr Ala His Gln Ser Lys Phe Val Val Tyr Asp Ser
915 920 925
Met Phe Asp Asn Phe Ser Ile Asn Phe Trp Val Arg Thr Pro Lys Tyr

930 935 940
Asn Asn Asn Asp Ile Gln Thr Tyr Leu Gln Asn Glu Tyr Thr Ile Ile
945 950 955 960
Ser Cys Ile Lys Asn Asp Ser Gly Trp Lys Val Ser Ile Lys Gly Asn
965 970 975
Arg Ile Ile Trp Thr Leu Ile Asp Val Asn Ala Lys Ser Lys Ser Ile
980 985 990
Phe Phe Glu Tyr Ser Ile Lys Asp Asn Ile Ser Asp Tyr Ile Asn Lys
995 1000 1005
Trp Phe Ser Ile Thr Ile Thr Asn Asp Arg Leu Gly Asn Ala Asn
1010 1015 1020
Ile Tyr Ile Asn Gly Ser Leu Lys Lys Ser Glu Lys Ile Leu Asn
1025 1030 1035
Leu Asp Arg Ile Asn Ser Ser Asn Asp Ile Asp Phe Lys Leu Ile
1040 1045 1050
Asn Cys Thr Asp Thr Thr Lys Phe Val Trp Ile Lys Asp Phe Asn
1055 1060 1065
Ile Phe Gly Arg Glu Leu Asn Ala Thr Glu Val Ser Ser Leu Tyr
1070 1075 1080
Trp Ile Gln Ser Ser Thr Asn Thr Leu Lys Asp Phe Trp Gly Asn
1085 1090 1095
[0027] Pro Leu Arg Tyr Asp Thr Gln Tyr Tyr Leu Phe Asn Gln Gly Met
1100 1105 1110
Gln Asn Ile Tyr Ile Lys Tyr Phe Ser Lys Ala Ser Met Gly Glu
1115 1120 1125
Thr Ala Pro Arg Thr Asn Phe Asn Asn Ala Ala Ile Asn Tyr Gln
1130 1135 1140
Asn Leu Tyr Leu Gly Leu Arg Phe Ile Ile Lys Lys Ala Ser Asn
1145 1150 1155
Ser Arg Asn Ile Asn Asn Asp Asn Ile Val Arg Glu Gly Asp Tyr
1160 1165 1170
Ile Tyr Leu Asn Ile Asp Asn Ile Ser Asp Glu Ser Tyr Arg Val
1175 1180 1185
Tyr Val Leu Val Asn Ser Lys Glu Ile Gln Thr Gln Leu Phe Leu
1190 1195 1200
Ala Pro Ile Asn Asp Asp Pro Thr Phe Tyr Asp Val Leu Gln Ile
1205 1210 1215
Lys Lys Tyr Tyr Glu Lys Thr Thr Tyr Asn Cys Gln Ile Leu Cys
1220 1225 1230
Glu Lys Asp Thr Lys Thr Phe Gly Leu Phe Gly Ile Gly Lys Phe
1235 1240 1245
Val Lys Asp Tyr Gly Tyr Val Trp Asp Thr Tyr Asp Asn Tyr Phe
1250 1255 1260
Cys Ile Ser Gln Trp Tyr Leu Arg Arg Ile Ser Glu Asn Ile Asn

1265 1270 1275
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Gly Trp Thr Glu
1295
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20 25 30
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35 40 45
Arg Tyr Glu Phe Gly Thr Lys Pro Glu Asp Phe Asn Pro Pro Ser Ser
50 55 60
Leu Ile Glu Gly Ala Ser Glu Tyr Tyr Asp Pro Asn Tyr Leu Arg Thr
65 70 75 80
Asp Ser Asp Lys Asp Arg Phe Leu Gln Thr Met Val Lys Leu Phe Asn
[0028] 85 90 95
Arg Ile Lys Asn Asn Val Ala Gly Glu Ala Leu Leu Asp Lys Ile Ile
100 105 110
Asn Ala Ile Pro Tyr Leu Gly Asn Ser Tyr Ser Leu Leu Asp Lys Phe
115 120 125
Asp Thr Asn Ser Asn Ser Val Ser Phe Asn Leu Leu Glu Gln Asp Pro
130 135 140
Ser Gly Ala Thr Thr Lys Ser Ala Met Leu Thr Asn Leu Ile Ile Phe
145 150 155 160
Gly Pro Gly Pro Val Leu Asn Lys Asn Glu Val Arg Gly Ile Val Leu
165 170 175
Arg Val Asp Asn Lys Asn Tyr Phe Pro Cys Arg Asp Gly Phe Gly Ser
180 185 190
Ile Met Gln Met Ala Phe Cys Pro Glu Tyr Val Pro Thr Phe Asp Asn
195 200 205
Val Ile Glu Asn Ile Thr Ser Leu Thr Ile Gly Lys Ser Lys Tyr Phe
210 215 220
Gln Asp Pro Ala Leu Leu Leu Met His Glu Leu Ile His Val Leu His
225 230 235 240
Gly Leu Tyr Gly Met Gln Val Ser Ser His Glu Ile Ile Pro Ser Lys
245 250 255
Gln Glu Ile Tyr Met Gln His Thr Tyr Pro Ile Ser Ala Glu Glu Leu
260 265 270

Phe Thr Phe Gly Gly Gln Asp Ala Asn Leu Ile Ser Ile Asp Ile Lys
275 280 285
Asn Asp Leu Tyr Glu Lys Thr Leu Asn Asp Tyr Lys Ala Ile Ala Asn
290 295 300
Lys Leu Ser Gln Val Thr Ser Cys Asn Asp Pro Asn Ile Asp Ile Asp
305 310 315 320
Ser Tyr Lys Gln Ile Tyr Gln Gln Lys Tyr Gln Phe Asp Lys Asp Ser
325 330 335
Asn Gly Gln Tyr Ile Val Asn Glu Asp Lys Phe Gln Ile Leu Tyr Asn
340 345 350
Ser Ile Met Tyr Gly Phe Thr Glu Ile Glu Leu Gly Lys Lys Phe Asn
355 360 365
Ile Lys Thr Arg Leu Ser Tyr Phe Ser Met Asn His Asp Pro Val Lys
370 375 380
Ile Pro Asn Leu Leu Asp Asp Thr Ile Tyr Asn Asp Thr Glu Gly Phe
385 390 395 400
Asn Ile Glu Ser Lys Asp Leu Lys Ser Glu Tyr Lys Gly Gln Asn Met
405 410 415
Arg Val Asn Thr Asn Ala Phe Arg Asn Val Asp Gly Ser Gly Leu Val
420 425 430
Ser Lys Leu Ile Gly Leu Cys Lys Lys Ile Ile Pro Pro Thr Asn Ile
435 440 445
[0029] Arg Glu Asn Leu Tyr Asn Arg Thr Ala Ser Leu Thr Asp Leu Gly Gly
450 455 460
Glu Leu Cys Ile Lys Ile Lys Asn Glu Asp Leu Thr Phe Ile Ala Glu
465 470 475 480
Lys Asn Ser Phe Ser Glu Glu Pro Phe Gln Asp Glu Ile Val Ser Tyr
485 490 495
Asn Thr Lys Asn Lys Pro Leu Asn Phe Asn Tyr Ser Leu Asp Lys Ile
500 505 510
Ile Val Asp Tyr Asn Leu Gln Ser Lys Ile Thr Leu Pro Asn Asp Arg
515 520 525
Thr Thr Pro Val Thr Lys Gly Ile Pro Tyr Ala Pro Glu Tyr Lys Ser
530 535 540
Asn Ala Ala Ser Thr Ile Glu Ile His Asn Ile Asp Asp Asn Thr Ile
545 550 555 560
Tyr Gln Tyr Leu Tyr Ala Gln Lys Ser Pro Thr Thr Leu Gln Arg Ile
565 570 575
Thr Met Thr Asn Ser Val Asp Asp Ala Leu Ile Asn Ser Thr Lys Ile
580 585 590
Tyr Ser Tyr Phe Pro Ser Val Ile Ser Lys Val Asn Gln Gly Ala Gln
595 600 605
Gly Ile Leu Phe Leu Gln Trp Val Arg Asp Ile Ile Asp Asp Phe Thr
610 615 620

Asn Glu Ser Ser Gln Lys Thr Thr Ile Asp Lys Ile Ser Asp Val Ser
625 630 635 640
Thr Ile Val Pro Tyr Ile Gly Pro Ala Leu Asn Ile Val Lys Gln Gly
645 650 655
Tyr Glu Gly Asn Phe Ile Gly Ala Leu Glu Thr Thr Gly Val Val Leu
660 665 670
Leu Leu Glu Tyr Ile Pro Glu Ile Thr Leu Pro Val Ile Ala Ala Leu
675 680 685
Ser Ile Ala Glu Ser Ser Thr Gln Lys Glu Lys Ile Ile Lys Thr Ile
690 695 700
Asp Asn Phe Leu Glu Lys Arg Tyr Glu Lys Trp Ile Glu Val Tyr Lys
705 710 715 720
Leu Val Lys Ala Lys Trp Leu Gly Thr Val Asn Thr Gln Phe Gln Lys
725 730 735
Arg Ser Tyr Gln Met Tyr Arg Ser Leu Glu Tyr Gln Val Asp Ala Ile
740 745 750
Lys Lys Ile Ile Asp Tyr Glu Tyr Lys Ile Tyr Ser Gly Pro Asp Lys
755 760 765
Glu Gln Ile Ala Asp Glu Ile Asn Asn Leu Lys Asn Lys Leu Glu Glu
770 775 780
Lys Ala Asn Lys Ala Met Ile Asn Ile Asn Ile Phe Met Arg Glu Ser
[0030] 785 790 795 800
Ser Arg Ser Phe Leu Val Asn Gln Met Ile Asn Glu Ala Lys Lys Gln
805 810 815
Leu Leu Glu Phe Asp Thr Gln Ser Lys Asn Ile Leu Met Gln Tyr Ile
820 825 830
Lys Ala Asn Ser Lys Phe Ile Gly Ile Thr Glu Leu Lys Lys Leu Glu
835 840 845
Ser Lys Ile Asn Lys Val Phe Ser Thr Pro Ile Pro Phe Ser Tyr Ser
850 855 860
Lys Asn Leu Asp Cys Trp Val Asp Asn Glu Glu Asp Ile Asp Val Ile
865 870 875 880
Leu Lys Lys Ser Thr Ile Leu Asn Leu Asp Ile Asn Asn Asp Ile Ile
885 890 895
Ser Asp Ile Ser Gly Phe Asn Ser Ser Val Ile Thr Tyr Pro Asp Ala
900 905 910
Gln Leu Val Pro Gly Ile Asn Gly Lys Ala Ile His Leu Val Asn Asn
915 920 925
Glu Ser Ser Glu Val Ile Val His Lys Ala Met Asp Ile Glu Tyr Asn
930 935 940
Asp Met Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val Pro Lys
945 950 955 960
Val Ser Ala Ser His Leu Glu Gln Tyr Gly Thr Asn Glu Tyr Ser Ile
965 970 975

Ile Ser Ser Met Lys Lys His Ser Leu Ser Ile Gly Ser Gly Trp Ser
980 985 990
Val Ser Leu Lys Gly Asn Asn Leu Ile Trp Thr Leu Lys Asp Ser Ala
995 1000 1005
Gly Glu Val Arg Gln Ile Thr Phe Arg Asp Leu Pro Asp Lys Phe
1010 1015 1020
Asn Ala Tyr Leu Ala Asn Lys Trp Val Phe Ile Thr Ile Thr Asn
1025 1030 1035
Asp Arg Leu Ser Ser Ala Asn Leu Tyr Ile Asn Gly Val Leu Met
1040 1045 1050
Gly Ser Ala Glu Ile Thr Gly Leu Gly Ala Ile Arg Glu Asp Asn
1055 1060 1065
Asn Ile Thr Leu Lys Leu Asp Arg Cys Asn Asn Asn Asn Gln Tyr
1070 1075 1080
Val Ser Ile Asp Lys Phe Arg Ile Phe Cys Lys Ala Leu Asn Pro
1085 1090 1095
Lys Glu Ile Glu Lys Leu Tyr Thr Ser Tyr Leu Ser Ile Thr Phe
1100 1105 1110
Leu Arg Asp Phe Trp Gly Asn Pro Leu Arg Tyr Asp Thr Glu Tyr
1115 1120 1125
Tyr Leu Ile Pro Val Ala Ser Ser Ser Lys Asp Val Gln Leu Lys
1130 1135 1140
[0031] Asn Ile Thr Asp Tyr Met Tyr Leu Thr Asn Ala Pro Ser Tyr Thr
1145 1150 1155
Asn Gly Lys Leu Asn Ile Tyr Tyr Arg Arg Leu Tyr Asn Gly Leu
1160 1165 1170
Lys Phe Ile Ile Lys Arg Tyr Thr Pro Asn Asn Glu Ile Asp Ser
1175 1180 1185
Phe Val Lys Ser Gly Asp Phe Ile Lys Leu Tyr Val Ser Tyr Asn
1190 1195 1200
Asn Asn Glu His Ile Val Gly Tyr Pro Lys Asp Gly Asn Ala Phe
1205 1210 1215
Asn Asn Leu Asp Arg Ile Leu Arg Val Gly Tyr Asn Ala Pro Gly
1220 1225 1230
Ile Pro Leu Tyr Lys Lys Met Glu Ala Val Lys Leu Arg Asp Leu
1235 1240 1245
Lys Thr Tyr Ser Val Gln Leu Lys Leu Tyr Asp Asp Lys Asn Ala
1250 1255 1260
Ser Leu Gly Leu Val Gly Thr His Asn Gly Gln Ile Gly Asn Asp
1265 1270 1275
Pro Asn Arg Asp Ile Leu Ile Ala Ser Asn Trp Tyr Phe Asn His
1280 1285 1290
Leu Lys Asp Lys Ile Leu Gly Cys Asp Trp Tyr Phe Val Pro Thr
1295 1300 1305

Asp Glu Gly Trp Thr Asn Asp
1310 1315
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Pro Gly Gly Gly Pro Pro Gly Pro Pro Pro Asn Thr Thr Ser Asn Arg
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Arg Leu Gln Gln Thr Gln Ala Gln Val Glu Glu Val Val Asp Ile Met
35 40 45
Arg Val Asn Val Asp Lys Val Leu Glu Arg Asp Gln Lys Leu Ser Glu
50 55 60
Leu Asp Asp Arg Ala Asp Ala Leu Gln Ala Gly Ala Ser Val Phe Glu
65 70 75 80
Ser Ser Ala Ala Lys Leu Lys Arg Lys Tyr Trp Trp Lys Asn Cys Lys
85 90 95
Met Met Ile Met Leu Gly Ala Ile Cys Ala Ile Ile Val Val Val Ile
100 105 110
[0032] Val Ile Tyr Ile Phe Thr
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<400> 10
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1 5 10 15
Pro Gly Gly Gly Pro Pro Gly Pro Pro Pro Asn Met Thr Ser Asn Arg
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Arg Leu Gln Gln Thr Gln Ala Gln Val Glu Glu Val Val Asp Ile Ile
35 40 45
Arg Val Asn Val Asp Lys Val Leu Glu Arg Asp Gln Lys Leu Ser Glu
50 55 60
Leu Asp Asp Arg Ala Asp Ala Leu Gln Ala Gly Ala Ser Gln Phe Glu
65 70 75 80
Ser Ser Ala Ala Lys Leu Lys Arg Lys Tyr Trp Trp Lys Asn Cys Lys
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Val Ile Tyr Phe Phe Thr
115

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

[0033] <210> 12
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Gln Gln Thr Gln Ala Gln Val Asp Glu Val Val Asp Ile Met Arg Val
35 40 45
Asn Val Asp Lys Val Leu Glu Arg Asp Gln Lys Leu Ser Glu Leu Asp
50 55 60
Asp Arg Ala Asp Ala Leu Gln Ala Gly Ala Ser Gln Phe Glu Thr Ser
65 70 75 80
Ala Ala Lys Leu Lys Arg Lys Tyr Trp Trp Lys Asn Leu Lys Met Met
85 90 95
Ile Ile Leu Gly Val Ile Cys Ala Ile Ile Leu Ile Ile Ile Ile Val
100 105 110
Tyr Phe Ser Thr
115
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<211> 103

<212> PRT
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20 25 30
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35 40 45
Glu Leu Asp Asp Arg Ala Asp Ala Leu Gln Ala Gly Ala Ser Gln Phe
50 55 60
Glu Thr Ser Ala Ala Lys Leu Lys Arg Lys Tyr Trp Trp Lys Asn Cys
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Lys Met Trp Ala Ile Gly Ile Ser Val Leu Val Ile Ile Val Ile Ile
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Ile Ile Val Trp Cys Val Ser
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<400> 14

[0034]
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Val Asp Lys Val Leu Glu Arg Asp Gln Lys Leu Ser Glu Leu Asp Asp
35 40 45
Arg Ala Asp Ala Leu Gln Ala Gly Ala Ser Gln Phe Glu Thr Ser Ala
50 55 60
Ala Lys Leu Lys Arg Lys Tyr Trp Trp Lys Asn Cys Lys Met Trp Ala
65 70 75 80
Ile Gly Ile Thr Val Leu Val Ile Phe Ile Ile Ile Ile Ile Val Trp
85 90 95
Val Val Ser Ser
100
<210> 15
<211> 13
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<213> 人工序列
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<223> VAMP 表位
<400> 15
Lys Leu Ser Glu Leu Asp Asp Arg Ala Asp Ala Leu Gln

1 5 10
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<223> VAMP 表位
<400> 16
Gln Lys Leu Ser Glu Leu Asp Asp Arg Ala Asp Ala Leu Gln
1 5 10
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<400> 17
Lys Leu Ser Glu Leu Asp Asp Arg Ala Asp
1 5 10
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<211> 17
[0035] <212> PRT
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<223> VAMP 表位
<400> 18
Lys Leu Ser Glu Leu Asp Asp Arg Ala Asp Ala Leu Gln Ala Gly Ala
1 5 10 15
Ser

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<211> 12
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<213> 人工序列
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<223> VAMP 表位
<400> 19
Leu Ser Glu Leu Asp Asp Arg Ala Asp Ala Leu Gln
1 5 10
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<212> PRT
<213> 人工序列
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<223> VAMP 表位
<400> 20
Leu Ser Glu Leu Asp Asp Arg Ala Asp Ala
1 5 10

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<211> 16
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<213> 人工序列
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<223> VAMP 表位
<400> 21
Leu Ser Glu Leu Asp Asp Arg Ala Asp Ala Leu Gln Ala Gly Ala Ser
1 5 10 15
<210> 22
<211> 13
<212> PRT
<213> 人工序列
<220>
<223> VAMP 表位
<400> 22
[0036] Phe Glu Thr Ser Ala Ala Lys Leu Lys Arg Lys Tyr Trp
1 5 10
<210> 23
<211> 13
<212> PRT
<213> 人工序列
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<223> VAMP 表位
<400> 23
Phe Glu Ser Ser Ala Ala Lys Leu Lys Arg Lys Tyr Trp
1 5 10
<210> 24
<211> 14
<212> PRT
<213> 人工序列
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<223> VAMP 表位
<400> 24
Gln Phe Glu Thr Ser Ala Ala Lys Leu Lys Arg Lys Tyr Trp
1 5 10
<210> 25
<211> 10
<212> PRT

<213> 人工序列
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<223> VAMP 表位
<400> 25
Phe Glu Thr Ser Ala Ala Lys Leu Lys Arg
1 5 10
<210> 26
<211> 16
<212> PRT
<213> 人工序列
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<223> VAMP 表位
<400> 26
Phe Glu Thr Ser Ala Ala Lys Leu Lys Arg Lys Tyr Trp Trp Lys Asn
1 5 10 15
<210> 27
<211> 11
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<213> 人工序列
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<223> VAMP 表位
[0037] <400> 27
Ala Lys Leu Lys Arg Lys Tyr Trp Trp Lys Asn
1 5 10
<210> 28
<211> 12
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<223> VAMP 表位
<400> 28
Ala Ala Lys Leu Lys Arg Lys Tyr Trp Trp Lys Asn
1 5 10
<210> 29
<211> 14
<212> PRT
<213> 人工序列
<220>
<223> VAMP 表位
<400> 29
Ala Lys Leu Lys Arg Lys Tyr Trp Trp Lys Asn Cys Lys Met
1 5 10
<210> 30
<211> 14

<212> PRT
<213> 人工序列
<220>
<223> VAMP 表位
<400> 30
Ala Lys Leu Lys Arg Lys Tyr Trp Trp Lys Asn Leu Lys Met
1 5 10
<210> 31
<211> 15
<212> PRT
<213> 人工序列
<220>
<223> VAMP 表位
<400> 31
Asp Gln Lys Leu Ser Glu Leu Asp Asp Arg Ala Asp Ala Leu Gln
1 5 10 15
<210> 32
<211> 13
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<213> 人工序列
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[0038] <223> VAMP 表位
<400> 32

Glu Arg Asp Gln Lys Leu Ser Glu Leu Asp Asp Arg Ala
1 5 10
<210> 33
<211> 14
<212> PRT
<213> 人工序列
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<223> VAMP 表位
<400> 33
Leu Glu Arg Asp Gln Lys Leu Ser Glu Leu Asp Asp Arg Ala
1 5 10
<210> 34
<211> 15
<212> PRT
<213> 人工序列
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<223> VAMP 表位
<400> 34
Val Leu Glu Arg Asp Gln Lys Leu Ser Glu Leu Asp Asp Arg Ala
1 5 10 15

<210> 35
<211> 888
<212> PRT
<213> 人工序列
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<223> LHND
<400> 35

Met Gly Ser Met Thr Trp Pro Val Lys Asp Phe Asn Tyr Ser Asp Pro
1 5 10 15
Val Asn Asp Asn Asp Ile Leu Tyr Leu Arg Ile Pro Gln Asn Lys Leu
20 25 30
Ile Thr Thr Pro Val Lys Ala Phe Met Ile Thr Gln Asn Ile Trp Val
35 40 45
Ile Pro Glu Arg Phe Ser Ser Asp Thr Asn Pro Ser Leu Ser Lys Pro
50 55 60
Pro Arg Pro Thr Ser Lys Tyr Gln Ser Tyr Tyr Asp Pro Ser Tyr Leu
65 70 75 80
Ser Thr Asp Glu Gln Lys Asp Thr Phe Leu Lys Gly Ile Ile Lys Leu
85 90 95
Phe Lys Arg Ile Asn Glu Arg Asp Ile Gly Lys Lys Leu Ile Asn Tyr
100 105 110
[0039] Leu Val Val Gly Ser Pro Phe Met Gly Asp Ser Ser Thr Pro Glu Asp
115 120 125
Thr Phe Asp Phe Thr Arg His Thr Thr Asn Ile Ala Val Glu Lys Phe
130 135 140
Glu Asn Gly Ser Trp Lys Val Thr Asn Ile Ile Thr Pro Ser Val Leu
145 150 155 160
Ile Phe Gly Pro Leu Pro Asn Ile Leu Asp Tyr Thr Ala Ser Leu Thr
165 170 175
Leu Gln Gly Gln Gln Ser Asn Pro Ser Phe Glu Gly Phe Gly Thr Leu
180 185 190
Ser Ile Leu Lys Val Ala Pro Glu Phe Leu Leu Thr Phe Ser Asp Val
195 200 205
Thr Ser Asn Gln Ser Ser Ala Val Leu Gly Lys Ser Ile Phe Cys Met
210 215 220
Asp Pro Val Ile Ala Leu Met His Glu Leu Thr His Ser Leu His Gln
225 230 235 240
Leu Tyr Gly Ile Asn Ile Pro Ser Asp Lys Arg Ile Arg Pro Gln Val
245 250 255
Ser Glu Gly Phe Phe Ser Gln Asp Gly Pro Asn Val Gln Phe Glu Glu
260 265 270
Leu Tyr Thr Phe Gly Gly Leu Asp Val Glu Ile Ile Pro Gln Ile Glu
275 280 285
Arg Ser Gln Leu Arg Glu Lys Ala Leu Gly His Tyr Lys Asp Ile Ala

290 295 300

Lys Arg Leu Asn Asn Ile Asn Lys Thr Ile Pro Ser Ser Trp Ile Ser
305 310 315 320

Asn Ile Asp Lys Tyr Lys Lys Ile Phe Ser Glu Lys Tyr Asn Phe Asp
325 330 335

Lys Asp Asn Thr Gly Asn Phe Val Val Asn Ile Asp Lys Phe Asn Ser
340 345 350

Leu Tyr Ser Asp Leu Thr Asn Val Met Ser Glu Val Val Tyr Ser Ser
355 360 365

Gln Tyr Asn Val Lys Asn Arg Thr His Tyr Phe Ser Arg His Tyr Leu
370 375 380

Pro Val Phe Ala Asn Ile Leu Asp Asp Asn Ile Tyr Thr Ile Arg Asp
385 390 395 400

Gly Phe Asn Leu Thr Asn Lys Gly Phe Asn Ile Glu Asn Ser Gly Gln
405 410 415

Asn Ile Glu Arg Asn Pro Ala Leu Gln Lys Leu Ser Ser Glu Ser Val
420 425 430

Val Asp Leu Phe Thr Lys Val Cys Val Asp Lys Ser Glu Glu Lys Leu
435 440 445

Tyr Asp Asp Asp Asp Lys Asp Arg Trp Gly Ser Ser Leu Gln Cys Ile
450 455 460

[0040] Lys Val Lys Asn Asn Arg Leu Pro Tyr Val Ala Asp Lys Asp Ser Ile
465 470 475 480

Ser Gln Glu Ile Phe Glu Asn Lys Ile Ile Thr Asp Glu Thr Asn Val
485 490 495

Gln Asn Tyr Ser Asp Lys Phe Ser Leu Asp Glu Ser Ile Leu Asp Gly
500 505 510

Gln Val Pro Ile Asn Pro Glu Ile Val Asp Pro Leu Leu Pro Asn Val
515 520 525

Asn Met Glu Pro Leu Asn Leu Pro Gly Glu Glu Ile Val Phe Tyr Asp
530 535 540

Asp Ile Thr Lys Tyr Val Asp Tyr Leu Asn Ser Tyr Tyr Tyr Leu Glu
545 550 555 560

Ser Gln Lys Leu Ser Asn Asn Val Glu Asn Ile Thr Leu Thr Thr Ser
565 570 575

Val Glu Glu Ala Leu Gly Tyr Ser Asn Lys Ile Tyr Thr Phe Leu Pro
580 585 590

Ser Leu Ala Glu Lys Val Asn Lys Gly Val Gln Ala Gly Leu Phe Leu
595 600 605

Asn Trp Ala Asn Glu Val Val Glu Asp Phe Thr Thr Asn Ile Met Lys
610 615 620

Lys Asp Thr Leu Asp Lys Ile Ser Asp Val Ser Val Ile Ile Pro Tyr
625 630 635 640

Ile Gly Pro Ala Leu Asn Ile Gly Asn Ser Ala Leu Arg Gly Asn Phe

645 650 655

Asn Gln Ala Phe Ala Thr Ala Gly Val Ala Phe Leu Leu Glu Gly Phe
660 665 670

Pro Glu Phe Thr Ile Pro Ala Leu Gly Val Phe Thr Phe Tyr Ser Ser
675 680 685

Ile Gln Glu Arg Glu Lys Ile Ile Lys Thr Ile Glu Asn Cys Leu Glu
690 695 700

Gln Arg Val Lys Arg Trp Lys Asp Ser Tyr Gln Trp Met Val Ser Asn
705 710 715 720

Trp Leu Ser Arg Ile Thr Thr Gln Phe Asn His Ile Asn Tyr Gln Met
725 730 735

Tyr Asp Ser Leu Ser Tyr Gln Ala Asp Ala Ile Lys Ala Lys Ile Asp
740 745 750

Leu Glu Tyr Lys Lys Tyr Ser Gly Ser Asp Lys Glu Asn Ile Lys Ser
755 760 765

Gln Val Glu Asn Leu Lys Asn Ser Leu Asp Val Lys Ile Ser Glu Ala
770 775 780

Met Asn Asn Ile Asn Lys Phe Ile Arg Glu Cys Ser Val Thr Tyr Leu
785 790 795 800

Phe Lys Asn Met Leu Pro Lys Val Ile Asp Glu Leu Asn Lys Phe Asp
805 810 815

[0041] Leu Arg Thr Lys Thr Glu Leu Ile Asn Leu Ile Asp Ser His Asn Ile
820 825 830

Ile Leu Val Gly Glu Val Asp Arg Leu Lys Ala Lys Val Asn Glu Ser
835 840 845

Phe Glu Asn Thr Met Pro Phe Asn Ile Phe Ser Tyr Thr Asn Asn Ser
850 855 860

Leu Leu Lys Asp Ile Ile Asn Glu Tyr Phe Asn Leu Glu Ala His His
865 870 875 880

His His His His His His His His
885

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<212> PRT
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<400> 36

Met Lys Ile Glu Glu Gly Lys Leu Val Ile Trp Ile Asn Gly Asp Lys
1 5 10 15

Gly Tyr Asn Gly Leu Ala Glu Val Gly Lys Lys Phe Glu Lys Asp Thr
20 25 30

Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu Glu Lys Phe
35 40 45

Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile Phe Trp Ala
50 55 60
His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu Ala Glu Ile
65 70 75 80
Thr Pro Asp Lys Ala Phe Gln Asp Lys Leu Tyr Pro Phe Thr Trp Asp
85 90 95
Ala Val Arg Tyr Asn Gly Lys Leu Ile Ala Tyr Pro Ile Ala Val Glu
100 105 110
Ala Leu Ser Leu Ile Tyr Asn Lys Asp Leu Leu Pro Asn Pro Pro Lys
115 120 125
Thr Trp Glu Glu Ile Pro Ala Leu Asp Lys Glu Leu Lys Ala Lys Gly
130 135 140
Lys Ser Ala Leu Met Phe Asn Leu Gln Glu Pro Tyr Phe Thr Trp Pro
145 150 155 160
Leu Ile Ala Ala Asp Gly Gly Tyr Ala Phe Lys Tyr Glu Asn Gly Lys
165 170 175
Tyr Asp Ile Lys Asp Val Gly Val Asp Asn Ala Gly Ala Lys Ala Gly
180 185 190
Leu Thr Phe Leu Val Asp Leu Ile Lys Asn Lys His Met Asn Ala Asp
195 200 205
Thr Asp Tyr Ser Ile Ala Glu Ala Ala Phe Asn Lys Gly Glu Thr Ala
210 215 220
[0042] Met Thr Ile Asn Gly Pro Trp Ala Trp Ser Asn Ile Asp Thr Ser Lys
225 230 235 240
Val Asn Tyr Gly Val Thr Val Leu Pro Thr Phe Lys Gly Gln Pro Ser
245 250 255
Lys Pro Phe Val Gly Val Leu Ser Ala Gly Ile Asn Ala Ala Ser Pro
260 265 270
Asn Lys Glu Leu Ala Lys Glu Phe Leu Glu Asn Tyr Leu Leu Thr Asp
275 280 285
Glu Gly Leu Glu Ala Val Asn Lys Asp Lys Pro Leu Gly Ala Val Ala
290 295 300
Leu Lys Ser Tyr Glu Glu Glu Leu Ala Lys Asp Pro Arg Ile Ala Ala
305 310 315 320
Thr Met Glu Asn Ala Gln Lys Gly Glu Ile Met Pro Asn Ile Pro Gln
325 330 335
Met Ser Ala Phe Trp Tyr Ala Val Arg Thr Ala Val Ile Asn Ala Ala
340 345 350
Ser Gly Arg Gln Thr Val Asp Glu Ala Leu Lys Asp Ala Gln Thr Asn
355 360 365
Ser Ser Ser Asn Asn Asn Asn Asn Asn Asn Asn Asn Asn Leu Gly Ile
370 375 380

Glu Gly Arg Ile Ser Glu Phe Gly Ser Met Pro Val Ala Ile Asn Ser

385 390 395 400
Phe Asn Tyr Asn Asp Pro Val Asn Asp Asp Thr Ile Leu Tyr Met Gln
405 410 415
Ile Pro Tyr Glu Glu Lys Ser Lys Lys Tyr Tyr Lys Ala Phe Glu Ile
420 425 430
Met Arg Asn Val Trp Ile Ile Pro Glu Arg Asn Thr Ile Gly Thr Asn
435 440 445
Pro Ser Asp Phe Asp Pro Pro Ala Ser Leu Lys Asn Gly Ser Ser Ala
450 455 460
Tyr Tyr Asp Pro Asn Tyr Leu Thr Thr Asp Ala Glu Lys Asp Arg Tyr
465 470 475 480
Leu Lys Thr Thr Ile Lys Leu Phe Lys Arg Ile Asn Ser Asn Pro Ala
485 490 495
Gly Lys Val Leu Leu Gln Glu Ile Ser Tyr Ala Lys Pro Tyr Leu Gly
500 505 510
Asn Asp His Thr Pro Ile Asp Glu Phe Ser Pro Val Thr Arg Thr Thr
515 520 525
Ser Val Asn Ile Lys Leu Ser Thr Asn Val Glu Ser Ser Met Leu Leu
530 535 540
Asn Leu Leu Val Leu Gly Ala Gly Pro Asp Ile Phe Glu Ser Cys Cys
545 550 555 560
[0043] Tyr Pro Val Arg Lys Leu Ile Asp Pro Asp Val Val Tyr Asp Pro Ser
565 570 575
Asn Tyr Gly Phe Gly Ser Ile Asn Ile Val Thr Phe Ser Pro Glu Tyr
580 585 590
Glu Tyr Thr Phe Asn Asp Ile Ser Gly Gly His Asn Ser Ser Thr Glu
595 600 605
Ser Phe Ile Ala Asp Pro Ala Ile Ser Leu Ala His Glu Leu Ile His
610 615 620
Ala Leu His Gly Leu Tyr Gly Ala Arg Gly Val Thr Tyr Glu Glu Thr
625 630 635 640
Ile Glu Val Lys Gln Ala Pro Leu Met Ile Ala Glu Lys Pro Ile Arg
645 650 655
Leu Glu Glu Phe Leu Thr Phe Gly Gly Gln Asp Leu Asn Ile Ile Thr
660 665 670
Ser Ala Met Lys Glu Lys Ile Tyr Asn Asn Leu Leu Ala Asn Tyr Glu
675 680 685
Lys Ile Ala Thr Arg Leu Ser Glu Val Asn Ser Ala Pro Pro Glu Tyr
690 695 700
Asp Ile Asn Glu Tyr Lys Asp Tyr Phe Gln Trp Lys Tyr Gly Leu Asp
705 710 715 720
Lys Asn Ala Asp Gly Ser Tyr Thr Val Asn Glu Asn Lys Phe Asn Glu
725 730 735
Ile Tyr Lys Lys Leu Tyr Ser Phe Thr Glu Ser Asp Leu Ala Asn Lys

740 745 750
Phe Lys Val Lys Cys Arg Asn Thr Tyr Phe Ile Lys Tyr Glu Phe Leu
755 760 765
Lys Val Pro Asn Leu Leu Asp Asp Asp Ile Tyr Thr Val Ser Glu Gly
770 775 780
Phe Asn Ile Gly Asn Leu Ala Val Asn Asn Arg Gly Gln Ser Ile Lys
785 790 795 800
Leu Asn Pro Lys Ile Ile Asp Ser Ile Pro Asp Lys Gly Leu Val Glu
805 810 815
Lys Ile Val Lys Phe Ala Val Asp Lys Leu Ala Ala Ala Leu Glu His
820 825 830
His His His His His
835
<210> 37
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<223> VAMP2-GFP
<400> 37
Gly Pro Leu Gly Ser Ser Ala Thr Ala Ala Thr Ala Pro Pro Ala Ala
[0044] 1 5 10 15
Pro Ala Gly Glu Gly Gly Pro Pro Ala Pro Pro Pro Asn Leu Thr Ser
20 25 30
Asn Arg Arg Leu Gln Gln Thr Gln Ala Gln Val Asp Glu Val Val Asp
35 40 45
Ile Met Arg Val Asn Val Asp Lys Val Leu Glu Arg Asp Gln Lys Leu
50 55 60
Ser Glu Leu Asp Asp Arg Ala Asp Ala Leu Gln Ala Gly Ala Ser Gln
65 70 75 80
Phe Glu Thr Ser Ala Ala Lys Leu Lys Arg Lys Tyr Trp Trp Lys Asn
85 90 95
Leu Lys Leu Glu Asn Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val
100 105 110
Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe
115 120 125
Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr
130 135 140
Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr
145 150 155 160
Leu Val Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro
165 170 175
Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly
180 185 190

Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys
195 200 205
Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile
210 215 220
Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His
225 230 235 240
Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp
245 250 255
Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile
260 265 270
Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro
275 280 285
Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr
290 295 300
Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val
305 310 315 320
Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu
325 330 335
Leu Tyr Lys

[0045]
<210> 38
<211> 1304
<212> PRT
<213> 人工序列
<220>
<223> BoNT/FA
<400> 38
Met Pro Val Val Ile Asn Ser Phe Asn Tyr Asp Asp Pro Val Asn Asp
1 5 10 15
Asn Thr Ile Ile Tyr Ile Arg Pro Pro Tyr Tyr Glu Thr Ser Asn Thr
20 25 30
Tyr Phe Lys Ala Phe Gln Ile Met Asp Asn Val Trp Ile Ile Pro Glu
35 40 45
Arg Tyr Arg Leu Gly Ile Asp Pro Ser Leu Phe Asn Pro Pro Val Ser
50 55 60
Leu Lys Ala Gly Ser Asp Gly Tyr Phe Asp Pro Asn Tyr Leu Ser Thr
65 70 75 80
Asn Thr Glu Lys Asn Lys Tyr Leu Gln Ile Met Ile Lys Leu Phe Lys
85 90 95
Arg Ile Asn Ser Lys Pro Ala Gly Gln Ile Leu Leu Glu Glu Ile Lys
100 105 110
Asn Ala Ile Pro Tyr Leu Gly Asn Ser Tyr Thr Gln Glu Glu Gln Phe
115 120 125
Thr Thr Asn Asn Arg Thr Val Ser Phe Asn Val Lys Leu Ala Asn Gly

130 135 140

Asn Ile Val Gln Gln Met Ala Asn Leu Ile Ile Trp Gly Pro Gly Pro
145 150 155 160

Asp Leu Thr Thr Asn Lys Thr Gly Gly Ile Ile Tyr Ser Pro Tyr Gln
165 170 175

Ser Met Glu Ala Thr Pro Tyr Lys Asp Gly Phe Gly Ser Ile Met Thr
180 185 190

Val Glu Phe Ser Pro Glu Tyr Ala Thr Ala Phe Asn Asp Ile Ser Ile
195 200 205

Ala Ser His Ser Pro Ser Leu Phe Ile Lys Asp Pro Ala Leu Ile Leu
210 215 220

Met His Glu Leu Ile His Val Leu His Gly Leu Tyr Gly Thr Tyr Ile
225 230 235 240

Thr Glu Tyr Lys Ile Thr Pro Asn Val Val Gln Ser Tyr Met Lys Val
245 250 255

Thr Lys Pro Ile Thr Ser Ala Glu Phe Leu Thr Phe Gly Gly Arg Asp
260 265 270

Arg Asn Ile Val Pro Gln Ser Ile Gln Ser Gln Leu Tyr Asn Lys Val
275 280 285

Leu Ser Asp Tyr Lys Arg Ile Ala Ser Arg Leu Asn Lys Val Asn Thr
290 295 300

[0046] Ala Thr Ala Leu Ile Asn Ile Asp Glu Phe Lys Asn Leu Tyr Glu Trp
305 310 315 320

Lys Tyr Gln Phe Ala Lys Asp Ser Asn Gly Val Tyr Ser Val Asp Leu
325 330 335

Asn Lys Phe Glu Gln Leu Tyr Lys Lys Ile Tyr Ser Phe Thr Glu Phe
340 345 350

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

Glu Asn Phe Gly Pro Phe Tyr Leu Pro Asn Leu Leu Asp Asp Ser Ile
370 375 380

Tyr Thr Glu Val Asp Gly Phe Asn Ile Gly Ala Leu Ser Ile Asn Tyr
385 390 395 400

Gln Gly Gln Asn Ile Gly Ser Asp Ile Asn Ser Ile Lys Lys Leu Gln
405 410 415

Gly Gln Gly Val Val Ser Arg Val Val Arg Leu Cys Lys Ser Val Ile
420 425 430

Pro Arg Lys Gly Thr Lys Ala Pro Pro Arg Leu Cys Ile Thr Val Asn
435 440 445

Asn Arg Asp Leu Phe Phe Ile Ala Ser Gln Glu Ser Tyr Gly Glu Asn
450 455 460

Thr Ile Asn Thr Tyr Lys Glu Ile Asp Asp Thr Thr Thr Leu Asp Pro
465 470 475 480

Ser Phe Glu Asp Ile Leu Asp Lys Val Ile Leu Asn Phe Asn Glu Gln

485 490 495
Val Ile Pro Gln Met Pro Asn Arg Asn Val Ser Thr Asp Ile Gln Lys
500 505 510
Asp Asn Tyr Ile Pro Lys Tyr Asp Tyr Asn Arg Thr Asp Ile Ile Asp
515 520 525
Ser Tyr Glu Val Gly Arg Asn Tyr Asn Thr Phe Phe Tyr Leu Asn Ala
530 535 540
Gln Lys Phe Ser Pro Asn Glu Ser Asn Ile Thr Leu Thr Ser Ser Phe
545 550 555 560
Asp Thr Gly Leu Leu Glu Gly Ser Lys Val Tyr Thr Phe Phe Ser Ser
565 570 575
Asp Phe Ile Asn Asn Ile Asn Lys Pro Val Gln Ala Leu Leu Phe Ile
580 585 590
Glu Trp Val Lys Gln Val Ile Arg Asp Phe Thr Thr Glu Ala Thr Lys
595 600 605
Thr Ser Thr Val Asp Lys Leu Lys Asp Ile Ser Leu Val Val Pro Tyr
610 615 620
Ile Gly Leu Ala Leu Asn Ile Gly Asp Glu Ile Tyr Lys Gln His Phe
625 630 635 640
Ala Glu Ala Val Glu Leu Val Gly Ala Gly Leu Leu Leu Glu Phe Ser
645 650 655
[0047] Pro Glu Phe Leu Ile Pro Thr Leu Leu Ile Phe Thr Ile Lys Gly Tyr
660 665 670
Leu Thr Gly Ser Ile Arg Asp Lys Asp Lys Ile Ile Lys Thr Leu Asp
675 680 685
Asn Ala Leu Asn Val Arg Asp Gln Lys Trp Lys Glu Leu Tyr Arg Trp
690 695 700
Val Val Ser Lys Trp Leu Thr Thr Ile Asn Thr Gln Phe Asn Lys Arg
705 710 715 720
Lys Glu Gln Met Tyr Lys Ala Leu Lys Asn Gln Ala Thr Ala Ile Lys
725 730 735
Lys Ile Ile Glu Asn Lys Tyr Asn Asn Tyr Thr Thr Asp Glu Lys Ser
740 745 750
Lys Ile Asp Ser Ser Tyr Asn Ile Asn Glu Ile Glu Arg Thr Leu Asn
755 760 765
Glu Lys Ile Asn Leu Ala Met Lys Asn Ile Glu Gln Phe Ile Thr Glu
770 775 780
Ser Ser Ile Ala Tyr Leu Ile Asn Ile Ile Asn Asn Glu Thr Ile Gln
785 790 795 800
Lys Leu Lys Ser Tyr Asp Asp Leu Val Arg Arg Tyr Leu Leu Gly Tyr
805 810 815
Ile Arg Asn His Ser Ser Ile Leu Gly Asn Ser Val Glu Glu Leu Asn
820 825 830
Ser Lys Val Asn Asn His Leu Asp Asn Gly Ile Pro Phe Glu Leu Ser

835 840 845
Ser Tyr Thr Asn Asp Ser Leu Leu Ile Arg Tyr Phe Asn Lys Asn Tyr
850 855 860
Gly Glu Leu Lys Tyr Asn Cys Ile Leu Asn Ile Lys Tyr Glu Met Asp
865 870 875 880
Arg Asp Lys Leu Val Asp Ser Ser Gly Tyr Arg Ser Arg Ile Asn Ile
885 890 895
Gly Thr Gly Val Lys Phe Ser Glu Ile Asp Lys Asn Gln Val Gln Leu
900 905 910
Ser Asn Leu Glu Ser Ser Lys Ile Glu Val Ile Leu Asn Asn Gly Val
915 920 925
Ile Tyr Asn Ser Met Tyr Glu Asn Phe Ser Thr Ser Phe Trp Ile Arg
930 935 940
Ile Pro Lys Tyr Phe Arg Asn Ile Asn Asn Glu Tyr Lys Ile Ile Ser
945 950 955 960
Cys Met Gln Asn Asn Ser Gly Trp Glu Val Ser Leu Asn Phe Ser Asn
965 970 975
Met Asn Ser Lys Ile Ile Trp Thr Leu Gln Asp Thr Glu Gly Ile Lys
980 985 990
Lys Thr Val Val Phe Gln Tyr Thr Gln Asn Ile Asn Ile Ser Asp Tyr
995 1000 1005
[0048] Ile Asn Arg Trp Ile Phe Val Thr Ile Thr Asn Asn Arg Leu Ser
1010 1015 1020
Asn Ser Lys Ile Tyr Ile Asn Gly Arg Leu Ile Asn Glu Glu Ser
1025 1030 1035
Ile Ser Asp Leu Gly Asn Ile His Ala Ser Asn Asn Ile Met Phe
1040 1045 1050
Lys Leu Asp Gly Cys Arg Asp Pro His Arg Tyr Ile Trp Ile Lys
1055 1060 1065
Tyr Phe Asn Leu Phe Asp Lys Glu Leu Asn Lys Lys Glu Ile Lys
1070 1075 1080
Asp Leu Tyr Asp Asn Gln Ser Asn Ser Gly Ile Leu Lys Asp Phe
1085 1090 1095
Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro Tyr Tyr Met Leu Asn
1100 1105 1110
Leu Tyr Asp Pro Asn Lys Tyr Leu Asp Val Asn Asn Val Gly Ile
1115 1120 1125
Arg Gly Tyr Met Tyr Leu Lys Gly Pro Arg Gly Arg Ile Val Thr
1130 1135 1140
Thr Asn Ile Tyr Leu Asn Ser Thr Leu Tyr Met Gly Thr Lys Phe
1145 1150 1155
Ile Ile Lys Lys Tyr Ala Ser Gly Asn Lys Asp Asn Ile Val Arg
1160 1165 1170
Asn Asn Asp Arg Val Tyr Ile Asn Val Val Val Lys Asn Lys Glu

1175 1180 1185

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

1190 1195 1200

Leu Ser Ala Val Glu Ile Pro Asp Val Gly Asn Leu Ser Gln Val

1205 1210 1215

Val Val Met Lys Ser Glu Asn Asp Gln Gly Ile Arg Asn Lys Cys

1220 1225 1230

Lys Met Asn Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly Phe Ile

1235 1240 1245

Gly Phe His Gln Phe Asn Asn Ile Ala Lys Leu Val Ala Ser Asn

1250 1255 1260

Trp Tyr Asn Arg Gln Ile Gly Lys Ala Ser Arg Thr Phe Gly Cys

1265 1270 1275

Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Gly Glu Ser Ser

1280 1285 1290

Leu His His His His His His His His His His

1295 1300

<210> 39

<211> 15

<212> PRT

<213> 人工序列

[0049] <220>

<223> Pep1

<400> 39

Ser Asn Arg Arg Leu Gln Gln Thr Gln Ala Gln Val Asp Glu Cys

1 5 10 15

<210> 40

<211> 8

<212> PRT

<213> 人工序列

<220>

<223> Pep3

<400> 40

Cys Leu Gln Ala Gly Ala Ser Gln

1 5

<210> 41

<211> 1306

<212> PRT

<213> 肉毒杆菌

<400> 41

Met Lys Leu Glu Ile Asn Lys Phe Asn Tyr Asn Asp Pro Ile Asp Gly

1 5 10 15

Ile Asn Val Ile Thr Met Arg Pro Pro Arg His Ser Asp Lys Ile Asn

20 25 30

Lys Gly Lys Gly Pro Phe Lys Ala Phe Gln Val Ile Lys Asn Ile Trp
35 40 45
Ile Val Pro Glu Arg Tyr Asn Phe Thr Asn Asn Thr Asn Asp Leu Asn
50 55 60
Ile Pro Ser Glu Pro Ile Met Glu Ala Asp Ala Ile Tyr Asn Pro Asn
65 70 75 80
Tyr Leu Asn Thr Pro Ser Glu Lys Asp Glu Phe Leu Gln Gly Val Ile
85 90 95
Lys Val Leu Glu Arg Ile Lys Ser Lys Pro Glu Gly Glu Lys Leu Leu
100 105 110
Glu Leu Ile Ser Ser Ser Ile Pro Leu Pro Leu Val Ser Asn Gly Ala
115 120 125
Leu Thr Leu Ser Asp Asn Glu Thr Ile Ala Tyr Gln Glu Asn Asn Asn
130 135 140
Ile Val Ser Asn Leu Gln Ala Asn Leu Val Ile Tyr Gly Pro Gly Pro
145 150 155 160
Asp Ile Ala Asn Asn Ala Thr Tyr Gly Leu Tyr Ser Thr Pro Ile Ser
165 170 175
Asn Gly Glu Gly Thr Leu Ser Glu Val Ser Phe Ser Pro Phe Tyr Leu
180 185 190
Lys Pro Phe Asp Glu Ser Tyr Gly Asn Tyr Arg Ser Leu Val Asn Ile
195 200 205
[0050] Val Asn Lys Phe Val Lys Arg Glu Phe Ala Pro Asp Pro Ala Ser Thr
210 215 220
Leu Met His Glu Leu Val His Val Thr His Asn Leu Tyr Gly Ile Ser
225 230 235 240
Asn Arg Asn Phe Tyr Tyr Asn Phe Asp Thr Gly Lys Ile Glu Thr Ser
245 250 255
Arg Gln Gln Asn Ser Leu Ile Phe Glu Glu Leu Leu Thr Phe Gly Gly
260 265 270
Ile Asp Ser Lys Ala Ile Ser Ser Leu Ile Ile Lys Lys Ile Ile Glu
275 280 285
Thr Ala Lys Asn Asn Tyr Thr Thr Leu Ile Ser Glu Arg Leu Asn Thr
290 295 300
Val Thr Val Glu Asn Asp Leu Leu Lys Tyr Ile Lys Asn Lys Ile Pro
305 310 315 320
Val Gln Gly Arg Leu Gly Asn Phe Lys Leu Asp Thr Ala Glu Phe Glu
325 330 335
Lys Lys Leu Asn Thr Ile Leu Phe Val Leu Asn Glu Ser Asn Leu Ala
340 345 350
Gln Arg Phe Ser Ile Leu Val Arg Lys His Tyr Leu Lys Glu Arg Pro
355 360 365
Ile Asp Pro Ile Tyr Val Asn Ile Leu Asp Asp Asn Ser Tyr Ser Thr
370 375 380

Leu Glu Gly Phe Asn Ile Ser Ser Gln Gly Ser Asn Asp Phe Gln Gly
385 390 395 400
Gln Leu Leu Glu Ser Ser Tyr Phe Glu Lys Ile Glu Ser Asn Ala Leu
405 410 415
Arg Ala Phe Ile Lys Ile Cys Pro Arg Asn Gly Leu Leu Tyr Asn Ala
420 425 430
Ile Tyr Arg Asn Ser Lys Asn Tyr Leu Asn Asn Ile Asp Leu Glu Asp
435 440 445
Lys Lys Thr Thr Ser Lys Thr Asn Val Ser Tyr Pro Cys Ser Leu Leu
450 455 460
Asn Gly Cys Ile Glu Val Glu Asn Lys Asp Leu Phe Leu Ile Ser Asn
465 470 475 480
Lys Asp Ser Leu Asn Asp Ile Asn Leu Ser Glu Glu Lys Ile Lys Pro
485 490 495
Glu Thr Thr Val Phe Phe Lys Asp Lys Leu Pro Pro Gln Asp Ile Thr
500 505 510
Leu Ser Asn Tyr Asp Phe Thr Glu Ala Asn Ser Ile Pro Ser Ile Ser
515 520 525
Gln Gln Asn Ile Leu Glu Arg Asn Glu Glu Leu Tyr Glu Pro Ile Arg
530 535 540
Asn Ser Leu Phe Glu Ile Lys Thr Ile Tyr Val Asp Lys Leu Thr Thr
545 550 555 560
[0051]
Phe His Phe Leu Glu Ala Gln Asn Ile Asp Glu Ser Ile Asp Ser Ser
565 570 575
Lys Ile Arg Val Glu Leu Thr Asp Ser Val Asp Glu Ala Leu Ser Asn
580 585 590
Pro Asn Lys Val Tyr Ser Pro Phe Lys Asn Met Ser Asn Thr Ile Asn
595 600 605
Ser Ile Glu Thr Gly Ile Thr Ser Thr Tyr Ile Phe Tyr Gln Trp Leu
610 615 620
Arg Ser Ile Val Lys Asp Phe Ser Asp Glu Thr Gly Lys Ile Asp Val
625 630 635 640
Ile Asp Lys Ser Ser Asp Thr Leu Ala Ile Val Pro Tyr Ile Gly Pro
645 650 655
Leu Leu Asn Ile Gly Asn Asp Ile Arg His Gly Asp Phe Val Gly Ala
660 665 670
Ile Glu Leu Ala Gly Ile Thr Ala Leu Leu Glu Tyr Val Pro Glu Phe
675 680 685
Thr Ile Pro Ile Leu Val Gly Leu Glu Val Ile Gly Gly Glu Leu Ala
690 695 700
Arg Glu Gln Val Glu Ala Ile Val Asn Asn Ala Leu Asp Lys Arg Asp
705 710 715 720
Gln Lys Trp Ala Glu Val Tyr Asn Ile Thr Lys Ala Gln Trp Trp Gly
725 730 735

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Thr Ile His Leu Gln Ile Asn Thr Arg Leu Ala His Thr Tyr Lys Ala
            740                 745                 750
Leu Ser Arg Gln Ala Asn Ala Ile Lys Met Asn Met Glu Phe Gln Leu
        755                 760                 765
Ala Asn Tyr Lys Gly Asn Ile Asp Asp Lys Ala Lys Ile Lys Asn Ala
    770                 775                 780
Ile Ser Glu Thr Glu Ile Leu Leu Asn Lys Ser Val Glu Gln Ala Met
785                 790                 795                 800
Lys Asn Thr Glu Lys Phe Met Ile Lys Leu Ser Asn Ser Tyr Leu Thr
                805                 810                 815
Lys Glu Met Ile Pro Lys Val Gln Asp Asn Leu Lys Asn Phe Asp Leu
            820                 825                 830
Glu Thr Lys Lys Thr Leu Asp Lys Phe Ile Lys Glu Lys Glu Asp Ile
        835                 840                 845
Leu Gly Thr Asn Leu Ser Ser Ser Leu Arg Arg Lys Val Ser Ile Arg
    850                 855                 860
Leu Asn Lys Asn Ile Ala Phe Asp Ile Asn Asp Ile Pro Phe Ser Glu
865                 870                 875                 880
Phe Asp Asp Leu Ile Asn Gln Tyr Lys Asn Glu Ile Glu Asp Tyr Glu
                885                 890                 895
Val Leu Asn Leu Gly Ala Glu Asp Gly Lys Ile Lys Asp Leu Ser Gly
            900                 905                 910
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[0052]

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Thr Thr Ser Asp Ile Asn Ile Gly Ser Asp Ile Glu Leu Ala Asp Gly
        915                 920                 925
Arg Glu Asn Lys Ala Ile Lys Ile Lys Gly Ser Glu Asn Ser Thr Ile
    930                 935                 940
Lys Ile Ala Met Asn Lys Tyr Leu Arg Phe Ser Ala Thr Asp Asn Phe
945                 950                 955                 960
Ser Ile Ser Phe Trp Ile Lys His Pro Lys Pro Thr Asn Leu Leu Asn
                965                 970                 975
Asn Gly Ile Glu Tyr Thr Leu Val Glu Asn Phe Asn Gln Arg Gly Trp
            980                 985                 990
Lys Ile Ser Ile Gln Asp Ser Lys  Leu Ile Trp Tyr Leu  Arg Asp His
        995                 1000                 1005
Asn Asn  Ser Ile Lys Ile Val  Thr Pro Asp Tyr Ile  Ala Phe Asn
    1010                 1015                 1020
Gly Trp  Asn Leu Ile Thr Ile  Thr Asn Asn Arg Ser  Lys Gly Ser
    1025                 1030                 1035
Ile Val  Tyr Val Asn Gly Ser  Lys Ile Glu Glu Lys  Asp Ile Ser
    1040                 1045                 1050
Ser Ile  Trp Asn Thr Glu Val  Asp Asp Pro Ile Ile  Phe Arg Leu
    1055                 1060                 1065
Lys Asn  Asn Arg Asp Thr Gln  Ala Phe Thr Leu Leu  Asp Gln Phe
    1070                 1075                 1080
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Ser Ile Tyr Arg Lys Glu Leu Asn Gln Asn Glu Val Val Lys Leu
1085 1090 1095
Tyr Asn Tyr Tyr Phe Asn Ser Asn Tyr Ile Arg Asp Ile Trp Gly
1100 1105 1110
Asn Pro Leu Gln Tyr Asn Lys Lys Tyr Tyr Leu Gln Thr Gln Asp
1115 1120 1125
Lys Pro Gly Lys Gly Leu Ile Arg Glu Tyr Trp Ser Ser Phe Gly
1130 1135 1140
Tyr Asp Tyr Val Ile Leu Ser Asp Ser Lys Thr Ile Thr Phe Pro
1145 1150 1155
Asn Asn Ile Arg Tyr Gly Ala Leu Tyr Asn Gly Ser Lys Val Leu
1160 1165 1170
Ile Lys Asn Ser Lys Lys Leu Asp Gly Leu Val Arg Asn Lys Asp
1175 1180 1185
Phe Ile Gln Leu Glu Ile Asp Gly Tyr Asn Met Gly Ile Ser Ala
1190 1195 1200
Asp Arg Phe Asn Glu Asp Thr Asn Tyr Ile Gly Thr Thr Tyr Gly
1205 1210 1215
Thr Thr His Asp Leu Thr Thr Asp Phe Glu Ile Ile Gln Arg Gln
1220 1225 1230
Glu Lys Tyr Arg Asn Tyr Cys Gln Leu Lys Thr Pro Tyr Asn Ile
1235 1240 1245

[0053]

Phe His Lys Ser Gly Leu Met Ser Thr Glu Thr Ser Lys Pro Thr
1250 1255 1260
Phe His Asp Tyr Arg Asp Trp Val Tyr Ser Ser Ala Trp Tyr Phe
1265 1270 1275
Gln Asn Tyr Glu Asn Leu Asn Leu Arg Lys His Thr Lys Thr Asn
1280 1285 1290
Trp Tyr Phe Ile Pro Lys Asp Glu Gly Trp Asp Glu Asp
1295 1300 1305

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Ser Val Lys Ser Glu Arg Arg Asn Leu Leu Glu Asp Asp Ser Asp Glu
20 25 30
Glu Glu Asp Phe Phe Leu Arg Gly Pro Ser Gly Pro Arg Phe Gly Pro
35 40 45
Arg Asn Asp Lys Ile Lys His Val Gln Asn Gln Val Asp Glu Val Ile
50 55 60
Asp Val Met Gln Glu Asn Ile Thr Lys Val Ile Glu Arg Gly Glu Arg

65 70 75 80
Leu Asp Glu Leu Gln Asp Lys Ser Glu Ser Leu Ser Asp Asn Ala Thr
85 90 95
Ala Phe Ser Asn Arg Ser Lys Gln Leu Arg Arg Gln Met Trp Trp Arg
100 105 110
Gly Cys Lys Ile Lys Ala Ile Met Ala Leu Ala Ala Ala Ile Leu Leu
115 120 125
Leu Met Ile Ile Thr Gln Ile Ile Leu His Leu Lys Lys
130 135 140
<210> 43
<211> 141
<212> PRT
<213> 智人
<400> 43
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1 5 10 15
Ser Val Lys Ser Glu Arg Arg Asn Leu Leu Glu Asp Asp Ser Asp Glu
20 25 30
Glu Glu Asp Phe Phe Leu Arg Gly Pro Ser Gly Pro Arg Phe Gly Pro
35 40 45
Arg Asn Asp Lys Ile Lys His Val Gln Asn Gln Val Asp Glu Val Ile
50 55 60
[0054] Asp Val Met Gln Glu Asn Ile Thr Lys Val Ile Glu Arg Gly Glu Arg
65 70 75 80
Leu Asp Glu Leu Gln Asp Lys Ser Glu Ser Leu Ser Asp Asn Ala Thr
85 90 95
Ala Phe Ser Asn Arg Ser Lys Gln Leu Arg Arg Gln Met Trp Trp Arg
100 105 110
Gly Cys Lys Ile Lys Ala Ile Met Ala Leu Val Ala Ala Ile Leu Leu
115 120 125
Leu Val Ile Ile Ile Leu Ile Val Met Lys Tyr Arg Thr
130 135 140
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<213> 褐家鼠
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Thr Glu Ile Met Leu Asn Asn Phe Asp Lys Val Leu Glu Arg Asp Gly
20 25 30
Lys Leu Ser Glu Leu Gln Gln Arg Ser Asp Gln Leu Leu Asp Met Ser
35 40 45
Ser Ala Phe Ser Lys Thr Thr Lys Thr Leu Ala Gln Gln Lys Arg Trp

50 55 60
Glu Asn Ile Arg Cys Arg Val Tyr Leu Gly Leu Ala Val Ala Gly Gly
65 70 75 80
Leu Leu Leu Ile Leu Val Val Leu Leu Val Ile Phe Leu Pro Ser Gly
85 90 95
Glu Asp Ser Ser Lys Pro
100
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<211> 116
<212> PRT
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Met Ala Gly Ile Glu Leu Glu Arg Cys Gln Gln Gln Ala Asn Glu Val
1 5 10 15
Thr Glu Ile Met Arg Asn Asn Phe Gly Lys Val Leu Glu Arg Gly Val
20 25 30
Lys Leu Ala Glu Leu Gln Gln Arg Ser Asp Gln Leu Leu Asp Met Ser
35 40 45
Ser Thr Phe Asn Lys Thr Thr Gln Asn Leu Ala Gln Lys Lys Cys Trp
50 55 60
Glu Asn Ile Arg Tyr Arg Ile Cys Val Gly Leu Val Val Val Gly Val
65 70 75 80
[0055] Leu Leu Ile Ile Leu Ile Val Leu Leu Val Val Phe Leu Pro Gln Ser
85 90 95
Ser Asp Ser Ser Ser Ala Pro Arg Thr Gln Asp Ala Gly Ile Ala Ser
100 105 110
Gly Pro Gly Asn
115
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Met Lys Leu Tyr Ser Leu Ser Val Phe Tyr Lys Gly Glu Pro Lys Ala
1 5 10 15
Val Leu Leu Lys Ala Ala Tyr Asp Val Ser Ser Phe Ser Phe Phe Gln
20 25 30
Arg Ser Ser Val Gln Glu Phe Met Thr Phe Thr Ser Gln Leu Ile Val
35 40 45
Glu Arg Ser Ala Lys Gly Ser Arg Ala Ser Val Lys Glu Gln Glu Tyr
50 55 60
Leu Cys His Val Tyr Val Arg Ser Asp Ser Leu Ala Gly Val Val Ile
65 70 75 80
Ala Asp Ser Glu Tyr Pro Ser Arg Val Ala Phe Thr Leu Leu Glu Lys

85 90 95
Val Leu Asp Glu Phe Ser Lys Gln Val Asp Arg Ile Asp Trp Pro Val
100 105 110
Gly Ser Pro Ala Thr Ile His Tyr Thr Ala Leu Asp Gly His Leu Ser
115 120 125
Arg Tyr Gln Asn Pro Arg Glu Ala Asp Pro Met Ser Lys Val Gln Ala
130 135 140
Glu Leu Asp Glu Thr Lys Ile Ile Leu His Asn Thr Met Glu Ser Leu
145 150 155 160
Leu Glu Arg Gly Glu Lys Leu Asp Asp Leu Val Ser Lys Ser Glu Val
165 170 175
Leu Gly Thr Gln Ser Lys Ala Phe Tyr Lys Thr Ala Arg Lys Gln Asn
180 185 190
Ser Cys Cys Ala Ile Met
195
<210> 47
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<212> PRT
<213> 智人
<400> 47
Met Lys Leu Tyr Ser Leu Ser Val Leu Tyr Lys Gly Glu Ala Lys Val
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Val Leu Leu Lys Ala Ala Tyr Asp Val Ser Ser Phe Ser Phe Phe Gln
20 25 30
Arg Ser Ser Val Gln Glu Phe Met Thr Phe Thr Ser Gln Leu Ile Val
35 40 45
Glu Arg Ser Ser Lys Gly Thr Arg Ala Ser Val Lys Glu Gln Asp Tyr
50 55 60
Leu Cys His Val Tyr Val Arg Asn Asp Ser Leu Ala Gly Val Val Ile
65 70 75 80
Ala Asp Asn Glu Tyr Pro Ser Arg Val Ala Phe Thr Leu Leu Glu Lys
85 90 95
Val Leu Asp Glu Phe Ser Lys Gln Val Asp Arg Ile Asp Trp Pro Val
100 105 110
Gly Ser Pro Ala Thr Ile His Tyr Pro Ala Leu Asp Gly His Leu Ser
115 120 125
Arg Tyr Gln Asn Pro Arg Glu Ala Asp Pro Met Thr Lys Val Gln Ala
130 135 140
Glu Leu Asp Glu Thr Lys Ile Ile Leu His Asn Thr Met Glu Ser Leu
145 150 155 160
Leu Glu Arg Gly Glu Lys Leu Asp Asp Leu Val Ser Lys Ser Glu Val
165 170 175
Leu Gly Thr Gln Ser Lys Ala Phe Tyr Lys Thr Ala Arg Lys Gln Asn
180 185 190

Ser Cys Cys Ala Ile Met
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1 5 10

<210> 49
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Phe Glu Thr Ser Ala Ala Lys Leu Lys Arg Lys Tyr Trp Trp Lys
1 5 10 15

[0057]
<210> 50
<211> 14
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1 5 10

<210> 51
<211> 10
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<213> 人工序列
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<223> VAMP 表位
<400> 51
Phe Glu Ser Ser Ala Ala Lys Leu Lys Arg
1 5 10

<210> 52
<211> 15
<212> PRT
<213> 人工序列
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<223> VAMP 表位

<400> 52
Phe Glu Ser Ser Ala Ala Lys Leu Lys Arg Lys Tyr Trp Trp Lys
1 5 10 15
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<211> 11
<212> PRT
<213> 人工序列
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<223> VAMP 表位
<400> 53
Ala Asp Ala Leu Gln Ala Gly Ala Ser Gln Phe
1 5 10
<210> 54
<211> 10
<212> PRT
<213> 人工序列
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<223> VAMP 表位
<400> 54
Ala Asp Ala Leu Gln Ala Gly Ala Ser Gln
1 5 10
[0058] <210> 55
<211> 12
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<213> 人工序列
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<223> VAMP 表位
<400> 55
Arg Ala Asp Ala Leu Gln Ala Gly Ala Ser Gln Phe
1 5 10
<210> 56
<211> 12
<212> PRT
<213> 人工序列
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<223> VAMP 表位
<400> 56
Ala Asp Ala Leu Gln Ala Gly Ala Ser Gln Phe Glu
1 5 10
<210> 57
<211> 11
<212> PRT
<213> 人工序列
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<223> VAMP 表位

<400> 57

Ala Asp Ala Leu Gln Ala Gly Ala Ser Val Phe
1 5 10

<210> 58

<211> 10

<212> PRT

<213> 人工序列

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<400> 58

Ala Asp Ala Leu Gln Ala Gly Ala Ser Val
1 5 10

<210> 59

<211> 12

<212> PRT

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<223> VAMP 表位

<400> 59

Ala Asp Ala Leu Gln Ala Gly Ala Ser Val Phe Glu
1 5 10

[0059]

<210> 60

<211> 12

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<213> 人工序列

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<223> VAMP 表位

<400> 60

Arg Ala Asp Ala Leu Gln Ala Gly Ala Ser Val Phe
1 5 10

<210> 61

<211> 10

<212> PRT

<213> 人工序列

<220>

<223> VAMP 表位

<400> 61

Arg Ala Asp Ala Leu Gln Ala Gly Ala Ser
1 5 10

<210> 62

<211> 11

<212> PRT

<213> 人工序列

<220>
<223> VAMP 表位
<400> 62
Ser Glu Ser Leu Ser Asp Asn Ala Thr Ala Phe
1 5 10
<210> 63
<211> 10
<212> PRT
<213> 人工序列
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<223> VAMP 表位
<400> 63
Ser Glu Ser Leu Ser Asp Asn Ala Thr Ala
1 5 10
<210> 64
<211> 12
<212> PRT
<213> 人工序列
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<400> 64
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1 5 10
<210> 65
<211> 12
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<213> 人工序列
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<223> VAMP 表位
<400> 65
Ser Glu Ser Leu Ser Asp Asn Ala Thr Ala Phe Ser
1 5 10
<210> 66
<211> 11
<212> PRT
<213> 人工序列
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<223> VAMP 表位
<400> 66
Ser Asp Gln Leu Leu Asp Met Ser Ser Thr Phe
1 5 10
<210> 67
<211> 10
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<213> 人工序列
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<223> VAMP 表位
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Ser Asp Gln Leu Leu Asp Met Ser Ser Thr
1 5 10
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<211> 12
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<400> 68
Arg Ser Asp Gln Leu Leu Asp Met Ser Ser Thr Phe
1 5 10
<210> 69
<211> 12
<212> PRT
<213> 人工序列
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[0061] <400> 69
Ser Asp Gln Leu Leu Asp Met Ser Ser Thr Phe Asn
1 5 10
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<211> 11
<212> PRT
<213> 人工序列
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Ser Asp Gln Leu Leu Asp Met Ser Ser Ala Phe
1 5 10
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<211> 10
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<400> 71
Ser Asp Gln Leu Leu Asp Met Ser Ser Ala
1 5 10
<210> 72
<211> 12

<212> PRT
<213> 人工序列
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<223> VAMP 表位
<400> 72
Arg Ser Asp Gln Leu Leu Asp Met Ser Ser Ala Phe
1 5 10
<210> 73
<211> 12
<212> PRT
<213> 人工序列
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<223> VAMP 表位
<400> 73
Ser Asp Gln Leu Leu Asp Met Ser Ser Ala Phe Ser
1 5 10
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<211> 10
<212> PRT
<213> 人工序列
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[0062] <223> VAMP 表位
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Arg Ser Asp Gln Leu Leu Asp Met Ser Ser
1 5 10
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<211> 11
<212> PRT
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<400> 75
Ser Glu Val Leu Gly Thr Gln Ser Lys Ala Phe
1 5 10
<210> 76
<211> 10
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<213> 人工序列
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<223> VAMP 表位
<400> 76
Ser Glu Val Leu Gly Thr Gln Ser Lys Ala
1 5 10
<210> 77

<211> 12
<212> PRT
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<220>
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<400> 77
Lys Ser Glu Val Leu Gly Thr Gln Ser Lys Ala Phe
1 5 10

[0063]
<210> 78
<211> 12
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<213> 人工序列
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<400> 78
Ser Glu Val Leu Gly Thr Gln Ser Lys Ala Phe Tyr
1 5 10

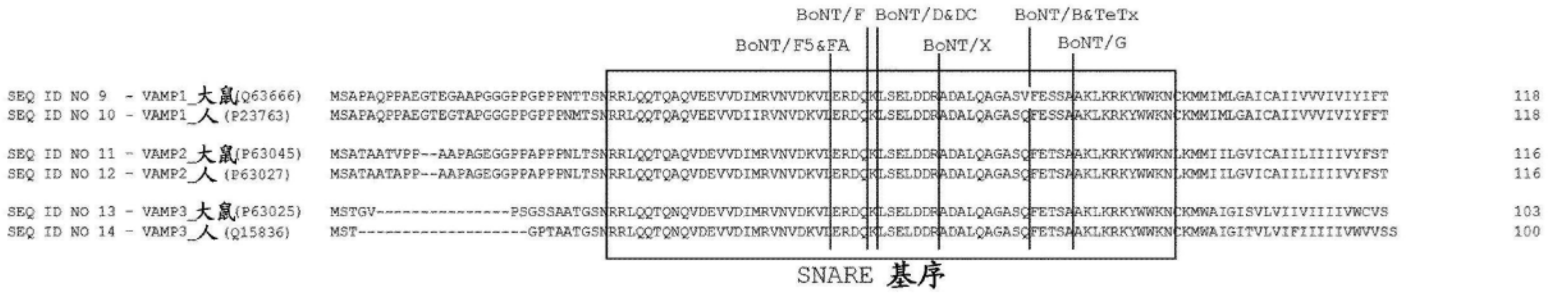
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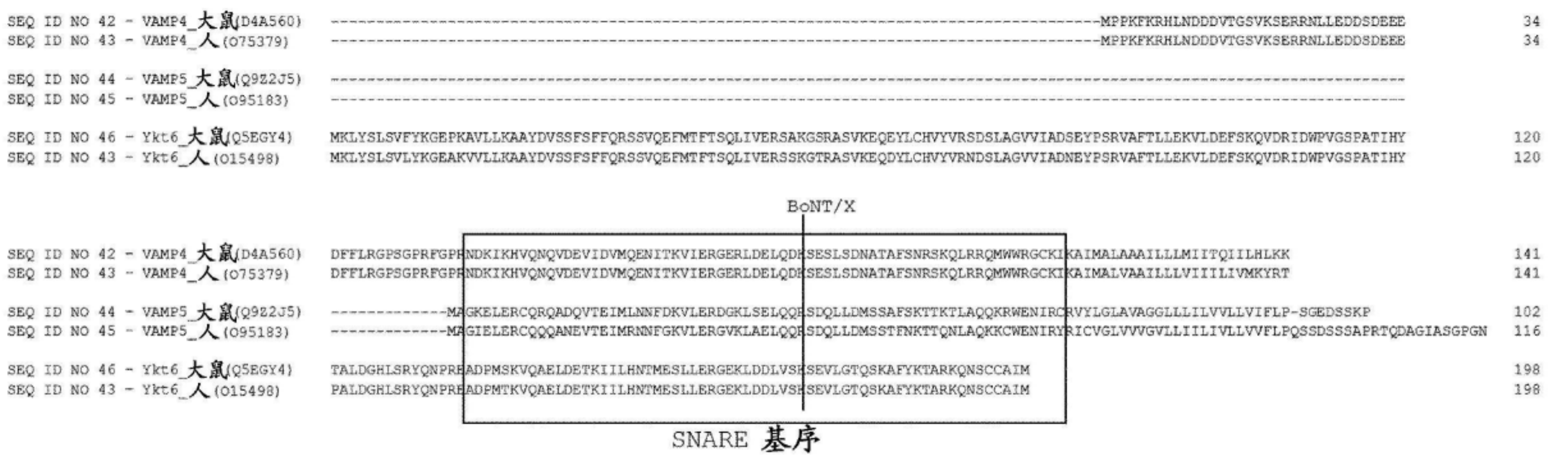
图1A



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SEQ ID NO 44 - VAMP5_大鼠(Q9Z2J5)    ------------------------------------------------------------------------------------------------------------------------
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SEQ ID NO 43 - VAMP4_人(O75379)      DFFLRGPSGPRFGPRNDKIKHVQNQVDEVIDVMQENITKVIERGERLDELQDKSESLSDNATAFSNRSKQLRRQMWWRGCKIKAIMALVAAILLLVIIILIVMKYRT    141

SEQ ID NO 44 - VAMP5_大鼠(Q9Z2J5)    -------------MAGKELERCQRQADQVTEIMLNNFDKVLERDGKLSELQQRSDQLLDMSSAFSKTTKTLAQQKRWENIRCRVYLGLAVAGGLLLILVVLLVIFLP-SGEDSSKP    102
SEQ ID NO 45 - VAMP5_人(O95183)      -------------MAGIELERCQQQANEVTEIMRNNFGKVLERGVKLAELQQRSDQLLDMSSTFNKTTQNLAQKKCWENIRYRICVGLVVVGVLLIILIVLLVVFLPQSSDSSSAPRTQDAGIASGPGN    116

SEQ ID NO 46 - Ykt6_大鼠(Q5EGY4)     TALDGHLSRYQNPREADPMSKVQAELDETKIILHNTMESLLERGEKLDDLVSKSEVLGTQSKAFYKTARKQNSCCAIM    198
SEQ ID NO 43 - Ykt6_人(O15498)       PALDGHLSRYQNPREADPMTKVQAELDETKIILHNTMESLLERGEKLDDLVSKSEVLGTQSKAFYKTARKQNSCCAIM    198
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图1B

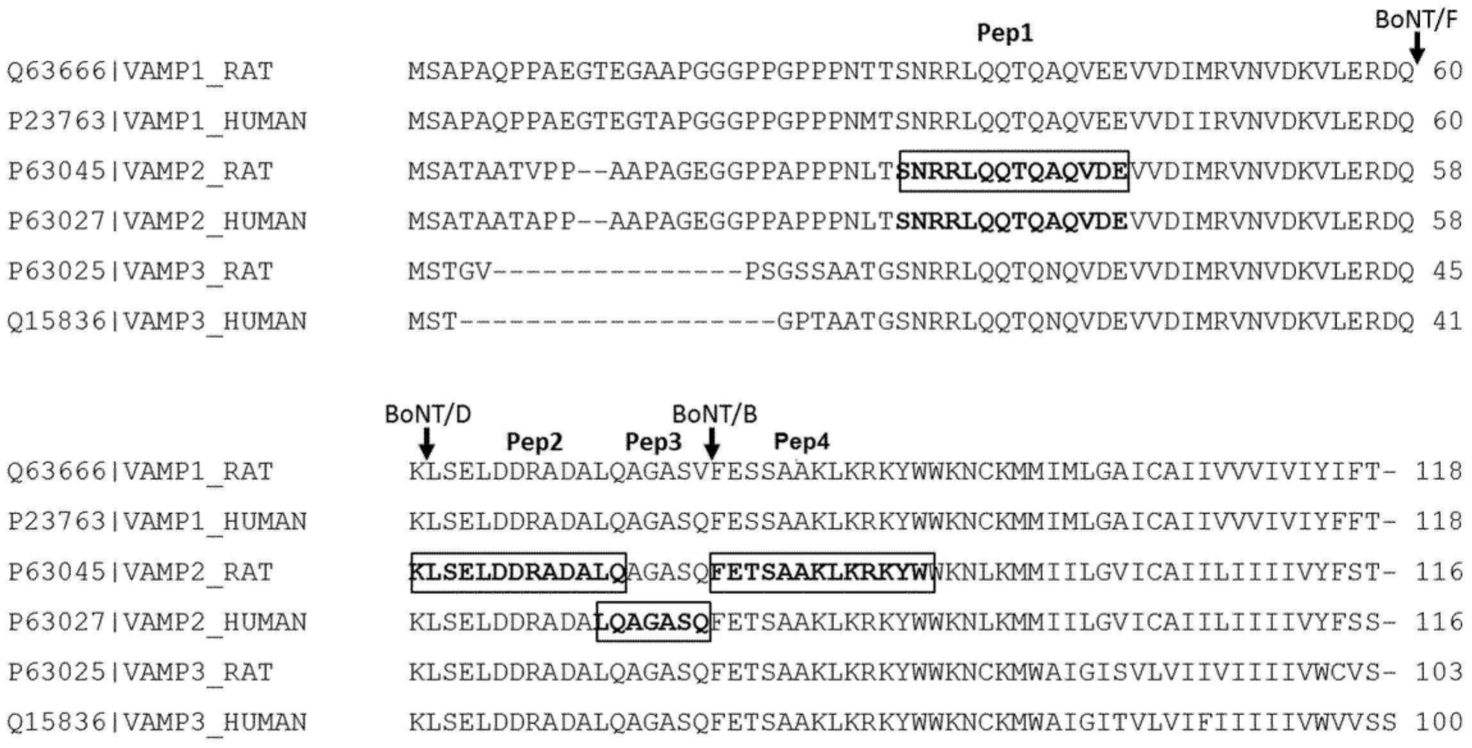
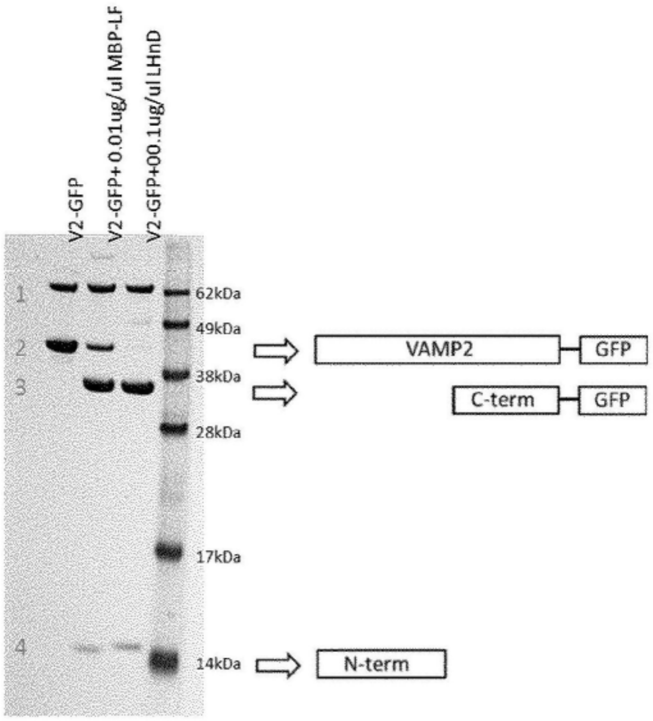
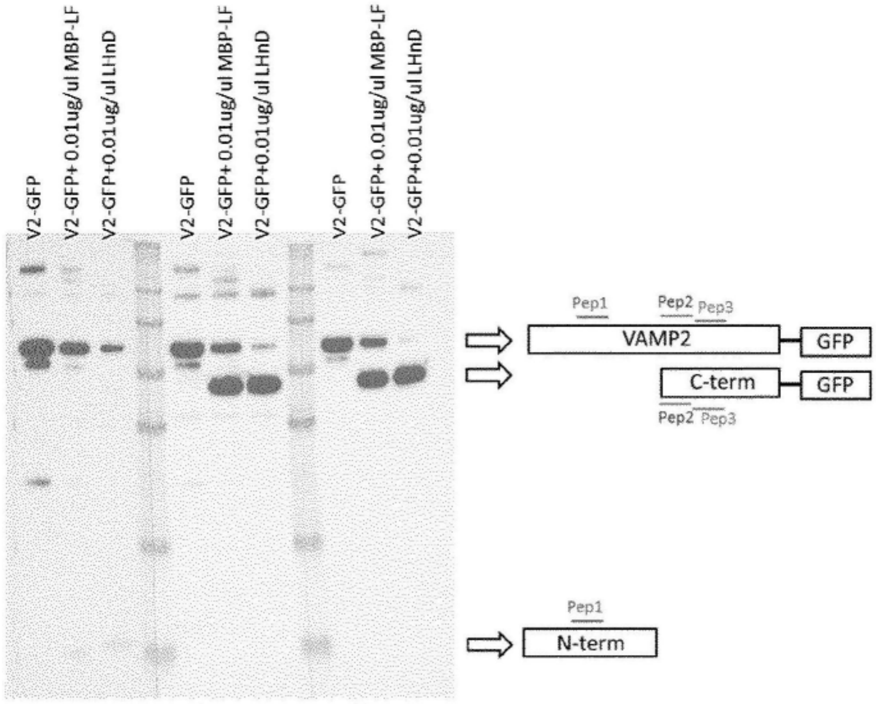

BoNT/F
Pep1
Q63666|VAMP1_RAT MSAPAQPPAEGTEGAAPGGGPPGPPPNTTSNRRLQQTQAQVEEVVDIMRVNVDKVLERDQ 60
P23763|VAMP1_HUMAN MSAPAQPPAEGTEGTAPGGGPPGPPPNMTSNRRLQQTQAQVEEVVDIIRVNVDKVLERDQ 60
P63045|VAMP2_RAT MSATAATVPP--AAPAGEGGPPAPPPNLTSNRRLQQTQAQVDEVVDIMRVNVDKVLERDQ 58
P63027|VAMP2_HUMAN MSATAATAPP--AAPAGEGGPPAPPPNLTSNRRLQQTQAQVDEVVDIMRVNVDKVLERDQ 58
P63025|VAMP3_RAT MSTGV---------------PSGSSAATGSNRRLQQTQNQVDEVVDIMRVNVDKVLERDQ 45
Q15836|VAMP3_HUMAN MST-------------------GPTAATGSNRRLQQTQNQVDEVVDIMRVNVDKVLERDQ 41
BoNT/D
Pep2
Pep3
BoNT/B
Pep4
Q63666|VAMP1_RAT KLSELDDRADALQAGASVFESSAAKLKRKYWWKNCKMMIMLGAICAIIVVVIVIYIFT- 118
P23763|VAMP1_HUMAN KLSELDDRADALQAGASQFESSAAKLKRKYWWKNCKMMIMLGAICAIIVVVIVIYFFT- 118
P63045|VAMP2_RAT KLSELDDRADALQAGASQFETSAAKLKRKYWWKNLKMMIILGVICAIILIIIIVYFST- 116
P63027|VAMP2_HUMAN KLSELDDRADALQAGASQFETSAAKLKRKYWWKNLKMMIILGVICAIILIIIIVYFSS- 116
P63025|VAMP3_RAT KLSELDDRADALQAGASQFETSAAKLKRKYWWKNCKMWAIGISVLVIIVIIIIVWCVS- 103
Q15836|VAMP3_HUMAN KLSELDDRADALQAGASQFETSAAKLKRKYWWKNCKMWAIGITVLVIFIIIIIVWVVSS 100

图2



考马斯



抗-Pep1　抗-Pep2　抗-Pep3

图3

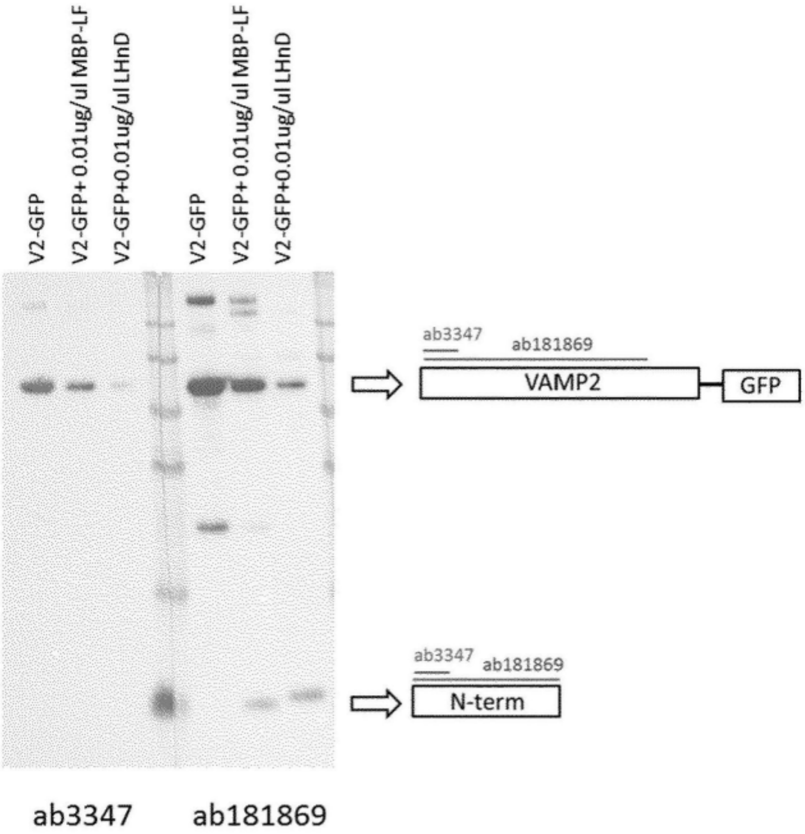

V2-GFP
V2-GFP+ 0.01ug/ul MBP-LF
V2-GFP+0.01ug/ul LHnD
V2-GFP
V2-GFP+ 0.01ug/ul MBP-LF
V2-GFP+0.01ug/ul LHnD
ab3347
ab181869
VAMP2
GFP
ab3347
ab181869
N-term
ab3347
ab181869

图3(续)

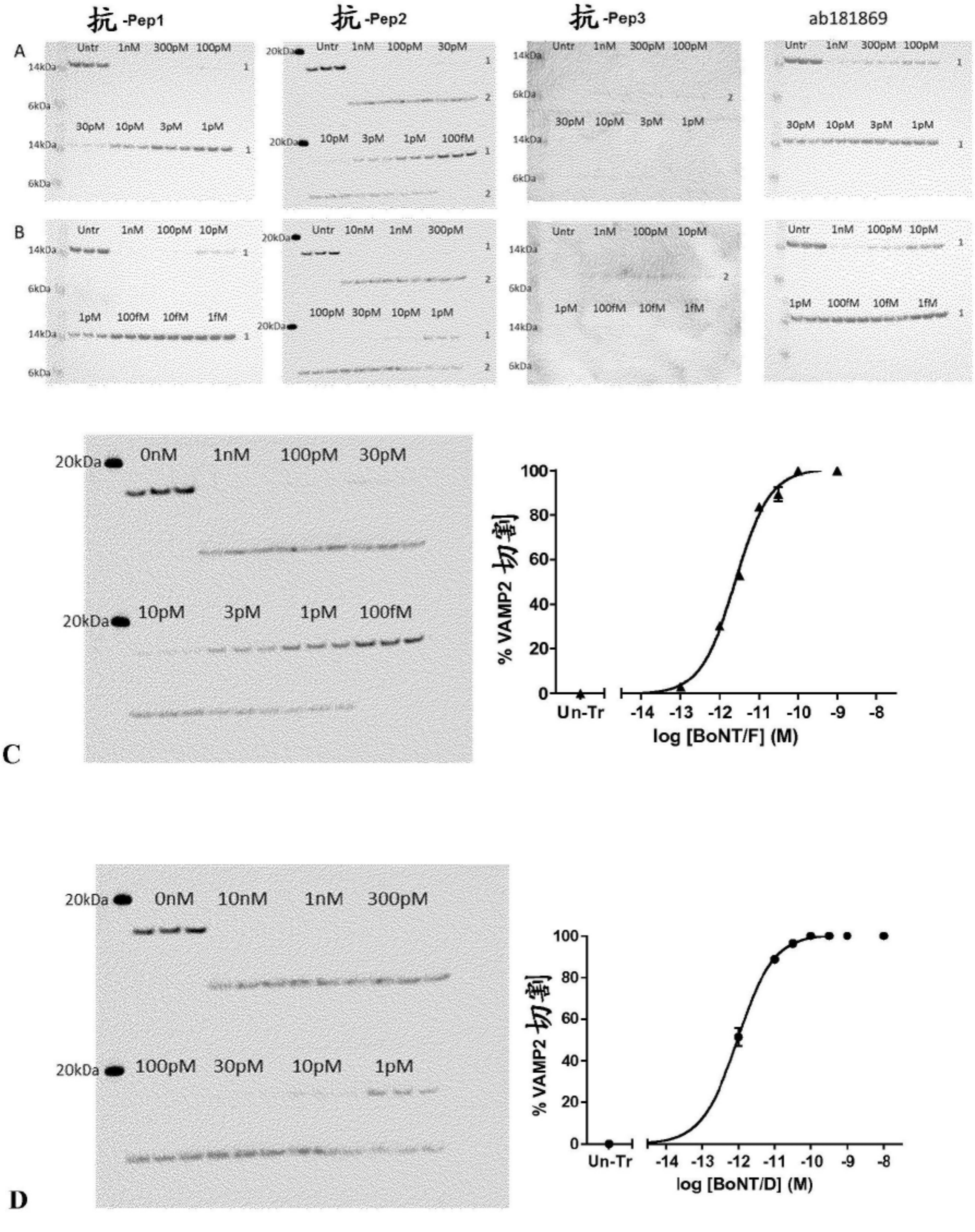
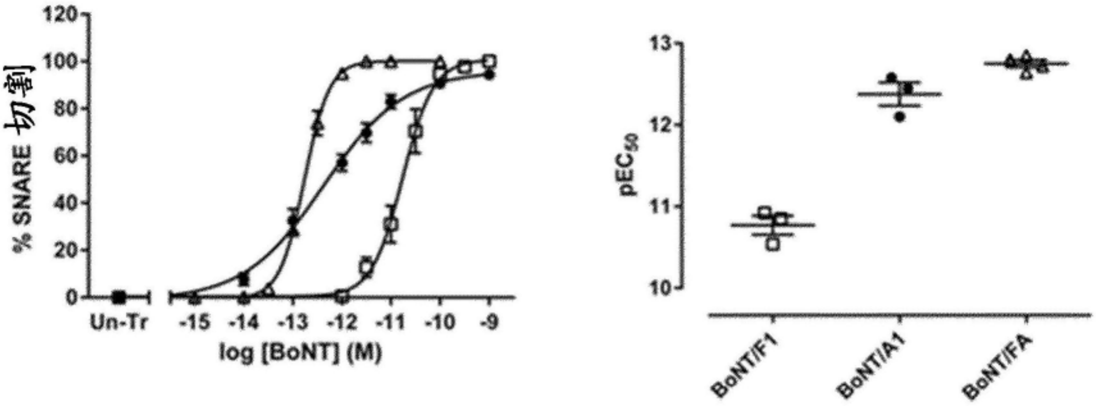
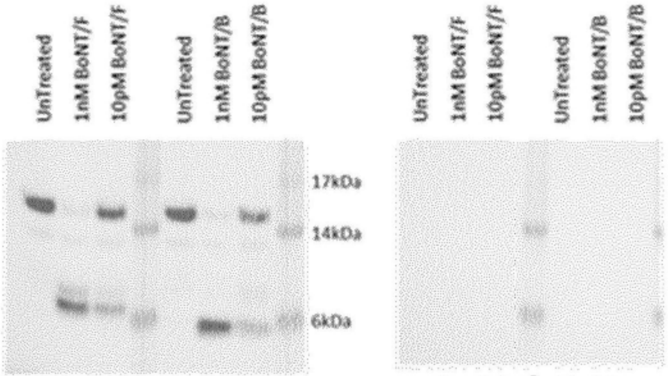

抗-Pep1
抗-Pep2
抗-Pep3
ab181869
A
Untr 1nM 300pM 100pM
14kDa
6kDa
30pM 10pM 3pM 1pM
Untr 1nM 100pM 30pM
20kDa
10pM 3pM 1pM 100fM
1
2
B
Untr 1nM 100pM 10pM
1pM 100fM 10fM 1fM
Untr 10nM 1nM 300pM
100pM 30pM 10pM 1pM
C
20kDa
0nM 1nM 100pM 30pM
10pM 3pM 1pM 100fM
% VAMP2 切割
100
80
60
40
20
0
Un-Tr
-14 -13 -12 -11 -10 -9 -8
log [BoNT/F] (M)
D
0nM 10nM 1nM 300pM
100pM 30pM 10pM 1pM
log [BoNT/D] (M)

图4

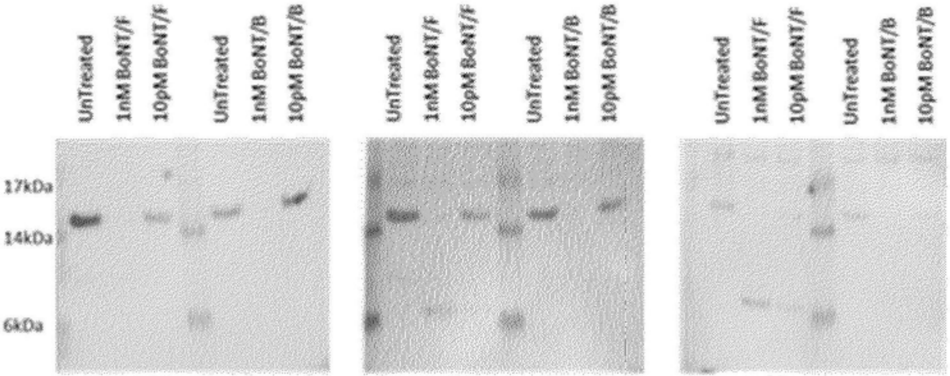


毒素	pEC$_{50}$	*n*
BoNT/A1	12.38 ± 0.14	3
BoNT/F1	10.77 ± 0.12	3
BoNT/FA	12.75 ± 0.09	4

图5



抗-Pep4 切割特异性



抗-Pep1 抗-Pep2 抗-Pep3

图6