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[54]发明名称 具有氨基酸取代的丝氨酸蛋白酶变体

[57]摘要

本发明涉及丝氨酸蛋白酶的变体,该变体的免疫原性相对于它们对应的野生型蛋白酶而言有所减少。更具体地说,本发明涉及具有野生型氨基酸序列的修饰的氨基酸序列的变体,其中修饰的氨基酸序列包括相应于枯草杆菌蛋白酶 BPN' 的一个或多个具体鉴定位置的取代。本发明还涉及编码这类变体的突变基因及包含这类变体的清洗和个人护理组合物。

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权利要求书

1. 一种丝氨酸蛋白酶变体，其特征在于野生型氨基酸序列的修饰的氨基酸序列包括在相应于枯草杆菌蛋白酶BPN'的第70-84位的一个位置上被取代氨基酸的取代，其中：

(a) 当取代发生于第70位时，取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Glu, Phe, His, Ile, Lys, Leu, Met, Pro, Gln, Ser, Thr, Trp, Tyr, 和Val；

(b) 当取代发生于第71位时，取代氨基酸选自Ala, Arg, Asn, Cys, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Gln, Ser, Trp, Tyr, 和Val；

(c) 当取代发生于第72位时，取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Gln, Ser, Thr, Trp, 和Tyr；

(d) 当取代发生于第73位时，取代氨基酸选自Arg, Asn, Asp, Cys, Glu, Gly, Phe, His, Ile, Lys, Met, Pro, Ser, Thr, Trp, Tyr, 和Val；

(e) 当取代发生于第74位时，取代氨基酸选自Arg, Asn, Asp, Cys, Glu, Gly, Phe, His, Ile, Lys, Met, Gln, Ser, Thr, Trp, Tyr, 和Val；

(f) 当取代发生于第75位时，取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Glu, Gly, Phe, His, Ile, Lys, Met, Gln, Ser, Thr, Trp, Tyr, 和Val；

(g) 当取代发生于第76位时，取代氨基酸选自Ala, Arg, Cys, Ile, Leu, Met, Gln, Ser, Thr, Trp, Tyr, 和Val；

(h) 当取代发生于第77位时，取代氨基酸选自Ala, Arg, Cys, Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Gln, Ser, Thr, Trp, Tyr, 和Val；

(i) 当取代发生于第78位时，取代氨基酸选自Ala, Arg, Cys, Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Gln, Thr, Trp, Tyr, 和Val；

(j) 当取代发生于第79位时，取代氨基酸选自Ala, Arg, Cys, Gly, Phe, His, Lys, Leu, Met, Pro, Gln, Ser, Thr, Trp, Tyr, 和Val；

(k) 当取代发生于第80位时，取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Glu, Phe, His, Ile, Lys, Leu, Met, Pro, Gln, Ser, Thr, Trp, Tyr,

和Val;

(l) 当取代发生于第81位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Gln, Ser, Thr, Trp, 和Val;

(m) 当取代发生于第82位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Glu, Gly, Phe, His, Ile, Lys, Met, Pro, Gln, Ser, Thr, Trp, Tyr, 和Val;

(n) 当取代发生于第83位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Glu, Phe, His, Ile, Lys, Leu, Met, Pro, Gln, Ser, Thr, Trp, Tyr, 和Val;和

(o) 当取代发生于第84位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Gln, Ser, Thr, Trp, 和Tyr.

2. 一种丝氨酸蛋白酶变体, 其特征在于野生型氨基酸序列的修饰的氨基酸序列包括在相应于枯草杆菌蛋白酶BPN' 的第70 - 84位的两个或多个位置上被取代氨基酸的取代, 其中:

(a) 当取代发生于第70位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Gln, Glu, Phe, His, Ile, Lys, Leu, Met, Pro, Ser, Thr, Trp, Tyr, 和Val;

(b) 当取代发生于第71位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Ser, Trp, Tyr, 和Val;

(c) 当取代发生于第72位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Ser, Thr, Trp, 和Tyr;

(d) 当取代发生于第73位时, 取代氨基酸选自Arg, Asn, Asp, Cys, Gln, Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Ser, Thr, Trp, Tyr, 和Val;

(e) 当取代发生于第74位时, 取代氨基酸选自Arg, Asn, Asp, Cys, Gln,

Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Ser, Thr, Trp, Tyr, 和Val;

(f) 当取代发生于第75位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, Phe, His, Ile, Lys, Met, Pro, Ser, Thr, Trp, Tyr, 和Val;

(g) 当取代发生于第76位时, 取代氨基酸选自Ala, Arg, Asp, Cys, Gln, Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Ser, Thr, Trp, Tyr, 和Val;

(h) 当取代发生于第77位时, 取代氨基酸选自Ala, Arg, Asp, Cys, Gln, Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Ser, Thr, Trp, Tyr, 和Val;

(i) 当取代发生于第78位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Thr, Trp, Tyr, 和Val;

(j) 当取代发生于第79位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, Phe, His, Lys, Leu, Met, Pro, Ser, Thr, Trp, Tyr, 和Val;

(k) 当取代发生于第80位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Gln, Glu, Phe, His, Ile, Lys, Leu, Met, Pro, Ser, Thr, Trp, Tyr, 和Val;

(l) 当取代发生于第81位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Ser, Thr, Trp, 和Val;

(m) 当取代发生于第82位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, Phe, His, Ile, Lys, Met, Pro, Ser, Thr, Trp, Tyr, 和Val;

(n) 当取代发生于第83位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Gln, Glu, Phe, His, Ile, Lys, Leu, Met, Pro, Ser, Thr, Trp, Tyr, 和Val;和



(o) 当取代发生于第84位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Ser, Thr, Trp, 和Tyr;

其中该变体不是Asn 76 Asp+Asn 77 Asp; Asn 76 Asp+Ile 79 Glu;或Asn 76 Asp+Ser 78 Asp.

3. 根据上述任一权利要求所述的变体, 其中所述的丝氨酸蛋白酶选自枯草杆菌蛋白酶BPN', 枯草杆菌蛋白酶Carlsberg, 枯草杆菌蛋白酶DY, 枯草杆菌蛋白酶309, 蛋白酶K和铝热酶。

4. 根据上述任一权利要求所述的变体, 其中所述的取代位于与枯草杆菌蛋白酶BPN' 第73-83位对应的一个或多个位置上。

5. 根据上述任一权利要求所述的变体, 其中所述的取代位于与枯草杆菌蛋白酶BPN' 第70和75-85位对应的一个或多个位置上。

6. 根据上述任一权利要求所述的变体, 其进一步的特征在于一个或多个稳定化突变。

7. 根据权利要求6所述的变体, 其中稳定化突变选自I107V, K213R, Y213R, Y217L, Y217K, N218S, G169A, M50F, Q19E, P5A, I31L, E156S, G169A, N212G, S188P, T254A, S3C + Q206C, 和Q271E。

8. 一种个人护理用组合物, 该组合物包含上述任一项权利要求中所述的变体以及一种个人护理用载体。

9. 一种洗涤组合物, 该组合物包含上述任一项权利要求中所述的变体以及一种洗涤组合物载体。

10. 一种编码上述任一项权利要求中所述变体的突变丝氨酸蛋白酶基因。

说明书

具有氨基酸取代的丝氨酸蛋白酶变体

发明领域

本发明涉及丝氨酸蛋白酶变体以及含有所述变体的组合物，与其对应野生型丝氨酸蛋白酶相比该变体免疫原性已减弱。

发明背景

酶是最大一类天然生成蛋白质。其中的一类是能催化水解其它蛋白质的蛋白酶。通过将天然生成及蛋白质工程蛋白酶掺加到清洗组合物中，尤其是洗衣用的那些清洗组合物中已使得这种水解蛋白质的能力得以开发利用。

清洗领域中，最常用的是丝氨酸蛋白酶。大多数丝氨酸蛋白酶是细菌生成的，而其中的一小部分是由其它微生物生成的，例如真菌。参见，Siezen 等人“枯草杆菌蛋白酶的同源性模拟及蛋白质工程策略，枯草杆菌蛋白酶样丝氨酸蛋白酶家族”，*蛋白质工程*, Vol. 4, No. 7, pp. 719 - 737 (1991)。不幸的是，野生型蛋白酶在其天然环境中的功效往往不能被转移到非天然清洗组合物的环境中。具体地说，蛋白酶特性，例如，热稳定性、pH 稳定性、氧化稳定性以及底物特异性等，在该蛋白酶天然环境之外都未必能达到最佳。

现已用几种方法改变丝氨酸蛋白酶的野生型氨基酸序列，以增强蛋白酶在非天然洗涤环境中的功效。这些方法包括蛋白酶的遗传学重构以增强蛋白酶在迥然不同条件下的热稳定性和氧化稳定性。

但是，由于这类遗传工程蛋白酶对于哺乳动物是异源性的，所以它们是潜在抗原。作为抗原，这些蛋白酶会引起哺乳动物的免疫原和/或变应原应答（本发明中统称为免疫原应答）。实际上，在人类经常接触丝



氨酸蛋白酶的环境中已经观察到了对丝氨酸蛋白酶的致敏。这类环境包括生产车间，其中的雇员通过未控制的粉尘或烟雾化接触到蛋白酶。烟雾化能够由蛋白酶导入肺而产生，这种接触蛋白酶的途径能够引发最危险的应答。蛋白酶致敏还发生于市场，其中消费者反复使用含有蛋白酶的产品可引发变态反应。

另外，虽然在通过遗传工程开发用于洗涤的更高效蛋白酶的持续研究中已取得了显著的成效，但是遗传工程蛋白酶几乎还未用于个人护理组合物和精细织品洗涤剂中。肥皂、凝胶、浴液以及香波等产品中不添加工程蛋白酶的主要原因是由于上述的人体致敏会导致不良的免疫原应答的问题。因此，提供一种具有工程蛋白酶的清洁性能但又使所激发的免疫原应答降至最小的个人护理组合物将具有十分重要的意义。

减小蛋白酶免疫活性的一种方法就是重构该蛋白酶的一个或多个表位。表位是抗原中的氨基酸区域，它们能够通过抗体结合或者通过主要组织相容性复合物蛋白(MHC)将加工过的抗原提呈给T细胞来激发免疫应答。表位的变化能够影响它们作为抗原的功效。参见Walsh, B. J. 和 M. E. H. Howden, “在修饰表位图谱基础上检测变应原中IgE结合序列的方法”，*免疫学方法杂志*, Vol. 121, pp. 275 - 280(1989)。

本发明人已经发现那些通常称为枯草杆菌蛋白酶的丝氨酸蛋白酶，包括枯草杆菌蛋白酶BPN'，在与枯草杆菌蛋白酶BPN'对应的第70 - 84位氨基酸上具有突出的表位区域。在本发明中，本发明人已对该枯草杆菌蛋白酶进行了遗传重构，从而弱化了归因于这一表位区域的免疫原性。这样做，本发明人已发现了能激发减弱后的免疫应答但仍保持它们作为有效清洁蛋白酶的活性的枯草杆菌蛋白酶。因此，本发明所述的蛋白酶可以稳定地用于几种类型的组合物中，包括但不限于，洗衣、清洗餐具、清洗坚硬表面，护肤、护发、美容、口腔护理以及清洗接触透镜的组合物。

发明概述

本发明涉及丝氨酸蛋白酶变体，与它们相应的野生型蛋白酶相比其免疫原性减弱。更具体地说，本发明涉及具有野生型氨基酸序列的修饰的氨基酸序列的变体，其中修饰的氨基酸序列包括在相应于枯草杆菌蛋白酶BPN'第70-84位的一个或多个位置上的取代。本发明还涉及编码这类变体的突变基因和包含这类变体的清洗及个人护理组合物。

发明详述

本发明的主要组分如下所述。另外还包括对用于本发明实施方案中的各种任选及优选组分的非限制性描述。

本发明可以包括任意一种本发明所述的必需或任选组分和/或限度，或者由或基本由任意一种本发明所述的必需或任选组分和/或限度所组成。

除另有说明外，所有百分比和比率都以重量来计算。除另有说明外，所有百分比都是根据组合物总量来计算的。

所有组分或组成含量都以该组分或组成的活性物含量为参照，并且排除杂质，例如，可能存在于商业来源产品中的残留溶剂或副产物。

本发明中所提及的所有文献，包括所有专利、专利申请以及印刷出版物，均在此全文引入作为参考。

本发明使用缩写来描述氨基酸。表1中提供了本发明使用的一系列缩写：

表 I

<u>氨基酸</u>	<u>三字母缩写</u>	<u>单字母缩写</u>
丙氨酸	Ala	A
精氨酸	Arg	R
天冬酰胺	Asn	N
天冬氨酸	Asp	D
半胱氨酸	Cys	C
谷氨酰胺	Gln	Q
谷氨酸	Glu	E



甘氨酸	Gly	G
组氨酸	His	H
异亮氨酸	Ile	I
亮氨酸	Leu	L
赖氨酸	Lys	K
甲硫氨酸	Met	M
苯丙氨酸	Phe	F
脯氨酸	Pro	P
丝氨酸	Ser	S
苏氨酸	Thr	T
色氨酸	Trp	W
酪氨酸	Tyr	Y
缬氨酸	Val	V

定义

本发明使用的术语“突变”是指基因序列以及由该基因序列生成的氨基酸序列中的变化。突变包括野生型蛋白质序列中氨基酸残基的缺失、取代或添加。

本发明使用的术语“野生型”是指未突变的生物所产生的蛋白质，在本发明中具体为蛋白酶。

本发明使用的术语“变体”是指其氨基酸序列不同于对应的野生型酶的氨基酸序列的蛋白质，在本发明中具体为蛋白酶。

本发明所述的变体不只限于那些枯草杆菌蛋白酶BPN'的变体，所有氨基酸编号方式均参照由SEQ ID NO: 1表示的枯草杆菌蛋白酶BPN'的氨基酸序列。枯草杆菌蛋白酶BPN'的氨基酸序列的进一步描述见Wells, J. A., E. Ferrari, D. J. Henner, D. A. Estell, 和 E. Y. Chen, *核酸研究*, Vol. II, pp. 7911 - 7925 (1983), 在此引用作为参考。

本发明的变体

本发明人已经发现了丝氨酸蛋白酶中相应于枯草杆菌蛋白酶BPN'的第70-84位的表位区域。本发明人还发现了野生型丝氨酸蛋白酶的氨基酸序列中的一个或多个氨基酸取代可提供变体，该变体相对于相应的野生型丝氨酸蛋白酶而言可引起变应原和/或免疫应答减弱。

本发明所用的变体可通过提及表征该变体的被取代的氨基酸位置来标示。在本发明中通过提供野生型氨基酸残基，随后是位置编号，再随后是将被取代的氨基酸残基来表示取代。其中被取代的氨基酸残基可以是在该特定位置上允许的任何天然氨基酸，符号“*”被提供。包括变体的多个取代用符号“+”分开。为了说明，在第70位以缬氨酸取代甘氨酸标示为Gly70Val或G70V。在第70和72两个位置上都有取代的变体的实例可以标示为Gly70Val+Val72Ala或G70V+V72A。

本发明的变体是丝氨酸蛋白酶的变体。本发明使用的术语“丝氨酸蛋白酶”是指与一种或多种枯草杆菌蛋白酶样丝氨酸蛋白酶具有至少50%，优选80%氨基酸序列同源性的蛋白酶。关于枯草杆菌蛋白酶样丝氨酸蛋白酶及其同源性的讨论可参见Siezen等人，“枯草杆菌蛋白酶的同源性模拟和蛋白质工程，枯草杆菌蛋白酶样丝氨酸蛋白酶家族”，蛋白质工程，Vol. 4, No. 7, pp. 719 - 737 (1991)。优选的用于突变的丝氨酸蛋白酶包括枯草杆菌蛋白酶 BPN'，枯草杆菌蛋白酶Carlsberg，枯草杆菌蛋白酶 DY，枯草杆菌蛋白酶 309，蛋白酶K以及铝热酶。最优选的用于突变的丝氨酸蛋白酶是枯草杆菌蛋白酶BPN'。

本发明的变体是具有野生型氨基酸序列的修饰的氨基酸序列的丝氨酸蛋白酶变体，其中修饰的氨基酸序列包括相应于BPN'第70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83和84 (70-84)位的一个或多个位置上的取代的。更优选地，修饰的氨基酸序列包括这些位置中的两个或多个位置上的取代。在这些位置上的取代是通过用另一天然氨基酸残基如表I中所给出的氨基酸残基替代野生型氨基酸残基进行的。

更具体地说，其中该变体具有野生型氨基酸序列的修饰的氨基酸序列，该序列包括在相应于BPN'的第70-84位的一个位置上的取代：



(a) 当取代发生于第70位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Glu, Phe, His, Ile, Lys, Leu, Met, Pro, Gln, Ser, Thr, Trp, Tyr, 和Val;

(b) 当取代发生于第71位时, 取代氨基酸选自Ala, Arg, Asn, Cys, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Gln, Ser, Trp, Tyr, 和Val;

(c) 当取代发生于第72位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Gln, Ser, Thr, Trp, 和Tyr;

(d) 当取代发生于第73位时, 取代氨基酸选自Arg, Asn, Asp, Cys, Glu, Gly, Phe, His, Ile, Lys, Met, Pro, Ser, Thr, Trp, Tyr, 和Val;

(e) 当取代发生于第74位时, 取代氨基酸选自Arg, Asn, Asp, Cys, Glu, Gly, Phe, His, Ile, Lys, Met, Gln, Ser, Thr, Trp, Tyr, 和Val;

(f) 当取代发生于第75位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Glu, Gly, Phe, His, Ile, Lys, Met, Gln, Ser, Thr, Trp, Tyr, 和Val;

(g) 当取代发生于第76位时, 取代氨基酸选自Ala, Arg, Cys, Ile, Leu, Met, Gln, Ser, Thr, Trp, Tyr, 和Val;

(h) 当取代发生于第77位时, 取代氨基酸选自Ala, Arg, Cys, Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Gln, Ser, Thr, Trp, Tyr, 和Val;

(i) 当取代发生于第78位时, 取代氨基酸选自Ala, Arg, Cys, Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Gln, Thr, Trp, Tyr, 和Val;

(j) 当取代发生于第79位时, 取代氨基酸选自Ala, Arg, Cys, Gly, Phe, His, Lys, Leu, Met, Pro, Gln, Ser, Thr, Trp, Tyr, 和Val;

(k) 当取代发生于第80位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Glu, Phe, His, Ile, Lys, Leu, Met, Pro, Gln, Ser, Thr, Trp, Tyr, 和Val;

(l) 当取代发生于第81位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Gln, Ser, Thr, Trp, 和Val;

(m) 当取代发生于第82位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys,



Glu, Gly, Phe, His, Ile, Lys, Met, Pro, Gln, Ser, Thr, Trp, Tyr, 和Val;

(n) 当取代发生于第83位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Glu, Phe, His, Ile, Lys, Leu, Met, Pro, Gln, Ser, Thr, Trp, Tyr, 和Val;和

(o) 当取代发生于第84位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Gln, Ser, Thr, Trp, 和Tyr.

其中该变体具有野生型氨基酸序列的修饰的氨基酸序列, 该序列包括在相应于BPN'的第70-80位的两个或多个位置上的取代:

(a) 当取代发生于第70位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Gln, Glu, Phe, His, Ile, Lys, Leu, Met, Pro, Ser, Thr, Trp, Tyr, 和Val;

(b) 当取代发生于第71位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Ser, Trp, Tyr, 和Val;

(c) 当取代发生于第72位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Ser, Thr, Trp, 和Tyr;

(d) 当取代发生于第73位时, 取代氨基酸选自Arg, Asn, Asp, Cys, Gln, Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Ser, Thr, Trp, Tyr, 和Val;

(e) 当取代发生于第74位时, 取代氨基酸选自Arg, Asn, Asp, Cys, Gln, Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Ser, Thr, Trp, Tyr, 和Val;

(f) 当取代发生于第75位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, Phe, His, Ile, Lys, Met, Pro, Ser, Thr, Trp, Tyr, 和Val;

(g) 当取代发生于第76位时, 取代氨基酸选自Ala, Arg, Asp, Cys, Gln,



Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Ser, Thr, Trp, Tyr, 和Val;

(h) 当取代发生于第77位时, 取代氨基酸选自Ala, Arg, Asp, Cys, Gln, Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Ser, Thr, Trp, Tyr, 和Val;

(i) 当取代发生于第78位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Thr, Trp, Tyr, 和Val;

(j) 当取代发生于第79位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, Phe, His, Lys, Leu, Met, Pro, Ser, Thr, Trp, Tyr, 和Val;

(k) 当取代发生于第80位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Gln, Glu, Phe, His, Ile, Lys, Leu, Met, Pro, Ser, Thr, Trp, Tyr, 和Val;

(l) 当取代发生于第81位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Ser, Thr, Trp, 和Val;

(m) 当取代发生于第82位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, Phe, His, Ile, Lys, Met, Pro, Ser, Thr, Trp, Tyr, 和Val;

(n) 当取代发生于第83位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Gln, Glu, Phe, His, Ile, Lys, Leu, Met, Pro, Ser, Thr, Trp, Tyr, 和Val;和

(o) 当取代发生于第84位时, 取代氨基酸选自Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, Phe, His, Ile, Lys, Leu, Met, Pro, Ser, Thr, Trp, 和Tyr;

其中该变体不是Asn 76 Asp+Asn 77 Asp; Asn 76 Asp+Ile 79 Glu;或Asn 76 Asp+Ser 78 Asp.

更优选地, 本发明的变体包括在第73, 74, 75, 76, 77, 78, 79,



80, 81, 82, 83 (73-83) 位的一个或多个位置的缺失, 还更优选在第75, 76, 77, 78, 79, 80, 81, 82 (75-82) 位的一个或多个位置的缺失。

下表2-7举例说明了本发明的非限制性优选变体。关于这些表, 在描述具体的取代时, 首先给出的是野生型氨基酸残基, 第二个给出的是相应的位置编号, 第三个给出的是取代氨基酸。表3-7叙述了具有两个或多个取代的优选变体。为了说明, 表3的第一个实例内容是“VAL 81 THR LEU 82 PHE”。这个实例旨在举例说明变体Val 81 Thr+Leu 82 Phe。

使用相似的记法, 表8-13举例说明了本发明更优选的变体。

表2

单取代变体

GLY 83 VAL
LEU 82 PHE
LEU 82 HIS
LEU 82 TYR
LEU 82 ALA
VAL 81 THR
VAL 81 ALA
GLY 80 VAL
ILE 79 LEU
ILE 79 MET
ILE 79 THR
ILE 79 VAL
ILE 79 ALA
SER 78 ASN
SER 78 THR
SER 78 ARG
SER 78 GLN
SER 78 HIS
SER 78 LYS
SER 78 TYR
SER 78 ALA
ASN 77 ALA
ASN 76 ALA
LEU 75 ILE
LEU 75 MET
LEU 75 VAL
LEU 75 ALA
ALA 74 VAL
ALA 73 VAL
VAL 72 ALA
THR 71 ALA
GLY 70 VAL

表3

双取代变体

VAL 81 THR LEU 82 PHE
VAL 81 THR LEU 82 HIS
VAL 81 THR LEU 82 TYR
ILE 79 LEU LEU 82 PHE
ILE 79 LEU LEU 82 HIS
ILE 79 LEU LEU 82 TYR
ILE 79 MET LEU 82 PHE
ILE 79 MET LEU 82 HIS
ILE 79 MET LEU 82 TYR
ILE 79 THR LEU 82 PHE
ILE 79 THR LEU 82 HIS
ILE 79 THR LEU 82 TYR
ILE 79 VAL LEU 82 PHE
ILE 79 VAL LEU 82 HIS
ILE 79 VAL LEU 82 TYR

ILE 79 LEU VAL 81 THR
ILE 79 MET VAL 81 THR
ILE 79 THR VAL 81 THR
ILE 79 VAL VAL 81 THR
SER 78 ASN LEU 82 PHE
SER 78 ASN LEU 82 HIS
SER 78 ASN LEU 82 TYR
SER 78 THR LEU 82 PHE
SER 78 THR LEU 82 HIS
SER 78 THR LEU 82 TYR
SER 78 ARG LEU 82 PHE
SER 78 ARG LEU 82 HIS
SER 78 ARG LEU 82 TYR
SER 78 ASP LEU 82 PHE
SER 78 ASP LEU 82 HIS
SER 78 ASP LEU 82 TYR
SER 78 GLN LEU 82 PHE
SER 78 GLN LEU 82 HIS
SER 78 GLN LEU 82 TYR
SER 78 HIS LEU 82 PHE
SER 78 HIS LEU 82 HIS
SER 78 HIS LEU 82 TYR
SER 78 LYS LEU 82 PHE
SER 78 LYS LEU 82 HIS
SER 78 LYS LEU 82 TYR
SER 78 TYR LEU 82 PHE
SER 78 TYR LEU 82 HIS
SER 78 TYR LEU 82 TYR
SER 78 ASN VAL 81 THR
SER 78 THR VAL 81 THR
SER 78 ARG VAL 81 THR
SER 78 ASP VAL 81 THR
SER 78 GLN VAL 81 THR
SER 78 HIS VAL 81 THR
SER 78 LYS VAL 81 THR
SER 78 TYR VAL 81 THR
SER 78 ASN ILE 79 LEU
SER 78 ASN ILE 79 MET
SER 78 ASN ILE 79 THR
SER 78 ASN ILE 79 VAL
SER 78 THR ILE 79 LEU
SER 78 THR ILE 79 MET
SER 78 THR ILE 79 THR
SER 78 THR ILE 79 VAL
SER 78 ARG ILE 79 LEU
SER 78 ARG ILE 79 MET
SER 78 ARG ILE 79 THR
SER 78 ARG ILE 79 VAL
SER 78 ASP ILE 79 LEU
SER 78 ASP ILE 79 MET
SER 78 ASP ILE 79 THR
SER 78 ASP ILE 79 VAL
SER 78 GLN ILE 79 LEU
SER 78 GLN ILE 79 MET
SER 78 GLN ILE 79 THR
SER 78 GLN ILE 79 VAL

SER 78 HIS ILE 79 LEU
SER 78 HIS ILE 79 MET
SER 78 HIS ILE 79 THR
SER 78 HIS ILE 79 VAL
SER 78 LYS ILE 79 LEU
SER 78 LYS ILE 79 MET
SER 78 LYS ILE 79 THR
SER 78 LYS ILE 79 VAL
SER 78 TYR ILE 79 LEU
SER 78 TYR ILE 79 MET
SER 78 TYR ILE 79 THR
SER 78 TYR ILE 79 VAL
ASN 76 HIS LEU 82 PHE
ASN 76 HIS LEU 82 HIS
ASN 76 HIS LEU 82 TYR
ASN 76 HIS VAL 81 THR
ASN 76 HIS ILE 79 LEU
ASN 76 HIS ILE 79 MET
ASN 76 HIS ILE 79 THR
ASN 76 HIS ILE 79 VAL
ASN 76 HIS SER 78 ASN
ASN 76 HIS SER 78 THR
ASN 76 HIS SER 78 ARG
ASN 76 HIS SER 78 ASP
ASN 76 HIS SER 78 GLN
ASN 76 HIS SER 78 HIS
ASN 76 HIS SER 78 LYS
ASN 76 HIS SER 78 TYR
LEU 75 ILE LEU 82 PHE
LEU 75 ILE LEU 82 HIS
LEU 75 ILE LEU 82 TYR
LEU 75 MET LEU 82 PHE
LEU 75 MET LEU 82 HIS
LEU 75 MET LEU 82 TYR
LEU 75 VAL LEU 82 PHE
LEU 75 VAL LEU 82 HIS
LEU 75 VAL LEU 82 TYR
LEU 75 ILE VAL 81 THR
LEU 75 MET VAL 81 THR
LEU 75 VAL VAL 81 THR
LEU 75 ILE ILE 79 LEU
LEU 75 ILE ILE 79 MET
LEU 75 ILE ILE 79 THR
LEU 75 ILE ILE 79 VAL
LEU 75 MET ILE 79 LEU
LEU 75 MET ILE 79 MET
LEU 75 MET ILE 79 THR
LEU 75 MET ILE 79 VAL
LEU 75 VAL ILE 79 LEU
LEU 75 VAL ILE 79 MET
LEU 75 VAL ILE 79 THR
LEU 75 VAL ILE 79 VAL
LEU 75 ILE SER 78 ASN
LEU 75 ILE SER 78 THR
LEU 75 ILE SER 78 ARG
LEU 75 ILE SER 78 ASP

LEU 75 ILE SER 78 GLN
 LEU 75 ILE SER 78 HIS
 LEU 75 ILE SER 78 LYS
 LEU 75 ILE SER 78 TYR
 LEU 75 MET SER 78 ASN
 LEU 75 MET SER 78 THR
 LEU 75 MET SER 78 ARG
 LEU 75 MET SER 78 ASP
 LEU 75 MET SER 78 GLN
 LEU 75 MET SER 78 HIS
 LEU 75 MET SER 78 LYS
 LEU 75 MET SER 78 TYR
 LEU 75 VAL SER 78 ASN
 LEU 75 VAL SER 78 THR
 LEU 75 VAL SER 78 ARG
 LEU 75 VAL SER 78 ASP
 LEU 75 VAL SER 78 GLN
 LEU 75 VAL SER 78 HIS
 LEU 75 VAL SER 78 LYS
 LEU 75 VAL SER 78 TYR
 LEU 75 ILE ASN 76 HIS
 LEU 75 MET ASN 76 HIS
 LEU 75 VAL ASN 76 HIS

表4

三取代变体

ILE 79 LEU VAL 81 THR LEU 82 PHE
 ILE 79 LEU VAL 81 THR LEU 82 HIS
 ILE 79 LEU VAL 81 THR LEU 82 TYR
 ILE 79 MET VAL 81 THR LEU 82 PHE
 ILE 79 MET VAL 81 THR LEU 82 HIS
 ILE 79 MET VAL 81 THR LEU 82 TYR
 ILE 79 THR VAL 81 THR LEU 82 PHE
 ILE 79 THR VAL 81 THR LEU 82 HIS
 ILE 79 THR VAL 81 THR LEU 82 TYR
 ILE 79 VAL VAL 81 THR LEU 82 PHE
 ILE 79 VAL VAL 81 THR LEU 82 HIS
 ILE 79 VAL VAL 81 THR LEU 82 TYR
 SER 78 ASN VAL 81 THR LEU 82 PHE
 SER 78 ASN VAL 81 THR LEU 82 HIS
 SER 78 ASN VAL 81 THR LEU 82 TYR
 SER 78 THR VAL 81 THR LEU 82 PHE
 SER 78 THR VAL 81 THR LEU 82 HIS
 SER 78 THR VAL 81 THR LEU 82 TYR
 SER 78 ARG VAL 81 THR LEU 82 PHE
 SER 78 ARG VAL 81 THR LEU 82 HIS
 SER 78 ARG VAL 81 THR LEU 82 TYR
 SER 78 ASP VAL 81 THR LEU 82 PHE
 SER 78 ASP VAL 81 THR LEU 82 HIS
 SER 78 ASP VAL 81 THR LEU 82 TYR
 SER 78 GLN VAL 81 THR LEU 82 PHE
 SER 78 GLN VAL 81 THR LEU 82 HIS
 SER 78 GLN VAL 81 THR LEU 82 TYR
 SER 78 HIS VAL 81 THR LEU 82 PHE

SER 78 HIS VAL 81 THR LEU 82 HIS
SER 78 HIS VAL 81 THR LEU 82 TYR
SER 78 LYS VAL 81 THR LEU 82 PHE
SER 78 LYS VAL 81 THR LEU 82 HIS
SER 78 LYS VAL 81 THR LEU 82 TYR
SER 78 TYR VAL 81 THR LEU 82 PHE
SER 78 TYR VAL 81 THR LEU 82 HIS
SER 78 TYR VAL 81 THR LEU 82 TYR
SER 78 ASN ILE 79 LEU LEU 82 PHE
SER 78 ASN ILE 79 LEU LEU 82 HIS
SER 78 ASN ILE 79 LEU LEU 82 TYR
SER 78 ASN ILE 79 MET LEU 82 PHE
SER 78 ASN ILE 79 MET LEU 82 HIS
SER 78 ASN ILE 79 MET LEU 82 TYR
SER 78 ASN ILE 79 THR LEU 82 PHE
SER 78 ASN ILE 79 THR LEU 82 HIS
SER 78 ASN ILE 79 THR LEU 82 TYR
SER 78 ASN ILE 79 VAL LEU 82 PHE
SER 78 ASN ILE 79 VAL LEU 82 HIS
SER 78 ASN ILE 79 VAL LEU 82 TYR
SER 78 THR ILE 79 LEU LEU 82 PHE
SER 78 THR ILE 79 LEU LEU 82 HIS
SER 78 THR ILE 79 LEU LEU 82 TYR
SER 78 THR ILE 79 MET LEU 82 PHE
SER 78 THR ILE 79 MET LEU 82 HIS
SER 78 THR ILE 79 MET LEU 82 TYR
SER 78 THR ILE 79 THR LEU 82 PHE
SER 78 THR ILE 79 THR LEU 82 HIS
SER 78 THR ILE 79 THR LEU 82 TYR
SER 78 THR ILE 79 VAL LEU 82 PHE
SER 78 THR ILE 79 VAL LEU 82 HIS
SER 78 THR ILE 79 VAL LEU 82 TYR
SER 78 ARG ILE 79 LEU LEU 82 PHE
SER 78 ARG ILE 79 LEU LEU 82 HIS
SER 78 ARG ILE 79 LEU LEU 82 TYR
SER 78 ARG ILE 79 MET LEU 82 PHE
SER 78 ARG ILE 79 MET LEU 82 HIS
SER 78 ARG ILE 79 MET LEU 82 TYR
SER 78 ARG ILE 79 THR LEU 82 PHE
SER 78 ARG ILE 79 THR LEU 82 HIS
SER 78 ARG ILE 79 THR LEU 82 TYR
SER 78 ARG ILE 79 VAL LEU 82 PHE
SER 78 ARG ILE 79 VAL LEU 82 HIS
SER 78 ARG ILE 79 VAL LEU 82 TYR
SER 78 ASP ILE 79 LEU LEU 82 PHE
SER 78 ASP ILE 79 LEU LEU 82 HIS
SER 78 ASP ILE 79 LEU LEU 82 TYR
SER 78 ASP ILE 79 MET LEU 82 PHE
SER 78 ASP ILE 79 MET LEU 82 HIS
SER 78 ASP ILE 79 MET LEU 82 TYR
SER 78 ASP ILE 79 THR LEU 82 PHE
SER 78 ASP ILE 79 THR LEU 82 HIS
SER 78 ASP ILE 79 THR LEU 82 TYR
SER 78 ASP ILE 79 VAL LEU 82 PHE
SER 78 ASP ILE 79 VAL LEU 82 HIS
SER 78 ASP ILE 79 VAL LEU 82 TYR

SER 78 GLN ILE 79 LEU LEU 82 PHE
SER 78 GLN ILE 79 LEU LEU 82 HIS
SER 78 GLN ILE 79 LEU LEU 82 TYR
SER 78 GLN ILE 79 MET LEU 82 PHE
SER 78 GLN ILE 79 MET LEU 82 HIS
SER 78 GLN ILE 79 MET LEU 82 TYR
SER 78 GLN ILE 79 THR LEU 82 PHE
SER 78 GLN ILE 79 THR LEU 82 HIS
SER 78 GLN ILE 79 THR LEU 82 TYR
SER 78 GLN ILE 79 VAL LEU 82 PHE
SER 78 GLN ILE 79 VAL LEU 82 HIS
SER 78 GLN ILE 79 VAL LEU 82 TYR
SER 78 HIS ILE 79 LEU LEU 82 PHE
SER 78 HIS ILE 79 LEU LEU 82 HIS
SER 78 HIS ILE 79 LEU LEU 82 TYR
SER 78 HIS ILE 79 MET LEU 82 PHE
SER 78 HIS ILE 79 MET LEU 82 HIS
SER 78 HIS ILE 79 MET LEU 82 TYR
SER 78 HIS ILE 79 THR LEU 82 PHE
SER 78 HIS ILE 79 THR LEU 82 HIS
SER 78 HIS ILE 79 THR LEU 82 TYR
SER 78 HIS ILE 79 VAL LEU 82 PHE
SER 78 HIS ILE 79 VAL LEU 82 HIS
SER 78 HIS ILE 79 VAL LEU 82 TYR
SER 78 LYS ILE 79 LEU LEU 82 PHE
SER 78 LYS ILE 79 LEU LEU 82 HIS
SER 78 LYS ILE 79 LEU LEU 82 TYR
SER 78 LYS ILE 79 MET LEU 82 PHE
SER 78 LYS ILE 79 MET LEU 82 HIS
SER 78 LYS ILE 79 MET LEU 82 TYR
SER 78 LYS ILE 79 THR LEU 82 PHE
SER 78 LYS ILE 79 THR LEU 82 HIS
SER 78 LYS ILE 79 THR LEU 82 TYR
SER 78 LYS ILE 79 VAL LEU 82 PHE
SER 78 LYS ILE 79 VAL LEU 82 HIS
SER 78 LYS ILE 79 VAL LEU 82 TYR
SER 78 TYR ILE 79 LEU LEU 82 PHE
SER 78 TYR ILE 79 LEU LEU 82 HIS
SER 78 TYR ILE 79 LEU LEU 82 TYR
SER 78 TYR ILE 79 MET LEU 82 PHE
SER 78 TYR ILE 79 MET LEU 82 HIS
SER 78 TYR ILE 79 MET LEU 82 TYR
SER 78 TYR ILE 79 THR LEU 82 PHE
SER 78 TYR ILE 79 THR LEU 82 HIS
SER 78 TYR ILE 79 THR LEU 82 TYR
SER 78 TYR ILE 79 VAL LEU 82 PHE
SER 78 TYR ILE 79 VAL LEU 82 HIS
SER 78 TYR ILE 79 VAL LEU 82 TYR
SER 78 ASN ILE 79 LEU VAL 81 THR
SER 78 ASN ILE 79 MET VAL 81 THR
SER 78 ASN ILE 79 THR VAL 81 THR
SER 78 ASN ILE 79 VAL VAL 81 THR
SER 78 THR ILE 79 LEU VAL 81 THR
SER 78 THR ILE 79 MET VAL 81 THR
SER 78 THR ILE 79 THR VAL 81 THR
SER 78 THR ILE 79 VAL VAL 81 THR

SER 78 ARG ILE 79 LEU VAL 81 THR
SER 78 ARG ILE 79 MET VAL 81 THR
SER 78 ARG ILE 79 THR VAL 81 THR
SER 78 ARG ILE 79 VAL VAL 81 THR
SER 78 ASP ILE 79 LEU VAL 81 THR
SER 78 ASP ILE 79 MET VAL 81 THR
SER 78 ASP ILE 79 THR VAL 81 THR
SER 78 ASP ILE 79 VAL VAL 81 THR
SER 78 GLN ILE 79 LEU VAL 81 THR
SER 78 GLN ILE 79 MET VAL 81 THR
SER 78 GLN ILE 79 THR VAL 81 THR
SER 78 GLN ILE 79 VAL VAL 81 THR
SER 78 HIS ILE 79 LEU VAL 81 THR
SER 78 HIS ILE 79 MET VAL 81 THR
SER 78 HIS ILE 79 THR VAL 81 THR
SER 78 HIS ILE 79 VAL VAL 81 THR
SER 78 LYS ILE 79 LEU VAL 81 THR
SER 78 LYS ILE 79 MET VAL 81 THR
SER 78 LYS ILE 79 THR VAL 81 THR
SER 78 LYS ILE 79 VAL VAL 81 THR
SER 78 TYR ILE 79 LEU VAL 81 THR
SER 78 TYR ILE 79 MET VAL 81 THR
SER 78 TYR ILE 79 THR VAL 81 THR
SER 78 TYR ILE 79 VAL VAL 81 THR
ASN 76 HIS VAL 81 THR LEU 82 PHE
ASN 76 HIS VAL 81 THR LEU 82 HIS
ASN 76 HIS VAL 81 THR LEU 82 TYR
ASN 76 HIS ILE 79 LEU LEU 82 PHE
ASN 76 HIS ILE 79 LEU LEU 82 HIS
ASN 76 HIS ILE 79 LEU LEU 82 TYR
ASN 76 HIS ILE 79 MET LEU 82 PHE
ASN 76 HIS ILE 79 MET LEU 82 HIS
ASN 76 HIS ILE 79 MET LEU 82 TYR
ASN 76 HIS ILE 79 THR LEU 82 PHE
ASN 76 HIS ILE 79 THR LEU 82 HIS
ASN 76 HIS ILE 79 THR LEU 82 TYR
ASN 76 HIS ILE 79 VAL LEU 82 PHE
ASN 76 HIS ILE 79 VAL LEU 82 HIS
ASN 76 HIS ILE 79 VAL LEU 82 TYR
ASN 76 HIS ILE 79 LEU VAL 81 THR
ASN 76 HIS ILE 79 MET VAL 81 THR
ASN 76 HIS ILE 79 THR VAL 81 THR
ASN 76 HIS ILE 79 VAL VAL 81 THR
ASN 76 HIS SER 78 ASN LEU 82 PHE
ASN 76 HIS SER 78 ASN LEU 82 HIS
ASN 76 HIS SER 78 ASN LEU 82 TYR
ASN 76 HIS SER 78 THR LEU 82 PHE
ASN 76 HIS SER 78 THR LEU 82 HIS
ASN 76 HIS SER 78 THR LEU 82 TYR
ASN 76 HIS SER 78 ARG LEU 82 PHE
ASN 76 HIS SER 78 ARG LEU 82 HIS
ASN 76 HIS SER 78 ARG LEU 82 TYR
ASN 76 HIS SER 78 ASP LEU 82 PHE
ASN 76 HIS SER 78 ASP LEU 82 HIS
ASN 76 HIS SER 78 ASP LEU 82 TYR
ASN 76 HIS SER 78 GLN LEU 82 PHE

ASN 76 HIS SER 78 GLN LEU 82 HIS
ASN 76 HIS SER 78 GLN LEU 82 TYR
ASN 76 HIS SER 78 HIS LEU 82 PHE
ASN 76 HIS SER 78 HIS LEU 82 HIS
ASN 76 HIS SER 78 HIS LEU 82 TYR
ASN 76 HIS SER 78 LYS LEU 82 PHE
ASN 76 HIS SER 78 LYS LEU 82 HIS
ASN 76 HIS SER 78 LYS LEU 82 TYR
ASN 76 HIS SER 78 TYR LEU 82 PHE
ASN 76 HIS SER 78 TYR LEU 82 HIS
ASN 76 HIS SER 78 TYR LEU 82 TYR
ASN 76 HIS SER 78 ASN VAL 81 THR
ASN 76 HIS SER 78 THR VAL 81 THR
ASN 76 HIS SER 78 ARG VAL 81 THR
ASN 76 HIS SER 78 ASP VAL 81 THR
ASN 76 HIS SER 78 GLN VAL 81 THR
ASN 76 HIS SER 78 HIS VAL 81 THR
ASN 76 HIS SER 78 LYS VAL 81 THR
ASN 76 HIS SER 78 TYR VAL 81 THR
ASN 76 HIS SER 78 ASN ILE 79 LEU
ASN 76 HIS SER 78 ASN ILE 79 MET
ASN 76 HIS SER 78 ASN ILE 79 THR
ASN 76 HIS SER 78 ASN ILE 79 VAL
ASN 76 HIS SER 78 THR ILE 79 LEU
ASN 76 HIS SER 78 THR ILE 79 MET
ASN 76 HIS SER 78 THR ILE 79 THR
ASN 76 HIS SER 78 THR ILE 79 VAL
ASN 76 HIS SER 78 ARG ILE 79 LEU
ASN 76 HIS SER 78 ARG ILE 79 MET
ASN 76 HIS SER 78 ARG ILE 79 THR
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LEU 75 VAL ILE 79 VAL VAL 81 THR LEU 82 TYR
LEU 75 ILE SER 78 ASN VAL 81 THR LEU 82 PHE
LEU 75 ILE SER 78 ASN VAL 81 THR LEU 82 HIS
LEU 75 ILE SER 78 ASN VAL 81 THR LEU 82 TYR
LEU 75 ILE SER 78 THR VAL 81 THR LEU 82 PHE
LEU 75 ILE SER 78 THR VAL 81 THR LEU 82 HIS
LEU 75 ILE SER 78 THR VAL 81 THR LEU 82 TYR
LEU 75 ILE SER 78 ARG VAL 81 THR LEU 82 PHE
LEU 75 ILE SER 78 ARG VAL 81 THR LEU 82 HIS
LEU 75 ILE SER 78 ARG VAL 81 THR LEU 82 TYR
LEU 75 ILE SER 78 ASP VAL 81 THR LEU 82 PHE
LEU 75 ILE SER 78 ASP VAL 81 THR LEU 82 HIS
LEU 75 ILE SER 78 ASP VAL 81 THR LEU 82 TYR
LEU 75 ILE SER 78 GLN VAL 81 THR LEU 82 PHE
LEU 75 ILE SER 78 GLN VAL 81 THR LEU 82 HIS
LEU 75 ILE SER 78 GLN VAL 81 THR LEU 82 TYR
LEU 75 ILE SER 78 HIS VAL 81 THR LEU 82 PHE
LEU 75 ILE SER 78 HIS VAL 81 THR LEU 82 HIS
LEU 75 ILE SER 78 HIS VAL 81 THR LEU 82 TYR
LEU 75 ILE SER 78 LYS VAL 81 THR LEU 82 PHE
LEU 75 ILE SER 78 LYS VAL 81 THR LEU 82 HIS
LEU 75 ILE SER 78 LYS VAL 81 THR LEU 82 TYR
LEU 75 ILE SER 78 TYR VAL 81 THR LEU 82 PHE
LEU 75 ILE SER 78 TYR VAL 81 THR LEU 82 HIS

LEU 75 ILE SER 78 TYR VAL 81 THR LEU 82 TYR
LEU 75 MET SER 78 ASN VAL 81 THR LEU 82 PHE
LEU 75 MET SER 78 ASN VAL 81 THR LEU 82 HIS
LEU 75 MET SER 78 ASN VAL 81 THR LEU 82 TYR
LEU 75 MET SER 78 THR VAL 81 THR LEU 82 PHE
LEU 75 MET SER 78 THR VAL 81 THR LEU 82 HIS
LEU 75 MET SER 78 THR VAL 81 THR LEU 82 TYR
LEU 75 MET SER 78 ARG VAL 81 THR LEU 82 PHE
LEU 75 MET SER 78 ARG VAL 81 THR LEU 82 HIS
LEU 75 MET SER 78 ARG VAL 81 THR LEU 82 TYR
LEU 75 MET SER 78 ASP VAL 81 THR LEU 82 PHE
LEU 75 MET SER 78 ASP VAL 81 THR LEU 82 HIS
LEU 75 MET SER 78 ASP VAL 81 THR LEU 82 TYR
LEU 75 MET SER 78 GLN VAL 81 THR LEU 82 PHE
LEU 75 MET SER 78 GLN VAL 81 THR LEU 82 HIS
LEU 75 MET SER 78 GLN VAL 81 THR LEU 82 TYR
LEU 75 MET SER 78 HIS VAL 81 THR LEU 82 PHE
LEU 75 MET SER 78 HIS VAL 81 THR LEU 82 HIS
LEU 75 MET SER 78 HIS VAL 81 THR LEU 82 TYR
LEU 75 MET SER 78 LYS VAL 81 THR LEU 82 PHE
LEU 75 MET SER 78 LYS VAL 81 THR LEU 82 HIS
LEU 75 MET SER 78 LYS VAL 81 THR LEU 82 TYR
LEU 75 MET SER 78 TYR VAL 81 THR LEU 82 PHE
LEU 75 MET SER 78 TYR VAL 81 THR LEU 82 HIS
LEU 75 MET SER 78 TYR VAL 81 THR LEU 82 TYR
LEU 75 VAL SER 78 ASN VAL 81 THR LEU 82 PHE
LEU 75 VAL SER 78 ASN VAL 81 THR LEU 82 HIS
LEU 75 VAL SER 78 ASN VAL 81 THR LEU 82 TYR
LEU 75 VAL SER 78 THR VAL 81 THR LEU 82 PHE
LEU 75 VAL SER 78 THR VAL 81 THR LEU 82 HIS
LEU 75 VAL SER 78 THR VAL 81 THR LEU 82 TYR
LEU 75 VAL SER 78 ARG VAL 81 THR LEU 82 PHE
LEU 75 VAL SER 78 ARG VAL 81 THR LEU 82 HIS
LEU 75 VAL SER 78 ARG VAL 81 THR LEU 82 TYR
LEU 75 VAL SER 78 ASP VAL 81 THR LEU 82 PHE
LEU 75 VAL SER 78 ASP VAL 81 THR LEU 82 HIS
LEU 75 VAL SER 78 ASP VAL 81 THR LEU 82 TYR
LEU 75 VAL SER 78 GLN VAL 81 THR LEU 82 PHE
LEU 75 VAL SER 78 GLN VAL 81 THR LEU 82 HIS
LEU 75 VAL SER 78 GLN VAL 81 THR LEU 82 TYR
LEU 75 VAL SER 78 HIS VAL 81 THR LEU 82 PHE
LEU 75 VAL SER 78 HIS VAL 81 THR LEU 82 HIS
LEU 75 VAL SER 78 HIS VAL 81 THR LEU 82 TYR
LEU 75 VAL SER 78 LYS VAL 81 THR LEU 82 PHE
LEU 75 VAL SER 78 LYS VAL 81 THR LEU 82 HIS
LEU 75 VAL SER 78 LYS VAL 81 THR LEU 82 TYR
LEU 75 VAL SER 78 TYR VAL 81 THR LEU 82 PHE
LEU 75 VAL SER 78 TYR VAL 81 THR LEU 82 HIS
LEU 75 VAL SER 78 TYR VAL 81 THR LEU 82 TYR
LEU 75 ILE SER 78 ASN ILE 79 LEU LEU 82 PHE
LEU 75 ILE SER 78 ASN ILE 79 LEU LEU 82 HIS
LEU 75 ILE SER 78 ASN ILE 79 LEU LEU 82 TYR
LEU 75 ILE SER 78 ASN ILE 79 MET LEU 82 PHE
LEU 75 ILE SER 78 ASN ILE 79 MET LEU 82 HIS
LEU 75 ILE SER 78 ASN ILE 79 MET LEU 82 TYR
LEU 75 ILE SER 78 ASN ILE 79 THR LEU 82 PHE

LEU 75 MET SER 78 LYS ILE 79 THR LEU 82 HIS
LEU 75 MET SER 78 LYS ILE 79 THR LEU 82 TYR
LEU 75 MET SER 78 LYS ILE 79 VAL LEU 82 PHE
LEU 75 MET SER 78 LYS ILE 79 VAL LEU 82 HIS
LEU 75 MET SER 78 LYS ILE 79 VAL LEU 82 TYR
LEU 75 MET SER 78 TYR ILE 79 LEU LEU 82 PHE
LEU 75 MET SER 78 TYR ILE 79 LEU LEU 82 HIS
LEU 75 MET SER 78 TYR ILE 79 LEU LEU 82 TYR
LEU 75 MET SER 78 TYR ILE 79 MET LEU 82 PHE
LEU 75 MET SER 78 TYR ILE 79 MET LEU 82 HIS
LEU 75 MET SER 78 TYR ILE 79 MET LEU 82 TYR
LEU 75 MET SER 78 TYR ILE 79 THR LEU 82 PHE
LEU 75 MET SER 78 TYR ILE 79 THR LEU 82 HIS
LEU 75 MET SER 78 TYR ILE 79 THR LEU 82 TYR
LEU 75 MET SER 78 TYR ILE 79 VAL LEU 82 PHE
LEU 75 MET SER 78 TYR ILE 79 VAL LEU 82 HIS
LEU 75 MET SER 78 TYR ILE 79 VAL LEU 82 TYR
LEU 75 VAL SER 78 ASN ILE 79 LEU LEU 82 PHE
LEU 75 VAL SER 78 ASN ILE 79 LEU LEU 82 HIS
LEU 75 VAL SER 78 ASN ILE 79 LEU LEU 82 TYR
LEU 75 VAL SER 78 ASN ILE 79 MET LEU 82 PHE
LEU 75 VAL SER 78 ASN ILE 79 MET LEU 82 HIS
LEU 75 VAL SER 78 ASN ILE 79 MET LEU 82 TYR
LEU 75 VAL SER 78 ASN ILE 79 THR LEU 82 PHE
LEU 75 VAL SER 78 ASN ILE 79 THR LEU 82 HIS
LEU 75 VAL SER 78 ASN ILE 79 THR LEU 82 TYR
LEU 75 VAL SER 78 ASN ILE 79 VAL LEU 82 PHE
LEU 75 VAL SER 78 ASN ILE 79 VAL LEU 82 HIS
LEU 75 VAL SER 78 ASN ILE 79 VAL LEU 82 TYR
LEU 75 VAL SER 78 THR ILE 79 LEU LEU 82 PHE
LEU 75 VAL SER 78 THR ILE 79 LEU LEU 82 HIS
LEU 75 VAL SER 78 THR ILE 79 LEU LEU 82 TYR
LEU 75 VAL SER 78 THR ILE 79 MET LEU 82 PHE
LEU 75 VAL SER 78 THR ILE 79 MET LEU 82 HIS
LEU 75 VAL SER 78 THR ILE 79 MET LEU 82 TYR
LEU 75 VAL SER 78 THR ILE 79 THR LEU 82 PHE
LEU 75 VAL SER 78 THR ILE 79 THR LEU 82 HIS
LEU 75 VAL SER 78 THR ILE 79 THR LEU 82 TYR
LEU 75 VAL SER 78 THR ILE 79 VAL LEU 82 PHE
LEU 75 VAL SER 78 THR ILE 79 VAL LEU 82 HIS
LEU 75 VAL SER 78 THR ILE 79 VAL LEU 82 TYR
LEU 75 VAL SER 78 ARG ILE 79 LEU LEU 82 PHE
LEU 75 VAL SER 78 ARG ILE 79 LEU LEU 82 HIS
LEU 75 VAL SER 78 ARG ILE 79 LEU LEU 82 TYR
LEU 75 VAL SER 78 ARG ILE 79 MET LEU 82 PHE
LEU 75 VAL SER 78 ARG ILE 79 MET LEU 82 HIS
LEU 75 VAL SER 78 ARG ILE 79 MET LEU 82 TYR
LEU 75 VAL SER 78 ARG ILE 79 THR LEU 82 PHE
LEU 75 VAL SER 78 ARG ILE 79 THR LEU 82 HIS
LEU 75 VAL SER 78 ARG ILE 79 THR LEU 82 TYR
LEU 75 VAL SER 78 ARG ILE 79 VAL LEU 82 PHE
LEU 75 VAL SER 78 ARG ILE 79 VAL LEU 82 HIS
LEU 75 VAL SER 78 ARG ILE 79 VAL LEU 82 TYR
LEU 75 VAL SER 78 ASP ILE 79 LEU LEU 82 PHE
LEU 75 VAL SER 78 ASP ILE 79 LEU LEU 82 HIS
LEU 75 VAL SER 78 ASP ILE 79 LEU LEU 82 TYR

LEU 75 MET SER 78 HIS ILE 79 VAL VAL 81 THR
 LEU 75 MET SER 78 LYS ILE 79 LEU VAL 81 THR
 LEU 75 MET SER 78 LYS ILE 79 MET VAL 81 THR
 LEU 75 MET SER 78 LYS ILE 79 THR VAL 81 THR
 LEU 75 MET SER 78 LYS ILE 79 VAL VAL 81 THR
 LEU 75 MET SER 78 TYR ILE 79 LEU VAL 81 THR
 LEU 75 MET SER 78 TYR ILE 79 MET VAL 81 THR
 LEU 75 MET SER 78 TYR ILE 79 THR VAL 81 THR
 LEU 75 MET SER 78 TYR ILE 79 VAL VAL 81 THR
 LEU 75 VAL SER 78 ASN ILE 79 LEU VAL 81 THR
 LEU 75 VAL SER 78 ASN ILE 79 MET VAL 81 THR
 LEU 75 VAL SER 78 ASN ILE 79 THR VAL 81 THR
 LEU 75 VAL SER 78 ASN ILE 79 VAL VAL 81 THR
 LEU 75 VAL SER 78 THR ILE 79 LEU VAL 81 THR
 LEU 75 VAL SER 78 THR ILE 79 MET VAL 81 THR
 LEU 75 VAL SER 78 THR ILE 79 THR VAL 81 THR
 LEU 75 VAL SER 78 THR ILE 79 VAL VAL 81 THR
 LEU 75 VAL SER 78 ARG ILE 79 LEU VAL 81 THR
 LEU 75 VAL SER 78 ARG ILE 79 MET VAL 81 THR
 LEU 75 VAL SER 78 ARG ILE 79 THR VAL 81 THR
 LEU 75 VAL SER 78 ARG ILE 79 VAL VAL 81 THR
 LEU 75 VAL SER 78 ASP ILE 79 LEU VAL 81 THR
 LEU 75 VAL SER 78 ASP ILE 79 MET VAL 81 THR
 LEU 75 VAL SER 78 ASP ILE 79 THR VAL 81 THR
 LEU 75 VAL SER 78 ASP ILE 79 VAL VAL 81 THR
 LEU 75 VAL SER 78 GLN ILE 79 LEU VAL 81 THR
 LEU 75 VAL SER 78 GLN ILE 79 MET VAL 81 THR
 LEU 75 VAL SER 78 GLN ILE 79 THR VAL 81 THR
 LEU 75 VAL SER 78 GLN ILE 79 VAL VAL 81 THR
 LEU 75 VAL SER 78 HIS ILE 79 LEU VAL 81 THR
 LEU 75 VAL SER 78 HIS ILE 79 MET VAL 81 THR
 LEU 75 VAL SER 78 HIS ILE 79 THR VAL 81 THR
 LEU 75 VAL SER 78 HIS ILE 79 VAL VAL 81 THR
 LEU 75 VAL SER 78 LYS ILE 79 LEU VAL 81 THR
 LEU 75 VAL SER 78 LYS ILE 79 MET VAL 81 THR
 LEU 75 VAL SER 78 LYS ILE 79 THR VAL 81 THR
 LEU 75 VAL SER 78 LYS ILE 79 VAL VAL 81 THR
 LEU 75 VAL SER 78 TYR ILE 79 LEU VAL 81 THR
 LEU 75 VAL SER 78 TYR ILE 79 MET VAL 81 THR
 LEU 75 VAL SER 78 TYR ILE 79 THR VAL 81 THR
 LEU 75 VAL SER 78 TYR ILE 79 VAL VAL 81 THR
 LEU 75 ILE ASN 76 HIS VAL 81 THR LEU 82 PHE
 LEU 75 ILE ASN 76 HIS VAL 81 THR LEU 82 HIS
 LEU 75 ILE ASN 76 HIS VAL 81 THR LEU 82 TYR
 LEU 75 MET ASN 76 HIS VAL 81 THR LEU 82 PHE
 LEU 75 MET ASN 76 HIS VAL 81 THR LEU 82 HIS
 LEU 75 MET ASN 76 HIS VAL 81 THR LEU 82 TYR
 LEU 75 VAL ASN 76 HIS VAL 81 THR LEU 82 PHE
 LEU 75 VAL ASN 76 HIS VAL 81 THR LEU 82 HIS
 LEU 75 VAL ASN 76 HIS VAL 81 THR LEU 82 TYR
 LEU 75 ILE ASN 76 HIS ILE 79 LEU LEU 82 PHE
 LEU 75 ILE ASN 76 HIS ILE 79 LEU LEU 82 HIS
 LEU 75 ILE ASN 76 HIS ILE 79 LEU LEU 82 TYR
 LEU 75 ILE ASN 76 HIS ILE 79 MET LEU 82 PHE
 LEU 75 ILE ASN 76 HIS ILE 79 MET LEU 82 HIS
 LEU 75 ILE ASN 76 HIS ILE 79 MET LEU 82 TYR

LEU 75 ILE ASN 76 HIS ILE 79 THR LEU 82 PHE
 LEU 75 ILE ASN 76 HIS ILE 79 THR LEU 82 HIS
 LEU 75 ILE ASN 76 HIS ILE 79 THR LEU 82 TYR
 LEU 75 ILE ASN 76 HIS ILE 79 VAL LEU 82 PHE
 LEU 75 ILE ASN 76 HIS ILE 79 VAL LEU 82 HIS
 LEU 75 ILE ASN 76 HIS ILE 79 VAL LEU 82 TYR
 LEU 75 MET ASN 76 HIS ILE 79 LEU LEU 82 PHE
 LEU 75 MET ASN 76 HIS ILE 79 LEU LEU 82 HIS
 LEU 75 MET ASN 76 HIS ILE 79 LEU LEU 82 TYR
 LEU 75 MET ASN 76 HIS ILE 79 MET LEU 82 PHE
 LEU 75 MET ASN 76 HIS ILE 79 MET LEU 82 HIS
 LEU 75 MET ASN 76 HIS ILE 79 MET LEU 82 TYR
 LEU 75 MET ASN 76 HIS ILE 79 THR LEU 82 PHE
 LEU 75 MET ASN 76 HIS ILE 79 THR LEU 82 HIS
 LEU 75 MET ASN 76 HIS ILE 79 THR LEU 82 TYR
 LEU 75 MET ASN 76 HIS ILE 79 VAL LEU 82 PHE
 LEU 75 MET ASN 76 HIS ILE 79 VAL LEU 82 HIS
 LEU 75 MET ASN 76 HIS ILE 79 VAL LEU 82 TYR
 LEU 75 VAL ASN 76 HIS ILE 79 LEU LEU 82 PHE
 LEU 75 VAL ASN 76 HIS ILE 79 LEU LEU 82 HIS
 LEU 75 VAL ASN 76 HIS ILE 79 LEU LEU 82 TYR
 LEU 75 VAL ASN 76 HIS ILE 79 MET LEU 82 PHE
 LEU 75 VAL ASN 76 HIS ILE 79 MET LEU 82 HIS
 LEU 75 VAL ASN 76 HIS ILE 79 MET LEU 82 TYR
 LEU 75 VAL ASN 76 HIS ILE 79 THR LEU 82 PHE
 LEU 75 VAL ASN 76 HIS ILE 79 THR LEU 82 HIS
 LEU 75 VAL ASN 76 HIS ILE 79 THR LEU 82 TYR
 LEU 75 VAL ASN 76 HIS ILE 79 VAL LEU 82 PHE
 LEU 75 VAL ASN 76 HIS ILE 79 VAL LEU 82 HIS
 LEU 75 VAL ASN 76 HIS ILE 79 VAL LEU 82 TYR
 LEU 75 ILE ASN 76 HIS ILE 79 LEU VAL 81 THR
 LEU 75 ILE ASN 76 HIS ILE 79 MET VAL 81 THR
 LEU 75 ILE ASN 76 HIS ILE 79 THR VAL 81 THR
 LEU 75 ILE ASN 76 HIS ILE 79 VAL VAL 81 THR
 LEU 75 MET ASN 76 HIS ILE 79 LEU VAL 81 THR
 LEU 75 MET ASN 76 HIS ILE 79 MET VAL 81 THR
 LEU 75 MET ASN 76 HIS ILE 79 THR VAL 81 THR
 LEU 75 MET ASN 76 HIS ILE 79 VAL VAL 81 THR
 LEU 75 VAL ASN 76 HIS ILE 79 LEU VAL 81 THR
 LEU 75 VAL ASN 76 HIS ILE 79 MET VAL 81 THR
 LEU 75 VAL ASN 76 HIS ILE 79 THR VAL 81 THR
 LEU 75 VAL ASN 76 HIS ILE 79 VAL VAL 81 THR
 LEU 75 ILE ASN 76 HIS SER 78 ASN LEU 82 PHE
 LEU 75 ILE ASN 76 HIS SER 78 ASN LEU 82 HIS
 LEU 75 ILE ASN 76 HIS SER 78 ASN LEU 82 TYR
 LEU 75 ILE ASN 76 HIS SER 78 THR LEU 82 PHE
 LEU 75 ILE ASN 76 HIS SER 78 THR LEU 82 HIS
 LEU 75 ILE ASN 76 HIS SER 78 THR LEU 82 TYR
 LEU 75 ILE ASN 76 HIS SER 78 ARG LEU 82 PHE
 LEU 75 ILE ASN 76 HIS SER 78 ARG LEU 82 HIS
 LEU 75 ILE ASN 76 HIS SER 78 ARG LEU 82 TYR
 LEU 75 ILE ASN 76 HIS SER 78 ASP LEU 82 PHE
 LEU 75 ILE ASN 76 HIS SER 78 ASP LEU 82 HIS
 LEU 75 ILE ASN 76 HIS SER 78 ASP LEU 82 TYR
 LEU 75 ILE ASN 76 HIS SER 78 GLN LEU 82 PHE
 LEU 75 ILE ASN 76 HIS SER 78 GLN LEU 82 HIS

LEU 75 VAL ASN 76 HIS SER 78 TYR LEU 82 HIS
LEU 75 VAL ASN 76 HIS SER 78 TYR LEU 82 TYR
LEU 75 ILE ASN 76 HIS SER 78 ASN VAL 81 THR
LEU 75 ILE ASN 76 HIS SER 78 THR VAL 81 THR
LEU 75 ILE ASN 76 HIS SER 78 ARG VAL 81 THR
LEU 75 ILE ASN 76 HIS SER 78 ASP VAL 81 THR
LEU 75 ILE ASN 76 HIS SER 78 GLN VAL 81 THR
LEU 75 ILE ASN 76 HIS SER 78 HIS VAL 81 THR
LEU 75 ILE ASN 76 HIS SER 78 LYS VAL 81 THR
LEU 75 ILE ASN 76 HIS SER 78 TYR VAL 81 THR
LEU 75 MET ASN 76 HIS SER 78 ASN VAL 81 THR
LEU 75 MET ASN 76 HIS SER 78 THR VAL 81 THR
LEU 75 MET ASN 76 HIS SER 78 ARG VAL 81 THR
LEU 75 MET ASN 76 HIS SER 78 ASP VAL 81 THR
LEU 75 MET ASN 76 HIS SER 78 GLN VAL 81 THR
LEU 75 MET ASN 76 HIS SER 78 HIS VAL 81 THR
LEU 75 MET ASN 76 HIS SER 78 LYS VAL 81 THR
LEU 75 MET ASN 76 HIS SER 78 TYR VAL 81 THR
LEU 75 VAL ASN 76 HIS SER 78 ASN VAL 81 THR
LEU 75 VAL ASN 76 HIS SER 78 THR VAL 81 THR
LEU 75 VAL ASN 76 HIS SER 78 ARG VAL 81 THR
LEU 75 VAL ASN 76 HIS SER 78 ASP VAL 81 THR
LEU 75 VAL ASN 76 HIS SER 78 GLN VAL 81 THR
LEU 75 VAL ASN 76 HIS SER 78 HIS VAL 81 THR
LEU 75 VAL ASN 76 HIS SER 78 LYS VAL 81 THR
LEU 75 VAL ASN 76 HIS SER 78 TYR VAL 81 THR
LEU 75 ILE ASN 76 HIS SER 78 ASN ILE 79 LEU
LEU 75 ILE ASN 76 HIS SER 78 ASN ILE 79 MET
LEU 75 ILE ASN 76 HIS SER 78 ASN ILE 79 THR
LEU 75 ILE ASN 76 HIS SER 78 ASN ILE 79 VAL
LEU 75 ILE ASN 76 HIS SER 78 THR ILE 79 LEU
LEU 75 ILE ASN 76 HIS SER 78 THR ILE 79 MET
LEU 75 ILE ASN 76 HIS SER 78 THR ILE 79 THR
LEU 75 ILE ASN 76 HIS SER 78 THR ILE 79 VAL
LEU 75 ILE ASN 76 HIS SER 78 ARG ILE 79 LEU
LEU 75 ILE ASN 76 HIS SER 78 ARG ILE 79 MET
LEU 75 ILE ASN 76 HIS SER 78 ARG ILE 79 THR
LEU 75 ILE ASN 76 HIS SER 78 ARG ILE 79 VAL
LEU 75 ILE ASN 76 HIS SER 78 ASP ILE 79 LEU
LEU 75 ILE ASN 76 HIS SER 78 ASP ILE 79 MET
LEU 75 ILE ASN 76 HIS SER 78 ASP ILE 79 THR
LEU 75 ILE ASN 76 HIS SER 78 ASP ILE 79 VAL
LEU 75 ILE ASN 76 HIS SER 78 GLN ILE 79 LEU
LEU 75 ILE ASN 76 HIS SER 78 GLN ILE 79 MET
LEU 75 ILE ASN 76 HIS SER 78 GLN ILE 79 THR
LEU 75 ILE ASN 76 HIS SER 78 GLN ILE 79 VAL
LEU 75 ILE ASN 76 HIS SER 78 HIS ILE 79 LEU
LEU 75 ILE ASN 76 HIS SER 78 HIS ILE 79 MET
LEU 75 ILE ASN 76 HIS SER 78 HIS ILE 79 THR
LEU 75 ILE ASN 76 HIS SER 78 HIS ILE 79 VAL
LEU 75 ILE ASN 76 HIS SER 78 LYS ILE 79 LEU
LEU 75 ILE ASN 76 HIS SER 78 LYS ILE 79 MET
LEU 75 ILE ASN 76 HIS SER 78 LYS ILE 79 THR
LEU 75 ILE ASN 76 HIS SER 78 LYS ILE 79 VAL
LEU 75 ILE ASN 76 HIS SER 78 TYR ILE 79 LEU
LEU 75 ILE ASN 76 HIS SER 78 TYR ILE 79 MET

LEU 75 VAL ASN 76 HIS SER 78 HIS ILE 79 THR
 LEU 75 VAL ASN 76 HIS SER 78 HIS ILE 79 VAL
 LEU 75 VAL ASN 76 HIS SER 78 LYS ILE 79 LEU
 LEU 75 VAL ASN 76 HIS SER 78 LYS ILE 79 MET
 LEU 75 VAL ASN 76 HIS SER 78 LYS ILE 79 THR
 LEU 75 VAL ASN 76 HIS SER 78 LYS ILE 79 VAL
 LEU 75 VAL ASN 76 HIS SER 78 TYR ILE 79 LEU
 LEU 75 VAL ASN 76 HIS SER 78 TYR ILE 79 MET
 LEU 75 VAL ASN 76 HIS SER 78 TYR ILE 79 THR
 LEU 75 VAL ASN 76 HIS SER 78 TYR ILE 79 VAL

表6

五取代变体

ASN 76 HIS SER 78 ASN ILE 79 LEU VAL 81 THR LEU 82 PHE
 ASN 76 HIS SER 78 ASN ILE 79 LEU VAL 81 THR LEU 82 HIS
 ASN 76 HIS SER 78 ASN ILE 79 LEU VAL 81 THR LEU 82 TYR
 ASN 76 HIS SER 78 ASN ILE 79 MET VAL 81 THR LEU 82 PHE
 ASN 76 HIS SER 78 ASN ILE 79 MET VAL 81 THR LEU 82 HIS
 ASN 76 HIS SER 78 ASN ILE 79 MET VAL 81 THR LEU 82 TYR
 ASN 76 HIS SER 78 ASN ILE 79 THR VAL 81 THR LEU 82 PHE
 ASN 76 HIS SER 78 ASN ILE 79 THR VAL 81 THR LEU 82 HIS
 ASN 76 HIS SER 78 ASN ILE 79 THR VAL 81 THR LEU 82 TYR
 ASN 76 HIS SER 78 ASN ILE 79 VAL VAL 81 THR LEU 82 PHE
 ASN 76 HIS SER 78 ASN ILE 79 VAL VAL 81 THR LEU 82 HIS
 ASN 76 HIS SER 78 ASN ILE 79 VAL VAL 81 THR LEU 82 TYR
 ASN 76 HIS SER 78 THR ILE 79 LEU VAL 81 THR LEU 82 PHE
 ASN 76 HIS SER 78 THR ILE 79 LEU VAL 81 THR LEU 82 HIS
 ASN 76 HIS SER 78 THR ILE 79 LEU VAL 81 THR LEU 82 TYR
 ASN 76 HIS SER 78 THR ILE 79 MET VAL 81 THR LEU 82 PHE
 ASN 76 HIS SER 78 THR ILE 79 MET VAL 81 THR LEU 82 HIS
 ASN 76 HIS SER 78 THR ILE 79 MET VAL 81 THR LEU 82 TYR
 ASN 76 HIS SER 78 THR ILE 79 THR VAL 81 THR LEU 82 PHE
 ASN 76 HIS SER 78 THR ILE 79 THR VAL 81 THR LEU 82 HIS
 ASN 76 HIS SER 78 THR ILE 79 THR VAL 81 THR LEU 82 TYR
 ASN 76 HIS SER 78 THR ILE 79 VAL VAL 81 THR LEU 82 PHE
 ASN 76 HIS SER 78 THR ILE 79 VAL VAL 81 THR LEU 82 HIS
 ASN 76 HIS SER 78 THR ILE 79 VAL VAL 81 THR LEU 82 TYR
 ASN 76 HIS SER 78 ARG ILE 79 LEU VAL 81 THR LEU 82 PHE
 ASN 76 HIS SER 78 ARG ILE 79 LEU VAL 81 THR LEU 82 HIS
 ASN 76 HIS SER 78 ARG ILE 79 LEU VAL 81 THR LEU 82 TYR
 ASN 76 HIS SER 78 ARG ILE 79 MET VAL 81 THR LEU 82 PHE
 ASN 76 HIS SER 78 ARG ILE 79 MET VAL 81 THR LEU 82 HIS
 ASN 76 HIS SER 78 ARG ILE 79 MET VAL 81 THR LEU 82 TYR
 ASN 76 HIS SER 78 ARG ILE 79 THR VAL 81 THR LEU 82 PHE
 ASN 76 HIS SER 78 ARG ILE 79 THR VAL 81 THR LEU 82 HIS
 ASN 76 HIS SER 78 ARG ILE 79 THR VAL 81 THR LEU 82 TYR
 ASN 76 HIS SER 78 ARG ILE 79 VAL VAL 81 THR LEU 82 PHE
 ASN 76 HIS SER 78 ARG ILE 79 VAL VAL 81 THR LEU 82 HIS
 ASN 76 HIS SER 78 ARG ILE 79 VAL VAL 81 THR LEU 82 TYR
 ASN 76 HIS SER 78 ASP ILE 79 LEU VAL 81 THR LEU 82 PHE
 ASN 76 HIS SER 78 ASP ILE 79 LEU VAL 81 THR LEU 82 HIS
 ASN 76 HIS SER 78 ASP ILE 79 LEU VAL 81 THR LEU 82 TYR
 ASN 76 HIS SER 78 ASP ILE 79 MET VAL 81 THR LEU 82 PHE
 ASN 76 HIS SER 78 ASP ILE 79 MET VAL 81 THR LEU 82 HIS

LEU 75 VAL ASN 76 HIS SER 78 TYR ILE 79 MET VAL 81 THR LEU 82 HIS
 LEU 75 VAL ASN 76 HIS SER 78 TYR ILE 79 MET VAL 81 THR LEU 82 TYR
 LEU 75 VAL ASN 76 HIS SER 78 TYR ILE 79 THR VAL 81 THR LEU 82 PHE
 LEU 75 VAL ASN 76 HIS SER 78 TYR ILE 79 THR VAL 81 THR LEU 82 HIS
 LEU 75 VAL ASN 76 HIS SER 78 TYR ILE 79 THR VAL 81 THR LEU 82 TYR
 LEU 75 VAL ASN 76 HIS SER 78 TYR ILE 79 VAL VAL 81 THR LEU 82 PHE
 LEU 75 VAL ASN 76 HIS SER 78 TYR ILE 79 VAL VAL 81 THR LEU 82 HIS
 LEU 75 VAL ASN 76 HIS SER 78 TYR ILE 79 VAL VAL 81 THR LEU 82 TYR

表8

单取代变体

LEU 82 PHE
 LEU 82 HIS
 VAL 81 THR
 ILE 79 LEU
 ILE 79 MET
 ILE 79 THR
 SER 78 ASN
 SER 78 THR
 ASN 76 HIS
 LEU 75 ILE
 LEU 75 MET

表9

双取代变体

VAL 81 THR LEU 82 PHE
 VAL 81 THR LEU 82 HIS
 ILE 79 LEU LEU 82 PHE
 ILE 79 LEU LEU 82 HIS
 ILE 79 MET LEU 82 PHE
 ILE 79 MET LEU 82 HIS
 ILE 79 THR LEU 82 PHE
 ILE 79 THR LEU 82 HIS
 ILE 79 LEU VAL 81 THR
 ILE 79 MET VAL 81 THR
 ILE 79 THR VAL 81 THR
 SER 78 ASN LEU 82 PHE
 SER 78 ASN LEU 82 HIS
 SER 78 THR LEU 82 PHE
 SER 78 THR LEU 82 HIS
 SER 78 ASN VAL 81 THR
 SER 78 THR VAL 81 THR
 SER 78 ASN ILE 79 LEU
 SER 78 ASN ILE 79 MET
 SER 78 ASN ILE 79 THR
 SER 78 THR ILE 79 LEU
 SER 78 THR ILE 79 MET
 SER 78 THR ILE 79 THR
 ASN 76 HIS LEU 82 PHE
 ASN 76 HIS LEU 82 HIS
 ASN 76 HIS VAL 81 THR
 ASN 76 HIS ILE 79 LEU

ASN 76 HIS ILE 79 MET
 ASN 76 HIS ILE 79 THR
 ASN 76 HIS SER 78 ASN
 ASN 76 HIS SER 78 THR
 LEU 75 ILE LEU 82 PHE
 LEU 75 ILE LEU 82 HIS
 LEU 75 MET LEU 82 PHE
 LEU 75 MET LEU 82 HIS
 LEU 75 ILE VAL 81 THR
 LEU 75 MET VAL 81 THR
 LEU 75 ILE ILE 79 LEU
 LEU 75 ILE ILE 79 MET
 LEU 75 ILE ILE 79 THR
 LEU 75 MET ILE 79 LEU
 LEU 75 MET ILE 79 MET
 LEU 75 MET ILE 79 THR
 LEU 75 ILE SER 78 ASN
 LEU 75 ILE SER 78 THR
 LEU 75 MET SER 78 ASN
 LEU 75 MET SER 78 THR
 LEU 75 ILE ASN 76 HIS
 LEU 75 MET ASN 76 HIS

表10

三取代变体

ILE 79 LEU VAL 81 THR LEU 82 PHE
 ILE 79 LEU VAL 81 THR LEU 82 HIS
 ILE 79 MET VAL 81 THR LEU 82 PHE
 ILE 79 MET VAL 81 THR LEU 82 HIS
 ILE 79 THR VAL 81 THR LEU 82 PHE
 ILE 79 THR VAL 81 THR LEU 82 HIS
 SER 78 ASN VAL 81 THR LEU 82 PHE
 SER 78 ASN VAL 81 THR LEU 82 HIS
 SER 78 THR VAL 81 THR LEU 82 PHE
 SER 78 THR VAL 81 THR LEU 82 HIS
 SER 78 ASN ILE 79 LEU LEU 82 PHE
 SER 78 ASN ILE 79 LEU LEU 82 HIS
 SER 78 ASN ILE 79 MET LEU 82 PHE
 SER 78 ASN ILE 79 MET LEU 82 HIS
 SER 78 ASN ILE 79 THR LEU 82 PHE
 SER 78 ASN ILE 79 THR LEU 82 HIS
 SER 78 THR ILE 79 LEU LEU 82 PHE
 SER 78 THR ILE 79 LEU LEU 82 HIS
 SER 78 THR ILE 79 MET LEU 82 PHE
 SER 78 THR ILE 79 MET LEU 82 HIS
 SER 78 THR ILE 79 THR LEU 82 PHE
 SER 78 THR ILE 79 THR LEU 82 HIS
 SER 78 ASN ILE 79 LEU VAL 81 THR
 SER 78 ASN ILE 79 MET VAL 81 THR
 SER 78 ASN ILE 79 THR VAL 81 THR
 SER 78 THR ILE 79 LEU VAL 81 THR
 SER 78 THR ILE 79 MET VAL 81 THR
 SER 78 THR ILE 79 THR VAL 81 THR
 ASN 76 HIS VAL 81 THR LEU 82 PHE

ASN 76 HIS VAL 81 THR LEU 82 HIS
ASN 76 HIS ILE 79 LEU LEU 82 PHE
ASN 76 HIS ILE 79 LEU LEU 82 HIS
ASN 76 HIS ILE 79 MET LEU 82 PHE
ASN 76 HIS ILE 79 MET LEU 82 HIS
ASN 76 HIS ILE 79 THR LEU 82 PHE
ASN 76 HIS ILE 79 THR LEU 82 HIS
ASN 76 HIS ILE 79 LEU VAL 81 THR
ASN 76 HIS ILE 79 MET VAL 81 THR
ASN 76 HIS ILE 79 THR VAL 81 THR
ASN 76 HIS SER 78 ASN LEU 82 PHE
ASN 76 HIS SER 78 ASN LEU 82 HIS
ASN 76 HIS SER 78 THR LEU 82 PHE
ASN 76 HIS SER 78 THR LEU 82 HIS
ASN 76 HIS SER 78 ASN VAL 81 THR
ASN 76 HIS SER 78 THR VAL 81 THR
ASN 76 HIS SER 78 ASN ILE 79 LEU
ASN 76 HIS SER 78 ASN ILE 79 MET
ASN 76 HIS SER 78 ASN ILE 79 THR
ASN 76 HIS SER 78 THR ILE 79 LEU
ASN 76 HIS SER 78 THR ILE 79 MET
ASN 76 HIS SER 78 THR ILE 79 THR
LEU 75 ILE VAL 81 THR LEU 82 PHE
LEU 75 ILE VAL 81 THR LEU 82 HIS
LEU 75 MET VAL 81 THR LEU 82 PHE
LEU 75 MET VAL 81 THR LEU 82 HIS
LEU 75 ILE ILE 79 LEU LEU 82 PHE
LEU 75 ILE ILE 79 LEU LEU 82 HIS
LEU 75 ILE ILE 79 MET LEU 82 PHE
LEU 75 ILE ILE 79 MET LEU 82 HIS
LEU 75 ILE ILE 79 THR LEU 82 PHE
LEU 75 ILE ILE 79 THR LEU 82 HIS
LEU 75 MET ILE 79 LEU LEU 82 PHE
LEU 75 MET ILE 79 LEU LEU 82 HIS
LEU 75 MET ILE 79 MET LEU 82 PHE
LEU 75 MET ILE 79 MET LEU 82 HIS
LEU 75 MET ILE 79 THR LEU 82 PHE
LEU 75 MET ILE 79 THR LEU 82 HIS
LEU 75 ILE ILE 79 LEU VAL 81 THR
LEU 75 ILE ILE 79 MET VAL 81 THR
LEU 75 ILE ILE 79 THR VAL 81 THR
LEU 75 MET ILE 79 LEU VAL 81 THR
LEU 75 MET ILE 79 MET VAL 81 THR
LEU 75 MET ILE 79 THR VAL 81 THR
LEU 75 ILE SER 78 ASN LEU 82 PHE
LEU 75 ILE SER 78 ASN LEU 82 HIS
LEU 75 ILE SER 78 THR LEU 82 PHE
LEU 75 ILE SER 78 THR LEU 82 HIS
LEU 75 MET SER 78 ASN LEU 82 PHE
LEU 75 MET SER 78 ASN LEU 82 HIS
LEU 75 MET SER 78 THR LEU 82 PHE
LEU 75 MET SER 78 THR LEU 82 HIS
LEU 75 ILE SER 78 ASN VAL 81 THR
LEU 75 ILE SER 78 THR VAL 81 THR
LEU 75 MET SER 78 ASN VAL 81 THR
LEU 75 MET SER 78 THR VAL 81 THR

LEU 75 ILE SER 78 ASN ILE 79 LEU
 LEU 75 ILE SER 78 ASN ILE 79 MET
 LEU 75 ILE SER 78 ASN ILE 79 THR
 LEU 75 ILE SER 78 THR ILE 79 LEU
 LEU 75 ILE SER 78 THR ILE 79 MET
 LEU 75 ILE SER 78 THR ILE 79 THR
 LEU 75 MET SER 78 ASN ILE 79 LEU
 LEU 75 MET SER 78 ASN ILE 79 MET
 LEU 75 MET SER 78 ASN ILE 79 THR
 LEU 75 MET SER 78 THR ILE 79 LEU
 LEU 75 MET SER 78 THR ILE 79 MET
 LEU 75 MET SER 78 THR ILE 79 THR
 LEU 75 ILE ASN 76 HIS LEU 82 PHE
 LEU 75 ILE ASN 76 HIS LEU 82 HIS
 LEU 75 MET ASN 76 HIS LEU 82 PHE
 LEU 75 MET ASN 76 HIS LEU 82 HIS
 LEU 75 ILE ASN 76 HIS VAL 81 THR
 LEU 75 MET ASN 76 HIS VAL 81 THR
 LEU 75 ILE ASN 76 HIS ILE 79 LEU
 LEU 75 ILE ASN 76 HIS ILE 79 MET
 LEU 75 ILE ASN 76 HIS ILE 79 THR
 LEU 75 MET ASN 76 HIS ILE 79 LEU
 LEU 75 MET ASN 76 HIS ILE 79 MET
 LEU 75 MET ASN 76 HIS ILE 79 THR
 LEU 75 ILE ASN 76 HIS SER 78 ASN
 LEU 75 ILE ASN 76 HIS SER 78 THR
 LEU 75 MET ASN 76 HIS SER 78 ASN
 LEU 75 MET ASN 76 HIS SER 78 THR

表11

四取代变体

SER 78 ASN ILE 79 LEU VAL 81 THR LEU 82 PHE
 SER 78 ASN ILE 79 LEU VAL 81 THR LEU 82 HIS
 SER 78 ASN ILE 79 MET VAL 81 THR LEU 82 PHE
 SER 78 ASN ILE 79 MET VAL 81 THR LEU 82 HIS
 SER 78 ASN ILE 79 THR VAL 81 THR LEU 82 PHE
 SER 78 ASN ILE 79 THR VAL 81 THR LEU 82 HIS
 SER 78 THR ILE 79 LEU VAL 81 THR LEU 82 PHE
 SER 78 THR ILE 79 LEU VAL 81 THR LEU 82 HIS
 SER 78 THR ILE 79 MET VAL 81 THR LEU 82 PHE
 SER 78 THR ILE 79 MET VAL 81 THR LEU 82 HIS
 SER 78 THR ILE 79 THR VAL 81 THR LEU 82 PHE
 SER 78 THR ILE 79 THR VAL 81 THR LEU 82 HIS
 ASN 76 HIS ILE 79 LEU VAL 81 THR LEU 82 PHE
 ASN 76 HIS ILE 79 LEU VAL 81 THR LEU 82 HIS
 ASN 76 HIS ILE 79 MET VAL 81 THR LEU 82 PHE
 ASN 76 HIS ILE 79 MET VAL 81 THR LEU 82 HIS
 ASN 76 HIS ILE 79 THR VAL 81 THR LEU 82 PHE
 ASN 76 HIS ILE 79 THR VAL 81 THR LEU 82 HIS
 ASN 76 HIS SER 78 ASN VAL 81 THR LEU 82 PHE
 ASN 76 HIS SER 78 ASN VAL 81 THR LEU 82 HIS
 ASN 76 HIS SER 78 THR VAL 81 THR LEU 82 PHE
 ASN 76 HIS SER 78 THR VAL 81 THR LEU 82 HIS
 ASN 76 HIS SER 78 ASN ILE 79 LEU LEU 82 PHE

ASN 76 HIS SER 78 ASN ILE 79 LEU LEU 82 HIS
ASN 76 HIS SER 78 ASN ILE 79 MET LEU 82 PHE
ASN 76 HIS SER 78 ASN ILE 79 MET LEU 82 HIS
ASN 76 HIS SER 78 ASN ILE 79 THR LEU 82 PHE
ASN 76 HIS SER 78 ASN ILE 79 THR LEU 82 HIS
ASN 76 HIS SER 78 THR ILE 79 LEU LEU 82 PHE
ASN 76 HIS SER 78 THR ILE 79 LEU LEU 82 HIS
ASN 76 HIS SER 78 THR ILE 79 MET LEU 82 PHE
ASN 76 HIS SER 78 THR ILE 79 MET LEU 82 HIS
ASN 76 HIS SER 78 THR ILE 79 THR LEU 82 PHE
ASN 76 HIS SER 78 THR ILE 79 THR LEU 82 HIS
ASN 76 HIS SER 78 ASN ILE 79 LEU VAL 81 THR
ASN 76 HIS SER 78 ASN ILE 79 MET VAL 81 THR
ASN 76 HIS SER 78 ASN ILE 79 THR VAL 81 THR
ASN 76 HIS SER 78 THR ILE 79 LEU VAL 81 THR
ASN 76 HIS SER 78 THR ILE 79 MET VAL 81 THR
ASN 76 HIS SER 78 THR ILE 79 THR VAL 81 THR
LEU 75 ILE ILE 79 LEU VAL 81 THR LEU 82 PHE
LEU 75 ILE ILE 79 LEU VAL 81 THR LEU 82 HIS
LEU 75 ILE ILE 79 MET VAL 81 THR LEU 82 PHE
LEU 75 ILE ILE 79 MET VAL 81 THR LEU 82 HIS
LEU 75 ILE ILE 79 THR VAL 81 THR LEU 82 PHE
LEU 75 ILE ILE 79 THR VAL 81 THR LEU 82 HIS
LEU 75 MET ILE 79 LEU VAL 81 THR LEU 82 PHE
LEU 75 MET ILE 79 LEU VAL 81 THR LEU 82 HIS
LEU 75 MET ILE 79 MET VAL 81 THR LEU 82 PHE
LEU 75 MET ILE 79 MET VAL 81 THR LEU 82 HIS
LEU 75 MET ILE 79 THR VAL 81 THR LEU 82 PHE
LEU 75 MET ILE 79 THR VAL 81 THR LEU 82 HIS
LEU 75 ILE SER 78 ASN VAL 81 THR LEU 82 PHE
LEU 75 ILE SER 78 ASN VAL 81 THR LEU 82 HIS
LEU 75 ILE SER 78 THR VAL 81 THR LEU 82 PHE
LEU 75 ILE SER 78 THR VAL 81 THR LEU 82 HIS
LEU 75 MET SER 78 ASN VAL 81 THR LEU 82 PHE
LEU 75 MET SER 78 ASN VAL 81 THR LEU 82 HIS
LEU 75 MET SER 78 THR VAL 81 THR LEU 82 PHE
LEU 75 MET SER 78 THR VAL 81 THR LEU 82 HIS
LEU 75 ILE SER 78 ASN ILE 79 LEU LEU 82 PHE
LEU 75 ILE SER 78 ASN ILE 79 LEU LEU 82 HIS
LEU 75 ILE SER 78 ASN ILE 79 MET LEU 82 PHE
LEU 75 ILE SER 78 ASN ILE 79 MET LEU 82 HIS
LEU 75 ILE SER 78 ASN ILE 79 THR LEU 82 PHE
LEU 75 ILE SER 78 ASN ILE 79 THR LEU 82 HIS
LEU 75 ILE SER 78 THR ILE 79 LEU LEU 82 PHE
LEU 75 ILE SER 78 THR ILE 79 LEU LEU 82 HIS
LEU 75 ILE SER 78 THR ILE 79 MET LEU 82 PHE
LEU 75 ILE SER 78 THR ILE 79 MET LEU 82 HIS
LEU 75 ILE SER 78 THR ILE 79 THR LEU 82 PHE
LEU 75 ILE SER 78 THR ILE 79 THR LEU 82 HIS
LEU 75 MET SER 78 ASN ILE 79 LEU LEU 82 PHE
LEU 75 MET SER 78 ASN ILE 79 LEU LEU 82 HIS
LEU 75 MET SER 78 ASN ILE 79 MET LEU 82 PHE
LEU 75 MET SER 78 ASN ILE 79 MET LEU 82 HIS
LEU 75 MET SER 78 ASN ILE 79 THR LEU 82 PHE
LEU 75 MET SER 78 ASN ILE 79 THR LEU 82 HIS
LEU 75 MET SER 78 THR ILE 79 LEU LEU 82 PHE

LEU 75 MET SER 78 THR ILE 79 LEU LEU 82 HIS
 LEU 75 MET SER 78 THR ILE 79 MET LEU 82 PHE
 LEU 75 MET SER 78 THR ILE 79 MET LEU 82 HIS
 LEU 75 MET SER 78 THR ILE 79 THR LEU 82 PHE
 LEU 75 MET SER 78 THR ILE 79 THR LEU 82 HIS
 LEU 75 ILE SER 78 ASN ILE 79 LEU VAL 81 THR
 LEU 75 ILE SER 78 ASN ILE 79 MET VAL 81 THR
 LEU 75 ILE SER 78 ASN ILE 79 THR VAL 81 THR
 LEU 75 ILE SER 78 THR ILE 79 LEU VAL 81 THR
 LEU 75 ILE SER 78 THR ILE 79 MET VAL 81 THR
 LEU 75 ILE SER 78 THR ILE 79 THR VAL 81 THR
 LEU 75 MET SER 78 ASN ILE 79 LEU VAL 81 THR
 LEU 75 MET SER 78 ASN ILE 79 MET VAL 81 THR
 LEU 75 MET SER 78 ASN ILE 79 THR VAL 81 THR
 LEU 75 MET SER 78 THR ILE 79 LEU VAL 81 THR
 LEU 75 MET SER 78 THR ILE 79 MET VAL 81 THR
 LEU 75 MET SER 78 THR ILE 79 THR VAL 81 THR
 LEU 75 ILE ASN 76 HIS VAL 81 THR LEU 82 PHE
 LEU 75 ILE ASN 76 HIS VAL 81 THR LEU 82 HIS
 LEU 75 MET ASN 76 HIS VAL 81 THR LEU 82 PHE
 LEU 75 MET ASN 76 HIS VAL 81 THR LEU 82 HIS
 LEU 75 ILE ASN 76 HIS ILE 79 LEU LEU 82 PHE
 LEU 75 ILE ASN 76 HIS ILE 79 LEU LEU 82 HIS
 LEU 75 ILE ASN 76 HIS ILE 79 MET LEU 82 PHE
 LEU 75 ILE ASN 76 HIS ILE 79 MET LEU 82 HIS
 LEU 75 ILE ASN 76 HIS ILE 79 THR LEU 82 PHE
 LEU 75 ILE ASN 76 HIS ILE 79 THR LEU 82 HIS
 LEU 75 MET ASN 76 HIS ILE 79 LEU LEU 82 PHE
 LEU 75 MET ASN 76 HIS ILE 79 LEU LEU 82 HIS
 LEU 75 MET ASN 76 HIS ILE 79 MET LEU 82 PHE
 LEU 75 MET ASN 76 HIS ILE 79 MET LEU 82 HIS
 LEU 75 MET ASN 76 HIS ILE 79 THR LEU 82 PHE
 LEU 75 MET ASN 76 HIS ILE 79 THR LEU 82 HIS
 LEU 75 ILE ASN 76 HIS ILE 79 LEU VAL 81 THR
 LEU 75 ILE ASN 76 HIS ILE 79 MET VAL 81 THR
 LEU 75 ILE ASN 76 HIS ILE 79 THR VAL 81 THR
 LEU 75 MET ASN 76 HIS ILE 79 LEU VAL 81 THR
 LEU 75 MET ASN 76 HIS ILE 79 MET VAL 81 THR
 LEU 75 MET ASN 76 HIS ILE 79 THR VAL 81 THR
 LEU 75 ILE ASN 76 HIS SER 78 ASN LEU 82 PHE
 LEU 75 ILE ASN 76 HIS SER 78 ASN LEU 82 HIS
 LEU 75 ILE ASN 76 HIS SER 78 THR LEU 82 PHE
 LEU 75 ILE ASN 76 HIS SER 78 THR LEU 82 HIS
 LEU 75 MET ASN 76 HIS SER 78 ASN LEU 82 PHE
 LEU 75 MET ASN 76 HIS SER 78 ASN LEU 82 HIS
 LEU 75 MET ASN 76 HIS SER 78 THR LEU 82 PHE
 LEU 75 MET ASN 76 HIS SER 78 THR LEU 82 HIS
 LEU 75 ILE ASN 76 HIS SER 78 ASN VAL 81 THR
 LEU 75 ILE ASN 76 HIS SER 78 THR VAL 81 THR
 LEU 75 MET ASN 76 HIS SER 78 ASN VAL 81 THR
 LEU 75 MET ASN 76 HIS SER 78 THR VAL 81 THR
 LEU 75 ILE ASN 76 HIS SER 78 ASN ILE 79 LEU
 LEU 75 ILE ASN 76 HIS SER 78 ASN ILE 79 MET
 LEU 75 ILE ASN 76 HIS SER 78 ASN ILE 79 THR
 LEU 75 ILE ASN 76 HIS SER 78 THR ILE 79 LEU
 LEU 75 ILE ASN 76 HIS SER 78 THR ILE 79 MET

LEU 75 ILE ASN 76 HIS SER 78 THR ILE 79 THR
 LEU 75 MET ASN 76 HIS SER 78 ASN ILE 79 LEU
 LEU 75 MET ASN 76 HIS SER 78 ASN ILE 79 MET
 LEU 75 MET ASN 76 HIS SER 78 ASN ILE 79 THR
 LEU 75 MET ASN 76 HIS SER 78 THR ILE 79 LEU
 LEU 75 MET ASN 76 HIS SER 78 THR ILE 79 MET
 LEU 75 MET ASN 76 HIS SER 78 THR ILE 79 THR

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五取代变体

ASN 76 HIS SER 78 ASN ILE 79 LEU VAL 81 THR LEU 82 PHE
 ASN 76 HIS SER 78 ASN ILE 79 LEU VAL 81 THR LEU 82 HIS
 ASN 76 HIS SER 78 ASN ILE 79 MET VAL 81 THR LEU 82 PHE
 ASN 76 HIS SER 78 ASN ILE 79 MET VAL 81 THR LEU 82 HIS
 ASN 76 HIS SER 78 ASN ILE 79 THR VAL 81 THR LEU 82 PHE
 ASN 76 HIS SER 78 ASN ILE 79 THR VAL 81 THR LEU 82 HIS
 ASN 76 HIS SER 78 THR ILE 79 LEU VAL 81 THR LEU 82 PHE
 ASN 76 HIS SER 78 THR ILE 79 LEU VAL 81 THR LEU 82 HIS
 ASN 76 HIS SER 78 THR ILE 79 MET VAL 81 THR LEU 82 PHE
 ASN 76 HIS SER 78 THR ILE 79 MET VAL 81 THR LEU 82 HIS
 ASN 76 HIS SER 78 THR ILE 79 THR VAL 81 THR LEU 82 PHE
 LEU 75 ILE SER 78 ASN ILE 79 LEU VAL 81 THR LEU 82 PHE
 LEU 75 ILE SER 78 ASN ILE 79 LEU VAL 81 THR LEU 82 HIS
 LEU 75 ILE SER 78 ASN ILE 79 MET VAL 81 THR LEU 82 PHE
 LEU 75 ILE SER 78 ASN ILE 79 MET VAL 81 THR LEU 82 HIS
 LEU 75 ILE SER 78 ASN ILE 79 THR VAL 81 THR LEU 82 PHE
 LEU 75 ILE SER 78 ASN ILE 79 THR VAL 81 THR LEU 82 HIS
 LEU 75 ILE SER 78 THR ILE 79 LEU VAL 81 THR LEU 82 PHE
 LEU 75 ILE SER 78 THR ILE 79 LEU VAL 81 THR LEU 82 HIS
 LEU 75 ILE SER 78 THR ILE 79 MET VAL 81 THR LEU 82 PHE
 LEU 75 ILE SER 78 THR ILE 79 MET VAL 81 THR LEU 82 HIS
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 LEU 75 ILE SER 78 THR ILE 79 THR VAL 81 THR LEU 82 HIS
 LEU 75 MET SER 78 ASN ILE 79 LEU VAL 81 THR LEU 82 PHE
 LEU 75 MET SER 78 ASN ILE 79 LEU VAL 81 THR LEU 82 HIS
 LEU 75 MET SER 78 ASN ILE 79 MET VAL 81 THR LEU 82 PHE
 LEU 75 MET SER 78 ASN ILE 79 MET VAL 81 THR LEU 82 HIS
 LEU 75 MET SER 78 ASN ILE 79 THR VAL 81 THR LEU 82 PHE
 LEU 75 MET SER 78 ASN ILE 79 THR VAL 81 THR LEU 82 HIS
 LEU 75 MET SER 78 THR ILE 79 LEU VAL 81 THR LEU 82 PHE
 LEU 75 MET SER 78 THR ILE 79 LEU VAL 81 THR LEU 82 HIS
 LEU 75 MET SER 78 THR ILE 79 MET VAL 81 THR LEU 82 PHE
 LEU 75 MET SER 78 THR ILE 79 MET VAL 81 THR LEU 82 HIS
 LEU 75 MET SER 78 THR ILE 79 THR VAL 81 THR LEU 82 PHE
 LEU 75 MET SER 78 THR ILE 79 THR VAL 81 THR LEU 82 HIS
 LEU 75 ILE ASN 76 HIS ILE 79 LEU VAL 81 THR LEU 82 PHE
 LEU 75 ILE ASN 76 HIS ILE 79 LEU VAL 81 THR LEU 82 HIS
 LEU 75 ILE ASN 76 HIS ILE 79 MET VAL 81 THR LEU 82 PHE
 LEU 75 ILE ASN 76 HIS ILE 79 MET VAL 81 THR LEU 82 HIS
 LEU 75 ILE ASN 76 HIS ILE 79 THR VAL 81 THR LEU 82 PHE
 LEU 75 ILE ASN 76 HIS ILE 79 THR VAL 81 THR LEU 82 HIS
 LEU 75 MET ASN 76 HIS ILE 79 LEU VAL 81 THR LEU 82 PHE
 LEU 75 MET ASN 76 HIS ILE 79 LEU VAL 81 THR LEU 82 HIS

LEU 75 MET ASN 76 HIS ILE 79 MET VAL 81 THR LEU 82 PHE
 LEU 75 MET ASN 76 HIS ILE 79 MET VAL 81 THR LEU 82 HIS
 LEU 75 MET ASN 76 HIS ILE 79 THR VAL 81 THR LEU 82 PHE
 LEU 75 MET ASN 76 HIS ILE 79 THR VAL 81 THR LEU 82 HIS
 LEU 75 ILE ASN 76 HIS SER 78 ASN VAL 81 THR LEU 82 PHE
 LEU 75 ILE ASN 76 HIS SER 78 ASN VAL 81 THR LEU 82 HIS
 LEU 75 ILE ASN 76 HIS SER 78 THR VAL 81 THR LEU 82 PHE
 LEU 75 ILE ASN 76 HIS SER 78 THR VAL 81 THR LEU 82 HIS
 LEU 75 MET ASN 76 HIS SER 78 ASN VAL 81 THR LEU 82 PHE
 LEU 75 MET ASN 76 HIS SER 78 ASN VAL 81 THR LEU 82 HIS
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 LEU 75 ILE ASN 76 HIS SER 78 ASN ILE 79 LEU LEU 82 PHE
 LEU 75 ILE ASN 76 HIS SER 78 ASN ILE 79 LEU LEU 82 HIS
 LEU 75 ILE ASN 76 HIS SER 78 ASN ILE 79 MET LEU 82 PHE
 LEU 75 ILE ASN 76 HIS SER 78 ASN ILE 79 MET LEU 82 HIS
 LEU 75 ILE ASN 76 HIS SER 78 ASN ILE 79 THR LEU 82 PHE
 LEU 75 ILE ASN 76 HIS SER 78 ASN ILE 79 THR LEU 82 HIS
 LEU 75 ILE ASN 76 HIS SER 78 THR ILE 79 LEU LEU 82 PHE
 LEU 75 ILE ASN 76 HIS SER 78 THR ILE 79 LEU LEU 82 HIS
 LEU 75 ILE ASN 76 HIS SER 78 THR ILE 79 MET LEU 82 PHE
 LEU 75 ILE ASN 76 HIS SER 78 THR ILE 79 MET LEU 82 HIS
 LEU 75 ILE ASN 76 HIS SER 78 THR ILE 79 THR LEU 82 PHE
 LEU 75 ILE ASN 76 HIS SER 78 THR ILE 79 THR LEU 82 HIS
 LEU 75 MET ASN 76 HIS SER 78 ASN ILE 79 LEU LEU 82 PHE
 LEU 75 MET ASN 76 HIS SER 78 ASN ILE 79 LEU LEU 82 HIS
 LEU 75 MET ASN 76 HIS SER 78 ASN ILE 79 MET LEU 82 PHE
 LEU 75 MET ASN 76 HIS SER 78 ASN ILE 79 MET LEU 82 HIS
 LEU 75 MET ASN 76 HIS SER 78 ASN ILE 79 THR LEU 82 PHE
 LEU 75 MET ASN 76 HIS SER 78 ASN ILE 79 THR LEU 82 HIS
 LEU 75 MET ASN 76 HIS SER 78 THR ILE 79 LEU LEU 82 PHE
 LEU 75 MET ASN 76 HIS SER 78 THR ILE 79 LEU LEU 82 HIS
 LEU 75 MET ASN 76 HIS SER 78 THR ILE 79 MET LEU 82 PHE
 LEU 75 MET ASN 76 HIS SER 78 THR ILE 79 MET LEU 82 HIS
 LEU 75 MET ASN 76 HIS SER 78 THR ILE 79 THR LEU 82 PHE
 LEU 75 MET ASN 76 HIS SER 78 THR ILE 79 THR LEU 82 HIS
 LEU 75 ILE ASN 76 HIS SER 78 ASN ILE 79 LEU VAL 81 THR
 LEU 75 ILE ASN 76 HIS SER 78 ASN ILE 79 MET VAL 81 THR
 LEU 75 ILE ASN 76 HIS SER 78 ASN ILE 79 THR VAL 81 THR
 LEU 75 ILE ASN 76 HIS SER 78 THR ILE 79 LEU VAL 81 THR
 LEU 75 ILE ASN 76 HIS SER 78 THR ILE 79 MET VAL 81 THR
 LEU 75 ILE ASN 76 HIS SER 78 THR ILE 79 THR VAL 81 THR
 LEU 75 MET ASN 76 HIS SER 78 ASN ILE 79 LEU VAL 81 THR
 LEU 75 MET ASN 76 HIS SER 78 ASN ILE 79 MET VAL 81 THR
 LEU 75 MET ASN 76 HIS SER 78 ASN ILE 79 THR VAL 81 THR
 LEU 75 MET ASN 76 HIS SER 78 THR ILE 79 LEU VAL 81 THR
 LEU 75 MET ASN 76 HIS SER 78 THR ILE 79 MET VAL 81 THR
 LEU 75 MET ASN 76 HIS SER 78 THR ILE 79 THR VAL 81 THR

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六取代变体

LEU 75 ILE ASN 76 HIS SER 78 ASN ILE 79 LEU VAL 81 THR LEU 82 PHE
 LEU 75 ILE ASN 76 HIS SER 78 ASN ILE 79 LEU VAL 81 THR LEU 82 HIS
 LEU 75 ILE ASN 76 HIS SER 78 ASN ILE 79 MET VAL 81 THR LEU 82 PHE

LEU 75 ILE ASN 76 HIS SER 78 ASN ILE 79 MET VAL 81 THR LEU 82 HIS
 LEU 75 ILE ASN 76 HIS SER 78 ASN ILE 79 THR VAL 81 THR LEU 82 PHE
 LEU 75 ILE ASN 76 HIS SER 78 ASN ILE 79 THR VAL 81 THR LEU 82 HIS
 LEU 75 ILE ASN 76 HIS SER 78 THR ILE 79 LEU VAL 81 THR LEU 82 PHE
 LEU 75 ILE ASN 76 HIS SER 78 THR ILE 79 LEU VAL 81 THR LEU 82 HIS
 LEU 75 ILE ASN 76 HIS SER 78 THR ILE 79 MET VAL 81 THR LEU 82 PHE
 LEU 75 ILE ASN 76 HIS SER 78 THR ILE 79 MET VAL 81 THR LEU 82 HIS
 LEU 75 ILE ASN 76 HIS SER 78 THR ILE 79 THR VAL 81 THR LEU 82 PHE
 LEU 75 ILE ASN 76 HIS SER 78 THR ILE 79 THR VAL 81 THR LEU 82 HIS
 LEU 75 MET ASN 76 HIS SER 78 ASN ILE 79 LEU VAL 81 THR LEU 82 PHE
 LEU 75 MET ASN 76 HIS SER 78 ASN ILE 79 LEU VAL 81 THR LEU 82 HIS
 LEU 75 MET ASN 76 HIS SER 78 ASN ILE 79 MET VAL 81 THR LEU 82 PHE
 LEU 75 MET ASN 76 HIS SER 78 ASN ILE 79 MET VAL 81 THR LEU 82 HIS
 LEU 75 MET ASN 76 HIS SER 78 ASN ILE 79 THR VAL 81 THR LEU 82 PHE
 LEU 75 MET ASN 76 HIS SER 78 ASN ILE 79 THR VAL 81 THR LEU 82 HIS
 LEU 75 MET ASN 76 HIS SER 78 THR ILE 79 LEU VAL 81 THR LEU 82 PHE
 LEU 75 MET ASN 76 HIS SER 78 THR ILE 79 LEU VAL 81 THR LEU 82 HIS
 LEU 75 MET ASN 76 HIS SER 78 THR ILE 79 MET VAL 81 THR LEU 82 PHE
 LEU 75 MET ASN 76 HIS SER 78 THR ILE 79 MET VAL 81 THR LEU 82 HIS
 LEU 75 MET ASN 76 HIS SER 78 THR ILE 79 THR VAL 81 THR LEU 82 PHE
 LEU 75 MET ASN 76 HIS SER 78 THR ILE 79 THR VAL 81 THR LEU 82 HIS

制备方法

所述的变体通过突变野生型蛋白酶的核苷酸序列，由此产生具有修饰的氨基酸序列的变体来制备。这类方法为本领域所熟知；一种这样的方法如下所述：

将含有野生型枯草杆菌蛋白酶 BPN' 基因 (Mitchison, C. 和 J.A. Wells, “枯草杆菌蛋白酶 BPN' 中二硫键的蛋白质工程”，*生物化学*, Vol. 28, pp. 4807 - 4815 (1989)) 的噬菌粒 (pSS-5) 转化入大肠杆菌 *dut-ung* 菌株 CJ236，以及对 Yuckenberg 等人，“使用含尿嘧啶的 DNA 和噬菌粒载体的体外定点诱变”，定点诱变——一种实用的方法，McPherson, M. J. 编著，pp. 27 - 48 (1991) 一文中描述的方法改进后，用 VCSM13 辅助噬菌体 (Kunkel 等人，“不需表型选择的快速、有效的定点诱变”，*酶学方法*, Vol 154, pp. 367 -382 (1987)) 制备含尿嘧啶的单链 DNA 模板。从 Zoller 和 Smith 的方法 (Zoller, M. J. 和 M. Smith, “用 M13 衍生的载体进行寡核苷酸定点诱变：一种用于在任一 DNA 片段中产生点突变的有效

的通用方法”，核酸研究, Vol. 10, pp. 6487 - 6500 (1982)) 改进而来的引物定点诱变来制备所有的突变体 (基本上如 Yuckenberg 等人, 上述)。

用380B DNA合成仪 (Applied Biosystems Inc.) 制备寡核苷酸。将诱变反应产物转化入大肠杆菌MM294菌株 (美国典型培养物保藏中心大肠杆菌33625)。所有突变都经DNA测序证实, 并将分离的DNA转化入枯草杆菌表达菌株PG632 (Saunders 等人, “从枯草杆菌中分泌人甲状旁腺激素34-氨基酸片段的信号序列切割位点的优化”, 基因, Vol. 102, pp. 277 - 282 (1991) 以及 Yang 等人, “枯草杆菌中性蛋白酶基因的克隆以及该克隆基因在建立体外衍生的缺失突变中的应用”, 细菌学杂志, Vol. 160, pp. 15 - 21 (1984)。对变体活性的初步估价是由用突变质粒转化的PG632细胞水解酪蛋白的能力确定的。

按下述方法进行发酵。用一种含有10 g/L 葡萄糖的LB肉汤将含有目的变体的枯草杆菌细胞 (PG632) 培养至对数中期, 接种到总体积为9升的Biostat C 发酵罐 (Braun Biotech, Inc., Allentown, PA) 中。发酵培养基中含有酵母提取物、酪蛋白水解产物、水溶性部分水解的淀粉 (Maltrin M-250)、消泡剂、缓冲液、以及微量矿物质 (参见 “杆菌生物学: 工业应用”, Doi, R. H. 和 M. McGloughlin, 编著 (1992))。发酵过程中肉汤的pH恒定保持在7.5。加入卡那霉素 (500 μ g/mL) 对诱变质粒进行抗生素选择。细胞在37 $^{\circ}$ C下培养18小时直至 A_{600} 约为 60, 收获产物。

通过下述步骤处理发酵肉汤得到纯的变体。用0.16 μ m 滤膜通过切向流滤除肉汤中的枯草杆菌细胞。然后, 用8,000分子量切断滤膜超滤浓缩无细胞肉汤。用浓缩后的MES缓冲液 (2-(N-吗啉代) 乙磺酸) 将pH值调至5.5。用S-琼脂糖凝胶通过阳离子交换层析进一步纯化该变体, 并用NaCl 梯度洗脱 (参见Scopes, R. K. “蛋白纯化原理及操作”, Springer-Verlag, New York (1984))。

用pNA测定 (DelMar等人, 分析化学, Vol. 99, pp. 316 - 320 (1979)) 测定在梯度洗脱过程中收集的级分中的活性变体浓度。本测定可以测量

出变体水解可溶性合成底物琥珀酰-丙氨酸-丙氨酸-脯氨酸-苯丙氨酸-对硝基苯胺 (sAAPF-pNA) 时释放出对硝基苯胺的速率。用分光光度计在410 nm 处测量由水解反应产生黄色的速率, 该速率与活性酶的浓度成比例。此外, 用280 nm 处的吸光度测定总蛋白浓度。活性酶/总蛋白之比给出变体纯度, 并可用来鉴定收集到的原液中的级分。

为了避免存储过程中蛋白酶的自身溶解, 向从层析柱中得到的混合级分中加入等重量的丙二醇。当纯化过程完成后, 用SDS-PAGE (十二烷基硫酸钠聚丙烯酰胺凝胶电泳) 检测蛋白酶原液的纯度, 用II型胰蛋白酶抑制剂-T: 火鸡蛋白 (Sigma Chemical Company, St. Louis, Missouri) 通过活性位点滴定方法测定绝对的酶浓度。

在使用的制剂中, 通过Sephadex-G25 (Pharmacia, Piscataway, New Jersey) 大小排阻柱洗脱酶原液以除去丙二醇, 并更换缓冲液。将酶原液中的MES缓冲液更换成含0.01 M CaCl_2 且用HCl将pH调节至8.6约0.1M tris缓冲液 (三-羟甲基-氨基甲烷)。所有实验均在pH8.6下在恒温于25℃的tris缓冲液中进行。

分析方法

可以用下列方法测试本发明所述变体的酶促活性和免疫和/或变应原应答, 这两种方法都是本领域技术人员已知的。其它本领域熟知的方法也可以替代使用。

变体活性

可以用本领域熟知的方法来测定本发明所述变体的蛋白酶活性。两种这样的方法描述如下:

皮肤碎屑活性方法

用Scotch®#3750G带, 反复地从受试者腿部剥取人体皮肤碎屑直到带子上基本铺满碎屑。然后, 将带子切割成1平方英寸的正方形, 放在一边。在10 mm×35 mm 培替氏培养皿中, 将2mL 0.75 mg/mL的对照酶 (例如, 枯草杆菌蛋白酶 BPN') 或待测变体加入到0.01 M KH_2PO_4 pH 5.5 缓冲液中。

向该溶液中加入1mL 2.5% 月桂酸钠pH 8.6 溶液。将溶液置于平台振荡器上轻轻混合。将上述制得的正方形带在不断轻轻混合下浸泡于溶液中(碎屑面向上)10分钟。然后,用自来水轻轻漂洗正方形带15秒。将Stevenel 蓝染液 (3 mL, 购自Sigma Chemical Co., St. Louis, MO)吸移到一干净的培替氏培养皿中。在轻轻混合下将漂洗过的正方形带置于染液中(碎屑面向上)3分钟。从染液中取出正方形带, 在两个盛有300mL蒸馏水的烧杯中连续漂洗, 每次15秒。空气干燥该正方形带。视觉或用比色计比较从对照酶获得的正方形带与从变体获得的正方形带之间颜色强度的差异。与对照酶正方形带相比, 变体正方形带的颜色强度较弱, 这说明变体的活性较高。

着色胶原活性方法

将50m含有0.01M CaCl_2 的0.1 M tris缓冲液(三-羟甲基-氨基甲烷)(pH8.6)和0.5 g azocoll (偶氮颜料浸渍的胶原, 购自Sigma Chemical Co., St. Louis, MO)混合。25 °C下温育该混合物, 同时在平板振荡器上轻轻混合。用0.2微米注射滤器过滤2 mL该混合物, 读取该混合物在520 nm处的吸光度把分光光度计的调节器调整归零。向剩余的48ml tris/azocoll混合物中加入 1 ppm 对照酶(例如, 枯草杆菌蛋白酶 BPN')或待测变体。在总共10分钟的时间内, 每隔2分钟用0.2微米注射滤器过滤2 mL 含对照物/ 变体的溶液。对于每份过滤后的样品, 立即读取520 nm处的吸光度。绘制结果相对于时间的曲线。对照物及待测变体的斜率表明的是该样品的相对活性。斜率越大表示活性越高。可以将待测变体活性(斜率)表示为对照物活性(斜率)的百分比。

T-细胞增殖测定法

用T-细胞增殖测定法如下文所述的测定法测定本发明所述变体的免疫和/或变应原潜力。该测定法由下文中公开的测定法改进而来: Bungy Poor Fard 等人, “在体外和体内用重叠肽确定的黑麦草*Lolium perenne* (*Lolp I*)主要级分的T细胞表位”, 临床实验免疫学, Vol. 94, pp. 111 - 116 (1993)。

从对枯草杆菌蛋白酶 BPN' 过敏的受试者(刺痕试验阳性)和对照

受试者(刺痕试验阴性)取血,用于该测定。从每个受试者采血(约60ml),并用菲可-海帕克(可得自 Pharmacia, Piscataway, New Jersey)收获单核细胞。用RPMI 1640(可得自 Gibco, Grand Island, New York)洗涤细胞两次,然后重悬于补加了下列成分的完全培养基RPMI中:10%人AB-血清、2 mM L-谷氨酰胺和25 μ g / mL庆大霉素(可得自 Gibco)。用U型底的96-孔微量滴定板,以 2×10^5 细胞/孔的浓度用0.2mL完全培养基培养细胞。加入待测的潜在抗原(作为阳性对照的灭活的枯草杆菌蛋白酶BPN'或者本发明所述变体)至终浓度为至多约40 μ g / mL。37 $^{\circ}$ C下,在5% CO₂中温育培养物。5天后,按1 μ Ci /孔的量加入甲基-³H-胸腺嘧啶核苷,18小时后,收获细胞。通过液体闪烁计数测量T细胞增殖来评估细胞中³H-胸腺嘧啶核苷的掺入情况。

本发明的组合物

本发明的变体可以用于适合使用各自的野生型蛋白酶的任一情况。一个这样的实例包括洗涤组合物。由于本发明的变体合乎需要的减弱的变应原性和/或免疫原性,该变体还可以用于曾经从使用蛋白酶中受益最小的情况。这类用途的实例包括变体必定与哺乳动物皮肤(特别是人体皮肤)紧密接触的情况,例如其中使用个人护理组合物。

洗涤组合物

本发明的变体可用于洗涤组合物包括,但不限于,洗衣组合物、坚硬表面清洗组合物、精细织品清洗组合物包括餐具清洗组合物,以及自动洗碗机洗涤剂组合物。

本发明所述洗涤组合物包括有效量的一种或多种本发明所述变体以及洗涤组合物载体。

本发明使用的“有效量的变体”或者类似表述是指足以达到特定洗涤组合物中所需蛋白水解活性的变体的量。所述有效量可被本领域普通技术人员容易地确定,并取决于多种因素,例如所用变体的具体种类,清洗的用途,洗涤组合物的具体组成,以及需要使用的是液体还是干的(例如,颗粒状、棒状)组合物,等。优选地,所述洗涤组合物中包含约

0.0001%~约10%，更优选约0.001%~1%，以及最优选约0.01%~约0.1%的一种或多种本发明所述的变体。下面更详细地讨论了变体可以用于其中的不同洗涤组合物的几个实例。

除本发明所述的变体外，本发明洗涤组合物还包括其中含有一种或多种与变体相容的洗涤组合物材料的洗涤组合物载体。本发明使用的术语“洗涤组合物材料”是指任何选定的用于特定类型的所需的组合物和产品形式（例如，液体、颗粒、块、喷雾剂、棒、膏、凝胶）的材料，这些材料也是与用于组合物中的变体相容的。洗涤组合物材料的具体选择可通过考虑待清洗的表面材料、对于在使用过程中的清洗条件（例如，通过洗涤剂的使用）所需的组合物形式容易地进行。本发明所用的术语“相容的”是指洗涤组合物材料不会使变体的蛋白水解活性降低到这样的程度，即在正常使用情况下变体不像所期望的那样有效。下文中详细举例说明了具体的洗涤组合物材料。

本发明的变体可用于期望泡沫丰富且清洁效果良好的多种洗涤剂组合物中。因此，变体可以与各种常规成分一起使用，提供充分配制的坚硬表面清洗剂、餐具清洗组合物、织物清洗组合物等。这类组合物可以液体、颗粒、棒状等形式使用。这类组合物可以配制成“浓缩”洗涤剂，其中含有多达约30%~约60%（重量）的表面活性剂。

本发明所述的洗涤组合物可以任选地，以及优选地，包含各种表面活性剂（例如，阴离子、非离子或两性离子表面活性剂）。典型地，这类表面活性剂在组合物中的含量约5%~约35%。

用于本发明的表面活性剂的非限制性实例包括常规的 C_{11} - C_{18} 烷基苯磺酸盐以及伯和无规烷基硫酸盐；通式 $CH_3(CH_2)_x(CHOSO_3^-M^+)$ CH_3 和 $CH_3(CH_2)_y(CHOSO_3^-M^+)$ CH_2CH_3 的 C_{10} - C_{18} 仲(2,3)烷基硫酸盐，其中 x 和 $(y+1)$ 是至少约为7的整数，优选至少约为9，以及 M 是一种水溶性的阳离子，特别是钠； C_{10} - C_{18} 烷基烷氧基硫酸盐（特别是EO 1-5乙氧基硫酸盐）； C_{10} - C_{18} 烷基烷氧基羧酸盐（特别是EO 1-5乙氧基羧酸盐）； C_{10} - C_{18} 烷基聚葡萄糖苷及其相应的硫酸化聚葡萄糖苷； C_{12} - C_{18} α -磺化脂肪酸酯； C_{12} - C_{18} 烷基及烷基酚烷氧基化物（特别是乙氧基化物和混合的乙氧基/丙氧基）； C_{12} - C_{18} 甜

菜碱及磺基甜菜碱； C_{10} - C_{18} 胺氧化物等。本发明优选烷基烷氧基硫酸盐 (AES) 和烷基烷氧基羧酸盐 (AEC)。另外，还优选根据配方师的愿望将这类表面活性剂与胺氧化物和/或甜菜碱或磺基甜菜碱表面活性剂联用。其它常规的有用表面活性剂列于标准教科书中。特别有用的表面活性剂包括 C_{10} - C_{18} N-甲基葡糖胺，其描述见1993年3月16日颁布的授予Connor 等人的美国专利5,194,639。

本发明所述组合物中还可以包括多种能在洗涤剂洗涤组合物中发挥功效的其它成分，其中包括，例如，其它活性成分、载体、水溶助长剂、加工助剂、染料或色料以及用于液体制剂的溶剂。如果希望额外增加泡沫，则可以向组合物中加入 C_{10} - C_{16} 烷醇酰胺等增泡剂，典型地加入量为约1%~约10%。 C_{10} - C_{14} 一乙醇酰胺和二乙醇酰胺代表一类典型的这样的增泡剂。将这类增泡剂与高效发泡辅助表面活性剂，例如上述的胺氧化物、甜菜碱以及磺基甜菜碱联用也是有利的。如果需要，可以加入 $MgCl_2$ 、 $MgSO_4$ 等可溶性镁盐，典型地约0.1%~约2%，以提供额外的泡沫。

本发明所述的液态洗涤剂组合物可以包含水以及其它溶剂作为载体。低分子量的伯或仲醇的实例有甲醇、乙醇、丙醇以及异丙醇，它们都是合适的。优选一元醇来溶解表面活性剂，但是含有约2~约6个碳原子和约2~约6个羟基的多元醇（例如，1,3-丙二醇、乙二醇、丙三醇以及1,2-丙二醇）也可以使用。所述组合物可以包含约5%~约90%，典型地约10%~约50%的这类载体。

优选配制成洗涤剂组合物以便于在含水的清洗操作中使用期间，洗涤用水的 pH 值为约6.8~约11。因此，典型地将成品在该范围内配制。将pH值控制在推荐使用水平的技术包括，例如缓冲液、碱和酸的使用。这类技术为本领域技术人员所熟知。

当配制本发明所述的坚硬表面清洗组合物和织物清洗组合物时，配方师可以希望使用约5%~约50%(重量)的助洗剂。典型的助洗剂包括1-10微米沸石、聚羧酸盐例如柠檬酸盐和氧代丁二酸氢盐、层叠硅酸盐、磷酸盐等。其它常规助洗剂见标准配方手册。

同样，配方师可以在这类组合物中使用各种另外的酶，例如纤维素

酶、脂肪酶、淀粉酶和蛋白酶，典型用量为约 0.001% ~ 约1% (重量)。各种去污酶及织物护理酶都为洗衣洗涤剂领域所熟知。

各种漂白化合物，例如过碳酸盐、过硼酸盐等也可以用于此类组合物中，典型用量为约1%~约15%(重量)。如果需要，这类组合物还可以包含漂白活化剂，例如四乙酰乙二胺、壬酰羧苯磺酸盐等，这些也为本领域已知。典型用量为约1%~约10%(重量)。

去污剂，尤其是阴离子低聚酯型，螯合剂，尤其是氨基磷酸盐以及乙二胺丁二酸氢盐，粘土污垢去除剂，尤其是乙氧基化四亚乙基五胺，分散剂，尤其是聚丙烯酸盐和聚天冬氨酸盐，增白剂，尤其是阴离子增白剂，抑泡剂，尤其是硅氧烷及仲醇，织物软化剂，尤其是绿土粘土等都可以用于这类组合物中，用量为约1% ~ 35% (重量)。标准配方手册以及公开的专利中都包含了对这类常规材料的丰富详尽的描述。

酶稳定剂也可以用于洗涤组合物中。这类酶稳定剂包括丙二醇（优选为约1% ~ 约10%）、甲酸钠（优选为约0.1% ~ 约1%）以及甲酸钙（优选为约0.1% ~ 约1%）。

本发明所述的变体也可以用于坚硬表面清洗组合物中。本发明使用的“坚硬表面清洗组合物”是指用于清洗坚硬表面，例如地板、墙壁、浴室瓷砖等的液态及颗粒状的洗涤剂组合物。本发明所述的坚硬表面清洗组合物包含有效量的一种或多种本发明所述变体，优选组合物中的变体含量为约0.001% ~ 约 10%，更优选约0.01%~约5%，还更优选约0.05%~约1% (重量)。除含有一种或多种变体外，这类坚硬表面清洗组合物典型地还包括表面活性剂和水溶性的螯合助洗剂。但是，在某些专用产品，例如喷雾型窗户清洗剂中，有时并不使用所述表面活性剂，这是由于它们可能会在玻璃表面产生薄膜状和/或斑点状的残迹。

当含有表面活性剂组分时，其在本发明组合物中的含量可低至0.1%，但是，典型地，该组合物中表面活性剂的含量为约0.25%~约10%，更优选约1% ~ 约 5%。

典型地，所述组合物中包含约0.5% ~ 约 50%的去垢助剂，优选约1%~约10%。

优选的pH 范围应为约7~12。如果需要调节pH, 常用的pH调节剂, 例如氢氧化钠、碳酸钠或盐酸都可以使用。

溶剂也可以包含在所述组合物中。有用的溶剂包括, 但不限于, 乙二醇醚例如二甘醇单己基醚, 二甘醇单丁基醚, 乙二醇单丁基醚, 乙二醇单己基醚, 丙二醇单丁基醚, 二丙二醇单丁基醚, 以及二醇例如2, 2, 4-三甲基-1, 3-戊二醇和2-乙基-1, 3-己二醇。在使用时, 这类溶剂的典型用量为约0.5%~约15%, 更优选约3%~约11%。

此外, 当向要清洗的表面“全强度”施用本发明的组合物后不清洗该表面时, 可以在该组合物中使用高挥发性溶剂, 例如异丙醇或乙醇以加快表面上组合物的蒸发。使用时, 挥发性溶剂在组合物中的典型用量为约2%~约12%。

实施例1-6

液态坚硬表面清洗组合物

	实施例1	实施例2	实施例3	实施例4	实施例5	实施例6
G70*+S78*+I79*	0.05%	0.50%	0.02%	0.03%	0.30%	0.05%
EDTA	-	-	2.90%	2.90%	-	-
柠檬酸钠	-	-	-	-	2.90%	2.90%
十二烷基苯磺酸钠	1.95%	-	1.95%	-	1.95%	-
十二烷基硫酸钠	-	2.20%	-	2.20%	-	2.20%
C ₁₂ (乙氧基)硫酸钠	-	2.20%	-	2.20%	-	2.20%
C ₁₂ 二甲胺氧化物	-	0.50%	-	0.50%	-	0.50%
枯烯磺酸钠	1.30%	-	1.30%	-	1.30%	-
己基卡必醇	6.30%	6.30%	6.30%	6.30%	6.30%	6.30%
水	90.4%	88.3%	87.53%	85.87%	87.25%	85.85%

所有配方均调至 pH 7。

在实施例1-6中, 表2-10中所述的变体, 和本发明所述的优选变体都可以用来替代G70*+S78*+I79*, 结果基本上相似。

在本发明的另一个实施方案中，餐具清洗组合物包含一种或多种本发明所述的变体。本发明使用的“餐具清洗组合物”是指用于清洗餐具的所有形式的组合物，包括但不限于颗粒状的以及液态的形式。本发明的餐具清洗组合物用下列实施例说明。

实施例7 - 10

液态餐具洗涤剂

	实施例7	实施例8	实施例9	实施例10
G70*+L75*+N76*+N77*+S78*+I79*+ G80*+V81*+L82*	0.05 %	0.50 %	0.02 %	0.40 %
C ₁₂ - C ₁₄ N-甲基葡糖胺	0.90 %	0.90 %	0.90 %	0.90 %
C ₁₂ 乙氧基 (1)硫酸盐	12.0 %	12.0 %	12.0 %	12.0 %
2-甲基十一烷酸	4.50 %	4.50 %	4.50 %	4.50 %
C ₁₂ 乙氧基(2)羧酸盐	4.50 %	4.50 %	4.50 %	4.50 %
C ₁₂ 脂肪醇乙氧基化物(4)	3.00 %	3.00 %	3.00 %	3.00 %
C ₁₂ 氧化胺	3.00 %	3.00 %	3.00 %	3.00 %
枯烯磺酸钠	2.00 %	2.00 %	2.00 %	2.00 %
乙醇	4.00 %	4.00 %	4.00 %	4.00 %
Mg ²⁺ (作为 MgCl ₂)	0.20 %	0.20 %	0.20 %	0.20 %
Ca ²⁺ (作为 CaCl ₂)	0.40 %	0.40 %	0.40 %	0.40 %
水	65.45 %	65 %	65.48 %	65.1 %

所有制剂配方均调至pH 7。

在实施例7-10中，表2-10中所述的变体，和本发明所述的优选变体都用来替代G70*+L75*+N76*+N77*+S78*+I79*+G80*+V81*+L82*，结果基本上相似。

实施例11 - 13

液态织物清洗组合物

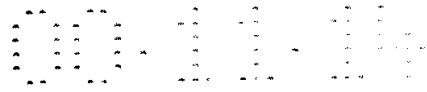
本发明的液体织物清洗组合物用下列实施例说明。

	实施例 11	实施例 12	实施例 13
G70*	0.05 %	0.03 %	0.30 %
C ₁₂ - C ₁₄ 烷基硫酸钠	20.0 %	20.0 %	20.0 %
2-丁基辛酸	5.0 %	5.0 %	5.0 %
柠檬酸钠	1.0%	1.0%	1.0%
C ₁₀ 脂肪醇乙氧基化物 (3)	13.0 %	13.0 %	13.0 %
一乙醇胺	2.50 %	2.50 %	2.50 %
水/丙二醇/乙醇(100:1:1)	58.45 %	58.47%	58.20 %

个人护理组合物

本发明所述的变体特别适合用于个人护理组合物，例如留存型和漂洗型护发剂，香波、留存型和漂洗型防治粉刺组合物、洗面乳及调理剂、淋浴凝胶、肥皂、泡沫及非泡沫型洁面剂、化妆品、手用、面用及体用润肤剂、保湿剂、贴敷剂和面膜，留存型面部保湿剂，化妆及清洁擦剂、口腔护理组合物、catamenials以及接触透镜护理组合物。本发明所述的个人护理组合物包含一种或多种本发明所述的变体和个人护理载体。

为了便于说明，本发明所述变体适合包含在下列参考文献中所述的组合物中：1997年6月24日颁布的授予Linares等人的美国专利5,641,479(皮肤清洁剂)；1997年2月4日颁布的授予Wivell等人的美国专利5,599,549(皮肤清洁剂)；1996年12月17日颁布的授予Ha等人的美国专利5,585,104(皮肤清洁剂)；1996年7月30日颁布的授予Kefauver等人的美国专利5,540,852(皮肤清洁剂)；1996年4月23日颁布的授予Dunbar等人的美国专利5,510,050(皮肤清洁剂)；1997年3月18日颁布的授予Guang Lin等人的美国专利5,612,324(抗粉刺制剂)；1996年12月24日颁布的授予Warren等人的美国专利5,587,176(抗粉刺制剂)；1996年8月27日颁布的授予Venkateswaran的美国专利5,549,888(抗粉刺



制剂); 1995年11月28日颁布的授予Corless 等人的美国专利5,470,884 (抗粉刺制剂); 1997年7月22日颁布的授予Gordon 等人的美国专利5,650,384 (淋浴凝胶); 1997年3月4日颁布的授予Moore 等人的美国专利5,607,678 (淋浴凝胶); 1997年4月29日颁布的授予Coffindaffer 等人的美国专利 5,624,666 (头发调理剂和/或洗发香波); 1997年4月8日颁布的授予Bolich等人的美国专利5,618,524 (头发调理剂和/或洗发香波); 1997年3月18日颁布的授予Inman的美国专利5,612,301, (头发调理剂和/或洗发香波); 1996年11月12日颁布的授予Wells的美国专利5,573,709 (头发调理剂和/或洗发香波); 1996年1月9日颁布的授予Pings的美国专利 5,482,703 (头发调理剂和/或洗发香波); 1994年4月12日再颁布的授予Grote 等人的美国专利 Re. 34,584 (头发调理剂和/或洗发香波); 1997年6月24日颁布的授予Date等人的美国专利 5,641,493 (化妆品); 1997年2月25日颁布的授予Blank 等人的美国专利5,605,894(化妆品); 1996年12月17日颁布的授予Yoshioka等人的美国专利5,585,090(化妆品); 1990年7月3日颁布的授予Cheney等人的美国专利4,949,179(手用、面用及体用润肤剂); 1997年3月4日颁布的授予McAtee等人的美国专利5,607,980 (手用、面用及体用润肤剂); 1977年8月30日颁布的授予Richter 等人的美国专利4,045,364 (化妆用或清洁用的擦剂); 1994年10月12日公开的Touchet 等人的欧洲专利申请, EP 0 619 074 (化妆用或清洁用的擦剂); 1990年12月4日颁布的授予Brown-Skrobot 等人的美国专利4,975,217 (化妆用或清洁用的擦剂); 1992年3月17日颁布的授予Seibel的美国专利5,096,700 (口腔清洁组合物); 1991年7月2日颁布的授予Sampathkumar的美国专利5,028,414 (口腔清洁组合物); 1991年7月2日颁布的授予Benedict 等人的美国专利 5,028,415 (口腔清洁组合物); 1989年9月5日颁布的授予Davies 等人的美国专利 4,863,627 (接触透镜清洗组合物); 1988年5月24日颁布的授予Huth等人的美国专利 Re. 32,672(接触透镜清洗组合物); 以及1986年9月2日颁布的授予Schafer的美国专利4,609,493 (接触透镜清洗组合物)。

为了进一步说明本发明所述的口腔清洁组合物, 可以向组合物中加

入药物上可接受量的一种或多种本发明所述变体用来清除牙齿或假牙上的蛋白性污渍。本发明使用的“口腔清洁组合物”是指洁齿剂、牙膏、牙粉、漱口水、口腔喷雾剂、口香糖、糖锭、香囊、片剂、生物凝胶、预防用糊剂、牙齿处理溶液等。优选地，所述的口腔清洁组合物包括约 0.0001% ~ 约 20% (重量) 的一种或多种本发明所述的变体，更优选约 0.001% ~ 约 10%，还更优选约 0.01% ~ 约 5%，以及药物上可接受的载体。本发明使用的“药物学上可接受的”是指该术语描述的药物、药剂或惰性成分适合用于与人和低等动物的组织接触而没有过度毒性、不相容性、不稳定性、刺激作用、过敏反应等，具有合理的效果/风险比。

典型地，所述口腔清洁组合物的药物上可接受的口腔清洁载体成分通常占组合物重量的约 50% ~ 约 99.99%，优选约 65% ~ 约 99.99%，更优选约 65% ~ 约 99%。

可加入到本发明所述口腔清洁组合物的口腔清洁组分中的药物上可接受的载体成分以及任选成分为本领域技术人员众所周知。多种多样的组合物类型、口腔清洁组合物中使用的载体成分以及任选成分都已在本发明上述的参考文献中公开。

在本发明的另一个实施方案中，用于在口腔外清洗假牙的假牙清洗组合物包含一种或多种本发明所述的变体。这类假牙清洗组合物中包含有效量的一种或多种变体以及假牙清洗载体，所述变体在组合物中的重量百分含量优选约 0.0001% ~ 约 50%，更优选约 0.001% ~ 约 35%，还更优选约 0.01% ~ 约 20%。各种假牙清洗组合物形式，例如泡腾片等都为本领域所熟知（参见，例如美国专利 5,055,305, Young），并且通常适合于掺入一种或多种变体用于清除假牙上的蛋白性污渍。

在本发明的另一个实施方案中，接触透镜清洗组合物中包括一种或多种本发明所述的变体。这类接触透镜清洗组合物中包含有效量的一种或多种变体以及接触透镜清洗载体，所述变体在组合物中所占重量百分比优选约 0.01% ~ 50%，更优选约 0.01% ~ 约 20%，还更优选约 1% ~ 约 5%。各种接触透镜清洗组合物形式，例如片剂、液体等都为本领域所熟知，并且通常适合于掺入一种或多种本发明的变体用于清除接触透镜上的蛋白

性污渍。

实施例14 -17

接触透镜清洗用组合物

	实施例14	实施例15	实施例16	实施例17
N76*	0.01 %	0.5 %	0.1 %	2.0 %
葡萄糖	50.0 %	50.0 %	50.0 %	50.0 %
非离子型表面活性剂 (聚氧乙烯-聚氧丙烯共聚物)	2.0 %	2.0 %	2.0 %	2.0 %
阴离子表面活性剂 (聚氧乙烯-烷基苯基醚硫酸酯钠)	1.0 %	1.0 %	1.0 %	1.0 %
氯化钠	1.0 %	1.0 %	1.0 %	1.0 %
硼砂	0.30 %	0.30 %	0.30 %	0.30 %
水	45.69 %	45.20 %	45.60 %	43.70 %

在实施例14-17中，表2-10中所述的变体，和本发明中所述的优选变体都可以用来替代N76*，结果基本上相似。

实施例18-21说明了本发明变体在洗浴产品中的应用。

实施例18 - 21

洗浴产品

	实施例 18	实施例19	实施例 20	实施例 21
水	62.62 %	65.72 %	57.72 %	60.72 %
EDTA二钠	0.2 %	0.2 %	0.2 %	0.2 %
丙三醇	3.0 %	3.0 %	3.0 %	3.0 %
Polyquatenium 10	0.4 %	0.4 %	0.4 %	0.4 %
月桂基醚硫酸钠	12.0 %	12.0 %	12.0 %	12.0 %
椰油酰胺 MEA	2.8 %	2.8 %	2.8 %	2.8 %
月桂酰两性乙酸钠	6.0 %	6.0 %	6.0 %	6.0 %
肉豆蔻酸	1.6 %	1.6 %	1.6 %	1.6 %



硫酸镁七水合物	0.3 %	0.3 %	0.3 %	0.3 %
三羟基硬脂	0.5 %	0.5 %	0.5 %	0.5 %
PEG-6辛酸/癸酸	3.0 %	-	-	-
甘油三酯				
棉籽酸脂肪酸蔗糖多酯	3.0 %	-	-	-
山萘酸脂肪酸蔗糖多酯	3.0 %	-	4.0 %	-
矿脂	-	4.0 %	8.0 %	-
矿物油	-	-	-	6.0 %
DMDM乙内酰脲	0.08 %	0.08 %	0.08 %	0.08 %
L75*+N76*+N77*+S78*+	0.1 %	2.0 %	2.0 %	5.0 %
I79*+G80*+V81*+L82*				
柠檬酸	1.40 %	1.40 %	1.40 %	1.40 %

在实施例18-21中,表2-10中所述的变体,和本发明所述的优选变体都可以用来替代L75*+N76*+N77*+S78*+I79*+G80*+V81*+L82*,结果基本上相似。

实施例22-25说明了本发明变体在洗面产品中的应用。

实施例22 - 25

洗面产品

	实施例22	实施例23	实施例24	实施例 25
水	66.52 %	65.17 %	68.47 %	68.72 %
EDTA二钠	0.1 %	0.1 %	0.2 %	0.2 %
柠檬酸	-	-	1.4 %	1.4 %
月桂基醚-3硫酸钠	3.0 %	3.5 %	-	-
月桂基醚-4羧酸钠	3.0 %	3.5 %	-	-
月桂基醚-12	1.0 %	1.2 %	-	-
Polyquaternium 10	-	-	0.4 %	0.4 %
Polyquaternium 25	0.3 %	0.3 %	-	-
甘油	3.0 %	3.0 %	3.0 %	3.0 %

月桂酰两性乙酸钠	-	-	6.0 %	6.0 %
月桂酸	6.0 %	6.0 %	3.0 %	3.0 %
肉豆蔻酸	-	-	3.0 %	3.0 %
硫酸镁七水合物	2.3 %	2.0 %	2.0 %	2.0 %
三乙醇胺	4.0 %	4.0 %	4.0 %	4.0 %
三羟基硬脂精	0.5 %	0.5 %	0.5 %	0.5 %
山萘酸脂肪酸蔗糖聚酯	2.0 %	2.0 %	-	-
棉籽酸脂肪酸蔗糖聚酯	3.0 %	2.0 %	-	-
PEG-6辛酸/癸酸	-	-	-	2.0 %
甘油三酯				
矿脂	-	-	4.0 %	-
矿物油	-	-	-	2.0 %
椰油酰氨基丙基甜菜碱	2.0 %	3.0 %	1.8 %	1.8 %
月桂基二甲胺氧化物	1.0 %	1.2 %	1.2 %	1.2 %
D-泛醇	1.0 %	0.25 %	0.25 %	-
DMDM乙内酰脲	0.08 %	0.08 %	0.08 %	0.08 %
L75*	1.0 %	2.0 %	0.5 %	0.5 %
日用香料	0.2 %	0.2 %	0.2 %	0.2 %

在实施例22-25中，表2-10中所述的变体，和本发明所述的优选变体都可以用来替代L75*，结果基本上相似。

实施例26-27说明了本发明变体在留存型皮肤保湿组合物中的应用。

实施例26 - 27

留存型皮肤保湿组合物

	实施例26	实施例27
甘油	5.0 %	-
硬脂酸	3.0 %	-
C ₁₁₋₁₃ 异链烷烃	2.0 %	-

乙二醇硬脂酸酯	1.5 %	-
丙二醇	-	3.0 %
矿物油	1.0 %	10.0 %
芝麻油	-	7.0 %
矿脂	-	1.8 %
三乙醇胺	0.7 %	-
乙酸鲸蜡酯	0.65 %	-
硬脂酸甘油酯	0.48 %	2.0 %
TEA硬脂酸酯	-	2.5 %
鲸蜡醇	0.47 %	-
羊毛脂醇	-	1.8 %
DEA -磷酸鲸蜡酯	0.25 %	-
对羟基苯甲酸甲酯	0.2 %	0.2 %
对羟基苯甲酸丙酯	0.12 %	0.1 %
Carbomer 934	0.11 %	-
EDTA二钠	0.1 %	-
L82*	0.1 %	0.5 %
水	84.32 %	71.1 %

在实施例26-27中，表2-10中所述的变体，和本发明所述的优选变体都可以用来替代L82*，结果基本上相似。

实施例28说明了本发明变体在擦净剂组合物中的应用。

实施例 34

擦净剂组合物

丙二醇	1.0 %
月桂基硫酸铵	0.6 %
琥珀酸	4.0 %
琥珀酸钠	3.2 %

Triclosan®	0.15 %
S78*	0.05 %
水	91.0 %

上述组合物被浸渍到一由纤维素和/或聚酯所组成的机织吸收层上，以吸收层重量计，组合物为约250%。

在实施例28中，表2-10中所述的变体，和本发明所述的优选变体都可以用来替代S78*，结果基本上相似。

序 列 表

(1) 一般信息:

(i) 申请人: RUBINGH, D.N. 等人

(ii) 发明名称: 免疫原性减少的丝氨酸蛋白酶变体

(iii) 序列数目: 1

(iv) 通讯地址:

(A) 地址: THE PROCTER & GAMBLE COMPANY

(B) 街道: 11810 EAST MIAMI RIVER ROAD

(C) 城市: ROSS

(D) 洲: OH

(E) 国家: 美国

(F) 邮编: 45061

(v) 电脑可读形式:

(A) 介质类型: 软盘

(B) 电脑: IBM PC兼容机

(C) 操作系统: PC-DOS/MS-DOS

(D) 软件: PatentIn Release #1.0, Version #1.30

(vi) 当前申请信息:

(A) 申请号:

(B) 申请日:

(C) 分类号:

(viii) 律师/代理人信息:

(A) 姓名: HERSKO, BART S.

(B) 注册号: 32, 572

(C) 律师文档号:

(ix) 电讯信息:

(A) 电话号码: 513-627-0633

(B) 传真: 513-627-0260

(2) SE ID NO: 1的信息:

(i) 序列特征:

(A) 长度: 275个氨基酸

(B) 类型: 氨基酸

(D) 拓扑学: 线型

(ii) 分子类型: 蛋白质

(xi) 序列描述: SEQ ID NO: 1:

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

Ser Ser Ser Thr Val Gly Tyr Pro Gly Lys Tyr Pro Ser Val Ile Ala
 165 170 175

Val Gly Ala Val Asp Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Val
 180 185 190

Gly Pro Glu Leu Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr
 195 200 205

Leu Pro Gly Asn Lys Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Ser
 210 215 220

Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Asn
 225 230 235 240

Trp Thr Asn Thr Gln Val Arg Ser Ser Leu Glu Asn Thr Thr Thr Lys
 245 250 255

Leu Gly Asp Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala
 260 265 270

Ala Ala Gln
 275