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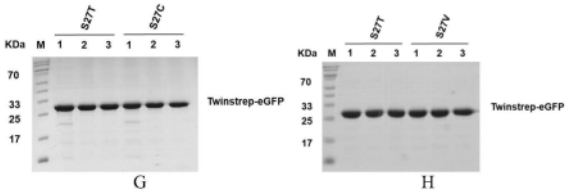
(54)发明名称

链霉亲和素第27位丝氨酸突变的突变蛋白及其应用

(57)摘要

本发明公开了链霉亲和素第27位丝氨酸突变的突变蛋白及其应用,突变蛋白为野生型链酶亲和素的44-47位氨基酸为VATR,以及在野生型链酶亲和素27位的丝氨酸突变为不同的氨基酸,突变后的蛋白与Strep tagⅡ结合力增加,提高了其对单Strep和TwinStrep融合蛋白的纯化能力,同时与Biotin的结合减弱,使得Biotin与其作用变得可逆,洗脱后进行洗涤再生可再次利用,因此能够更好的用于Biotin修饰蛋白和Strep tagⅡ标签蛋白纯化。



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1.链霉亲和素第27位丝氨酸突变的突变蛋白,其特征在于:所述突变蛋白为野生型链霉亲和素第44至47位氨基酸残基为VTAR,以及在野生型链霉亲和素的第27位丝氨酸突变为丙氨酸,记为S27A,氨基酸序列如SEQ ID NO.8所示。

2.权利要求1所述链霉亲和素第27位丝氨酸突变的突变蛋白与微球的固定复合物。

3.权利要求1所述链霉亲和素第27位丝氨酸突变的突变蛋白或权利要求2所述固定复合物在纯化Strep tagⅡ标签或Twinstrep标签蛋白中的应用。

4.权利要求1所述链霉亲和素第27位丝氨酸突变的突变蛋白或权利要求2所述固定复合物在纯化生物素修饰蛋白中的应用。

5.利用权利要求1所述链霉亲和素第27位丝氨酸突变的突变蛋白纯化Strep tagⅡ标签或Twinstrep标签蛋白的方法,其特征在于:将表达含Strep tagⅡ标签或Twinstrep标签蛋白的裂解液与所述突变蛋白的固定复合物结合,用缓冲液冲洗,最后用5-10mM Biotin缓冲液洗脱,收集洗脱液。

6.根据权利要求5所述链霉亲和素第27位丝氨酸突变的突变蛋白纯化Strep tagⅡ标签或Twinstrep标签蛋白的方法,其特征在于:Biotin缓冲液洗脱后还包括再生利用,具体为:向纯化过Strep tagⅡ标签或Twinstrep标签蛋白的固定复合物加入缓冲液洗涤即可再次使用。

链霉亲和素第27位丝氨酸突变的突变蛋白及其应用

技术领域

[0001] 本发明涉及链酶亲和突变的突变蛋白，经突变后能够可逆结合生物素，因此能够用于蛋白纯化，再生后并重复利用。

背景技术

[0002] 链霉亲和素(即Streptavidin)对生物素(Biotin)具有超强的非共价结合力，野生型链霉亲和素结合生物素的解离平衡常数K_d在10^{-14}mol/L，是目前自然界中已知的最强非共价相互作用，因此在分子生物学领域具有广泛运用，其应用领域包括：亲和层析、活细胞荧光成像、蛋白组学、生物素化酶的固定等。虽然链霉亲和素应用领域很广，但是具体到每一个应用，对链霉亲和素的性质更为细化的要求，例如亲和层析要求较低的亲和力和较高的解离常数，才能有效地将目的分子从含有链霉亲和素的微球上洗脱。由于野生型链霉亲和素会和生物素(Biotin)修饰的蛋白结合非常牢固，需要在非常剧烈的条件下，在含有高浓度的生物素(Biotin)的缓冲溶液中95℃加热才能将部分蛋白洗脱下来，因此其不能用于对生物素(Biotin)修饰的蛋白进行非变性亲和层析；野生型链霉亲和素的亲和纯化应用是利用其与长度为38个氨基酸的肽段SBP(氨基酸序列MDEKTTGWRGGHVVEGLAGELEQLRARLEHHPQGQREP)的强亲和力，解离平衡常数K_d为10^{-9}mol/L，将SBP肽段作为亲和纯化标签融合于目的蛋白N段或C端，并用Biotin进行竞争洗脱，但该应用的缺点仍在于Biotin与野生型链霉亲和素超强的结合力，导致野生型链霉亲和素无法有效再生，限制了SBP标签的应用。为了扩大对链霉亲和素的应用范围，需要对链霉亲和素进行改造以减弱其与Biotin的结合力。

[0003] Voss等对链霉亲和素的44-47位氨基酸突变得到StrepTactin突变体(44-47位氨基酸具有以下序列：Val-Thr-Ala-Arg，野生型链霉素亲和素在15位至139位的截短序列)，其能特异性结合短肽Strep tagⅡ(肽段序列：Trp-Ser-His-Pro-Gln-Phe-Glu-Lys)，其结合Strep tagⅡ的解离平衡常数K_d在10^{-7}mol/L，但其对生物素的解离平衡常数K_d仍在10^{-11}～10^{-12}mol/L，使得其与Biotin的结合仍不可逆；Wong等将野生型链霉亲和素的27位的丝氨酸(Ser)突变为丙氨酸(Ala)(S27A)，48位的甘氨酸(Gly)突变为苏氨酸(Thr)(G48T)得到SAVSBPM18突变体，该突变体对Biotin的解离平衡常数K_d升高10^{-8}mol/L，使得Biotin与SAVSBPM18的结合变得可逆。

[0004] 由于SBP标签长度较长，因此其应用有限，目前应用最广的是StrepTactin与Strep标签，但是单独的Strep tagⅡ与StrepTactin结合仍然不够强，因此实际使用过程中将两个Strep tagⅡ串联(即Twinstrep标签，肽段序列：Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(Gly\text{-}Gly\text{-}Gly\text{-}Ser)_3$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys，其编码的核苷酸如SEQ ID NO.1所示，氨基酸序列如SEQ ID NO.2所示)使得解离平衡常数K_d达到10^{-9}mol/L，大大增强其结合力。然而StrepTactin在用于纯化Strep tagⅡ标签的融合蛋白时，仍然具有以下缺点，特别是用生物素洗脱后会造成StrepTactin难以再生使用，并且用变性剂、强酸、强碱等进行重生，降低其使用寿命；洗脱目的蛋白时常常使用较为昂贵的脱硫生物素代替生物素对目的蛋白进行洗脱(Schmidt et al，2007)，但因为脱硫生物素的价格远高于生物素，使

用浓度较高,所以又造成了使用成本上升,不利于工业生产。

发明内容

[0005] 有鉴于此,本发明的目的之一在于提供链霉亲和素野生型链酶亲和素的44-47位氨基酸为VTAR,以及第27位丝氨酸突变的突变蛋白;本发明的目的之二在于提供含有所述链霉亲和素第27位丝氨酸突变的突变蛋白的固定复合物;本发明的目的之三在于提供所述链霉亲和素第27位丝氨酸突变的突变蛋白或所述固定复合物在纯化Strep tagⅡ标签或Twinstrep标签蛋白中的应用;本发明的目的之四在于提供所述链霉亲和素突变蛋白或所述固定复合物在纯化生物素修饰蛋白中的应用;本发明之五在于提供利用所述链霉亲和素第27位丝氨酸突变的突变蛋白纯化Strep tagⅡ标签或Twinstrep标签蛋白的方法;本发明目的之六在于提供利用所述链霉亲和素第27位丝氨酸突变的突变蛋白纯化生物素修饰蛋白的方法。

[0006] 为达到上述目的,本发明提供如下技术方案:

[0007] 1、链霉亲和素第27位丝氨酸突变的突变蛋白,所述突变蛋白为野生型链酶亲和素第44至47位氨基酸残基为VTAR,以及在野生型链酶亲和素的第27位丝氨酸突变为苏氨酸、丙氨酸、甘氨酸、谷氨酰胺、半胱氨酸、组氨酸、天冬酰胺、缬氨酸、甲硫氨酸、脯氨酸、亮氨酸、赖氨酸、异亮氨酸、精氨酸或苯丙氨酸,分别记为S27T、S27A、S27G、S27Q、S27C、S27H、S27V、S27M、S27P、S27L、S27K、S27I、S27R和S27F。

[0008] 本发明优选的,各突变氨基酸序列如下:S27T氨基酸序列如SEQ ID NO.4所示;S27A氨基酸序列如SEQ ID NO.8所示、S27G氨基酸序列如SEQ ID NO.6所示、S27Q氨基酸序列如SEQ ID NO.30所示、S27C氨基酸序列如SEQ ID NO.10所示、S27H氨基酸序列如SEQ ID NO.18所示、S27V氨基酸序列如SEQ ID NO.34所示、S27M氨基酸序列如SEQ ID NO.26所示、S27P氨基酸序列如SEQ ID NO.38所示、S27L氨基酸序列如SEQ ID NO.24所示、S27K氨基酸序列如SEQ ID NO.22所示、S27I氨基酸序列如SEQ ID NO.20所示、S27R氨基酸序列如SEQ ID NO.32所示和S27F氨基酸序列如SEQ ID NO.16所示。

[0009] 2、所述链霉亲和素第27位丝氨酸突变的突变蛋白与微球的固定复合物。

[0010] 3、所述链霉亲和素第27位丝氨酸突变的突变蛋白或所述固定复合物在纯化Strep tagⅡ标签或Twinstrep标签蛋白中的应用。

[0011] 4、所述链霉亲和素第27位丝氨酸突变的突变蛋白或所述固定复合物在纯化生物素修饰蛋白中的应用。

[0012] 5、利用所述链霉亲和素第27位丝氨酸突变的突变蛋白纯化Strep tagⅡ标签或Twinstrep标签蛋白的方法,将表达含Strep tagⅡ标签或Twinstrep标签蛋白的裂解液与所述突变蛋白的固定复合物结合,用缓冲液冲洗,最后用含5-10mM Biotin的缓冲液洗脱,收集洗脱液。

[0013] 本发明优选的,Biotin缓冲液洗脱后还包括再生利用,具体为:向纯化过Strep tagⅡ标签或Twinstrep标签蛋白的固定复合物加入缓冲液洗涤即可再次使用。

[0014] 6、利用所述链霉亲和素第27位丝氨酸突变的突变蛋白纯化生物素修饰蛋白的方法,将含链霉亲和素突变蛋白交联复合物平衡后,与含生物素修饰蛋白的裂解液结合,用含10-50mM Biotin的缓冲液洗脱,收集洗脱液。

[0015] 优选的，所述链霉亲和素第27位丝氨酸突变的突变蛋白纯化生物素修饰蛋白的方法，所述Biotin缓冲液洗脱后还包括再生利用，具体为：向纯化过生物素修饰蛋白的交联复合物加入缓冲液洗涤即可再次使用。

[0016] 本发明的有益效果在于：本发明提供了所述链霉亲和素第27位丝氨酸突变的突变蛋白，将野生型链酶亲和素第27位的丝氨酸(Ser)突变成不同氨基酸的突变体，突变后的蛋白与Strep tagⅡ结合力增加，提高了其对Strep和TwinStrep融合蛋白的纯化能力，同时与Biotin的结合减弱，使得Biotin与其作用变得可逆，洗脱后进行洗涤再生可再次利用，因此能够更好的用于Biotin修饰蛋白和Strep tagⅡ标签蛋白纯化，用Biotin对生物素修饰的蛋白进行洗脱，洗脱后用缓冲液对StrepTactin mut进行洗涤再生即可再次利用。

附图说明

[0017] 为了使本发明的目的、技术方案和有益效果更加清楚，本发明提供如下附图进行说明：

[0018] 图1为27位相关突变体富集Twinstrep修饰的蛋白的筛选(A:StepTactin Beads和S27T,S27A,S27G,S27E,S27Q,S27W,S27C,S27H Beads纯化Twinstrep-eGFP的效果单次对比;B:StepTactin Beads和S27T,S27N,S27V,S27M,S27P,S27L,S27K,Beads纯化Twinstrep-eGFP的效果单次对比;C:StepTactin Beads和S27T,S27Y,S27I,S27D,S27PR,S27F Beads纯化Twinstrep-eGFP的效果单次对比)。

[0019] 图2为27位相关突变体富集Twinstrep修饰的蛋白的重生3次结果(A:S27T Beads和S27A,S27G Beads纯化Twinstrep-eGFP的效果对比图(S27T 1-3:S27T Beads纯化Twinstrep-eGFP的重生三次实验;S27A 1-3:S27A Beads纯化Twinstrep-eGFP的重生三次实验;S27G 1-3:S27G Beads纯化Twinstrep-eGFP的重生三次实验);B:S27T Beads和S27H,S27Q Beads纯化Twinstrep-eGFP的效果对比图(S27T 1-3:S27T Beads纯化Twinstrep-eGFP的重生三次实验;S27H 1-3:S27H Beads纯化Twinstrep-eGFP的重生三次实验;S27Q 1-3:S27Q Beads纯化Twinstrep-eGFP的重生三次实验);C:S27T Beads和S27L,S27P Beads纯化Twinstrep-eGFP的效果对比图(S27T 1-3:S27T Beads纯化Twinstrep-eGFP的重生三次实验;S27L 1-3:S27LBeads纯化Twinstrep-eGFP的重生三次实验;S27P 1-3:S27P Beads纯化Twinstrep-eGFP的重生三次实验);D:S27T Beads和S27M,S27F Beads纯化Twinstrep-eGFP的效果对比图(S27T1-3:S27T Beads纯化Twinstrep-eGFP的重生三次实验;S27M 1-3:S27M Beads纯化Twinstrep-eGFP的重生三次实验;S27F 1-3:S27F Beads纯化Twinstrep-eGFP的重生三次实验);E:S27T Beads和S27N,S27K Beads纯化Twinstrep-eGFP的效果对比图(S27T 1-3:S27T Beads纯化Twinstrep-eGFP的重生三次实验;S27N 1-3:S27N Beads纯化Twinstrep-eGFP的重生三次实验;S27K 1-3:S27K Beads纯化Twinstrep-eGFP的重生三次实验);F:S27T Beads和S27R,S27I Beads纯化Twinstrep-eGFP的效果对比图(S27T 1-3:S27T Beads纯化Twinstrep-eGFP的重生三次实验;S27R 1-3:S27R Beads纯化Twinstrep-eGFP的重生三次实验;S27I 1-3:S27I Beads纯化Twinstrep-eGFP的重生三次实验);G:S27T Beads和S27C Beads纯化Twinstrep-eGFP的效果对比图(S27T 1-3:S27T Beads纯化Twinstrep-eGFP的重生三次实验;S27C 1-3:S27C Beads纯化Twinstrep-eGFP的重生三次实验);H:S27T Beads和S27VBeads纯化Twinstrep-eGFP的效果对比图(S27T 1-3:S27T

Beads纯化Twinstrep-eGFP的重生三次实验;S27 1-3:S27C Beads纯化Twinstrep-eGFP的重生三次实验))。

[0020] 图3为27位相关突变体富集Twinstrep修饰的蛋白重生六次结果(A:S27A Beads纯化Twinstrep-eGFP重复利用图(1-6:S27A Beads纯化Twinstrep-eGFP,并重生六次实验);B:S27C Beads纯化Twinstrep-eGFP重复利用图(1-6:S27C Beads纯化Twinstrep-eGFP,并重生六次实验);C:S27G Beads纯化Twinstrep-eGFP重复利用图(1-6:S27G Beads纯化Twinstrep-eGFP,并重生六次实验);D:S27Q Beads纯化Twinstrep-eGFP重复利用图(1-6:S27Q Beads纯化Twinstrep-eGFP,并重生六次实验);E:S27K Beads纯化Twinstrep-eGFP重复利用图(1-6:S27K Beads纯化Twinstrep-eGFP,并重生六次实验);F:S27L Beads纯化Twinstrep-eGFP重复利用图(1-6:S27L Beads纯化Twinstrep-eGFP,并重生六次实验);G:S27M Beads纯化Twinstrep-eGFP重复利用图(1-6:S27M Beads纯化Twinstrep-eGFP,并重生六次实验);H:S27P Beads纯化Twinstrep-eGFP重复利用图(1-6:S27P Beads纯化Twinstrep-eGFP,并重生六次实验);I:S27V Beads纯化Twinstrep-eGFP重复利用图(1-6:S27V Beads纯化Twinstrep-eGFP,并重生六次实验))。

[0021] 图4为27位相关突变体纯化Strep-eGFP的效果单次结果(A:S27T,S27A,S27V,S27P,S27L,S27H,S27G Beads纯化Strep-eGFP的效果单次对比;B:S27T,S27I,S27M,S27Q,S27C,S27K Beads纯化Strep-eGFP的效果单次对比)。

[0022] 图5为27位相关突变体富集Bio-eGFP和Bio-BSA对比图(A:S27T Beads和S27A,S27V,S27L,S27H,S27G Beads纯化富集Bio-eGFP和Bio-BSA对比图(In:加入的蛋白总量;S27T:S27T Beads洗脱下来的蛋白;S27A:S27A Beads洗脱下来的蛋白;S27V:S27V Beads洗脱下来的蛋白;S27L:S27L Beads洗脱下来的蛋白;S27H:S27H Beads洗脱下来的蛋白;S27G:S27G Beads洗脱下来的蛋白);B:S27T Beads和S27I,S27M,S27Q,S27C,S27K Beads纯化富集Bio-eGFP和Bio-BSA对比图(In:加入的蛋白总量;S27T:S27T Beads洗脱下来的蛋白;S27I:S27I Beads洗脱下来的蛋白;S27M:S27M Beads洗脱下来的蛋白;S27Q:S27QBeads洗脱下来的蛋白;S27C:S27C Beads洗脱下来的蛋白;S27K:S27K Beads洗脱下来的蛋白)。

具体实施方式

[0023] 下面结合附图和具体实施例对本发明作进一步说明,以使本领域的技术人员可以更好的理解本发明并能予以实施,但所举实施例不作为对本发明的限定。

[0024] 实施例1、27位相关突变体富集Twinstrep修饰的蛋白的筛选

[0025] 本发明中为了提升链霉亲和素(Streptavidin)与Strep tagⅡ的结合能力,降低StrepTactin与生物素(Biotin)的结合能力,综合StrepTactin与其配体的结合位点,对StrepTactin与生物素形成氢键的关键氨基酸进行定点突变,目的在于保留原来的产品优点的同时弥补其不足之处,减弱生物素与StrepTactin的结合,来获得可与生物素可逆结合的StrepTactin突变体(StrepTactin muts)。将27位进行了其他各个氨基酸突变,分别为S27T、S27G、S27A、S27C、S27D、S27E、S27F、S27H、S27I、S27K、S27L、S27M、S27N、S27Q、S27R、S27V、S27Y、S27P、S27W,其突变后的蛋白序列分别如SEQ ID NO.4、SEQ ID NO.6、SEQ ID NO.8、SEQ ID NO.10、SEQ ID NO.12、SEQ ID NO.14、SEQ ID NO.16、SEQ ID NO.18、SEQ ID NO.20、SEQ ID NO.22、SEQ ID NO.24、SEQ ID NO.26、SEQ ID NO.28、SEQ ID NO.30、SEQ ID

NO.32、SEQ ID NO.34、SEQ ID NO.36、SEQ ID NO.38和SEQ ID NO.40;基因序列分别如SEQ ID NO.3、SEQ ID NO.5、SEQ ID NO.7、SEQ ID NO.9、SEQ ID NO.11、SEQ ID NO.13、SEQ ID NO.15、SEQ ID NO.17、SEQ ID NO.19、SEQ ID NO.21、SEQ ID NO.23、SEQ ID NO.25、SEQ ID NO.27、SEQ ID NO.29、SEQ ID NO.31、SEQ ID NO.33、SEQ ID NO.35、SEQ ID NO.37和SEQ ID NO.37。

[0026] 实施例2、StrepTactin mut的制备

[0027] 1)取5μL抽提的含上述设计的含StrepTactin muts的质粒(含StrepTactin mut的质粒是以PIISA-His-StrepTactin为模板,设计引物对其进行定点突变,PIISA-His-StrepTactin是由公司合成而得,PIISA-His-StrepTactin如SEQ ID NO.45)加入至100μL的BL21 codon plus(DE3)感受态细胞中,冰浴30min后42℃热激90s,再在冰上静置2min,加入900μL的LB培养基在37℃、200rpm的摇床内复苏1h,涂布于含100μg/mL的氨苄抗性的平板上,37℃恒温过夜培养;

[0028] 2)第二天从过夜培养的平板上挑取一个单菌落至10mL含100μg/mL氨苄青霉素的LB培养基中,在37℃、200rpm的摇床中培养12h,将培养后的10mL菌液转接至1L含100μg/mL氨苄青霉素的LB培养基中,在37℃、200rpm的摇床中培养,当OD_{600}达到1.5时,将菌液冷却至0℃;冷却下来的菌液加入终浓度1mM的IPTG后于16℃、220rpm培养18h;培养结束后于大容量低温离心机内4℃、3500rpm离心20min收集所有大肠杆菌,倒净所有上清,用25mL 50mM PBS(pH 7.4)缓冲液重悬大肠杆菌,加入终浓度1mM的PMSF;

[0029] 3)将重悬的大肠杆菌使用超声破碎仪破碎,低温条件下以40%的功率,超声3s,停7s,超声20min,超声后的菌液在60℃加热15min,加热后的菌液在4℃、15000rpm、离心20min,取上清,并将上清液用0.45μm滤膜抽滤到一个干净50mL离心管内,放置于冰上;

[0030] 4)用50mL 50mM PBS(pH 7.4)缓冲液平衡处理好的Ni-IDA beads,平衡完毕加入上一步中抽滤好的大肠杆菌上清液,收集从柱内流出的裂解液重复上样一次;

[0031] 5)上样结束后,用含5mM咪唑的50mM PBS(pH 7.4)冲洗Ni柱,总计冲洗100mL;

[0032] 6)用含40mM咪唑的50mM PBS(pH 7.4)缓冲液冲洗Ni柱,总计冲洗50mL;

[0033] 7)用20mL的含250mM咪唑的50mM PBS(pH 7.4)缓冲液洗脱目的蛋白,洗脱结束将洗脱的蛋白放置于冰上;

[0034] 8)向洗脱后的蛋白加入8.72g硫酸铵,震荡溶解硫酸铵,待硫酸铵溶解后放置于冰上30min沉淀His-StrepTactin mut蛋白;

[0035] 9)将上一步中的蛋白在4℃、15000rpm、离心10min,离心结束弃上清,沉淀用2mL的含5mM EDTA的10mM $NaHCO_3$缓冲液溶解,溶解后的蛋白再次在4℃、15000rpm、离心10min,离心结束保留上清。

[0036] 实施例3、StrepTactin mut的交联固定

[0037] 1)取2mL纯化后的His-StrepTactin mut蛋白,将蛋白在2L的200mM $NaHCO_3$、500mM NaCl缓冲液中透析,每隔2h更换透析液一次,总计更换透析液两次;

[0038] 2)透析结束的His-StrepTactin mut蛋白测定其在280nm波长的紫外吸收值,按每毫克蛋白吸收值为2.84计算透析后的蛋白浓度和蛋白总质量;

[0039] 3)按每毫升NHS微球(NHS Beads)交联固定12mg StrepTactin mut蛋白量交联,用5倍Beads体积的1mM HCl溶液活化NHS Beads,活化后用5倍Beads体积的200mM $NaHCO_3$、

500mM NaCl缓冲液平衡NHS Beads，平衡结束加入透析好的蛋白，在4℃旋转交联12h；

[0040] 4)交联结束后，用5倍Beads体积的100mM Tris-HCl(pH 8.5)缓冲液冲洗Beads一次，再用5倍Beads体积的100mM Tris-HCl(pH 8.5)缓冲液封闭Beads上未反应的NHS基团，4℃旋转封闭12h；

[0041] 5)封闭结束后，用5倍Beads体积的50mM Tris-HCl(pH 7.4)、150mM NaCl、1mM EDTA缓冲液冲洗Beads一次，将交联后的StrepTactin mut保存于一倍Beads体积的50mM Tris-HCl(pH 7.4)、150mM NaCl、1mM EDTA、0.03％ NaN_3缓冲液中，存储于4℃。

[0042] 实施例4、TwinStrep-eGFP的表达及裂解液制备

[0043] 1)取5μL抽提的含TwinStrep-eGFP的质粒，TwinStrep-eGFP的核苷酸序列如SEQ ID NO.13所示(TwinStrep-eGFP的质粒由pIISA-TwinStrep质粒用限制性内切酶BsaI酶切，将eGFP通过BsaI酶切位点用T4连接酶与载体连接，pIISA-TwinStrep质粒由扩增的TwinStrep序列连入pIISA的BsaI酶切位点而得)，分别加入至100μL的BL21 codon plus(DE3)感受态细胞中，冰浴30min后42℃热激90s，再在冰上静置2min，加入900μL的LB培养基在37℃、200rpm的摇床内复苏1h，涂布于含100μg/mL的氨苄抗性的平板上，37℃恒温过夜培养；

[0044] 2)第二天从过夜培养的平板上挑取一个单菌落至10mL含100μg/mL氨苄青霉素的LB培养基中，在37℃、200rpm的摇床中培养12h，从培养后的10mL菌液取1mL菌液转接至100mL含100μg/mL氨苄青霉素的LB培养基中，在37℃、200rpm的摇床中培养，当OD_{600}达到0.6时，将菌液冷却至25℃；冷却下来的菌液加入终浓度1mM的IPTG后于25℃、220rpm培养10h；培养结束后于大容量低温离心机内4℃、3500rpm离心20min收集所有大肠杆菌，倒净所有上清，用10mL 50mM Tris-HCl(pH 7.4)、150mM NaCl缓冲液重悬大肠杆菌，加入终浓度1mM的PMSF；

[0045] 3)将重悬的大肠杆菌使用超声破碎仪破碎，低温条件下以40％的功率，超声3s，停7s，超声5min，超声后的菌液在4℃、15000rpm、离心20min，取上清，并将上清液用0.45μm滤膜抽滤到一个干净15mL离心管内，放置于冰上；得到TwinStrep-eGFP裂解液。

[0046] 实施例4、StrepTactin mut Beads对TwinStrep-eGFP的纯化

[0047] 1)取15μL StrepTactin mut Beads，用200μL 20mM Tris-HCl(pH 7.4)、150mM NaCl、1mM PMSF缓冲液平衡beads，4℃、3000rpm离心1min，弃上清；

[0048] 2)取100μL上述制备的TwinStrep-eGFP裂解液加入至StrepTactin mut交联固定的Beads中，4℃旋转结合30min；

[0049] 3)结合结束，用200μL 20mM Tris-HCl(pH 8.0)、150mM NaCl、1mM EDTA、0.5％ Triton-X100缓冲液洗涤beads 3次，再用200μL 20mM Tris-HCl(pH 7.4)、150mM NaCl、1mM EDTA缓冲液洗涤beads 2次，每次洗涤5min，然后在4℃、3000rpm离心1min，弃上清；

[0050] 4)用20μL 50mM Tris-HCl(pH 7.4)、150mM NaCl、5 mM Biotin缓冲液洗脱beads上的蛋白，洗脱液加入至beads中后，先静置5min，然后4℃、3000rpm离心1min，收集离出的液体，洗脱3次；

[0051] 5)纯化结束对每一步中的样品取样，加入5×SDS Loading Buffer(含DTT)，95℃加热5min后进行16.5％聚丙烯凝胶电泳，经考马斯亮蓝染色，结果如图1所示。结果显示S27T、S27G、S27A、S27C、S27F、S27H、S27I、S27K、S27L、S27M、S27N、S27Q、S27R、S27V、S27P对

Twinstrep单次结富集能力相当。本实施例中用含5-10mM Biotin的缓冲液洗脱均可。

[0052] 2、StrepTactin mut Beads纯化TwinStrep-eGFP后再生利用

[0053] 1)向纯化过TwinStrep-eGFP的15μL StrepTactin mut Beads中加入200μL 20mM Tris-HCl(pH 7.4)、150mM NaCl、1mM EDTA缓冲液洗涤beads 3次,每次洗涤5min,然后在4℃、3000rpm离心1min,弃上清,经过3次洗涤后StrepTactin mut Beads即可再次使用;

[0054] 2)按StrepTactin mut Beads对TwinStrep-eGFP的纯化步骤测试再生后的StrepTactin mut Beads的纯化效果,纯化结束按再生步骤进行再生,如此重复3次,结束后对每一次纯化的样品取样,加入5×SDS Loading Buffer(含DTT),95℃加热5min后进行16.5%聚丙烯凝胶电泳,经考马斯亮蓝染色,结果如图2所示。按照上述方法将S27T和W120H再生6次以上,结束后对每一次纯化的样品取样,加入5×SDS Loading Buffer(含DTT),95℃加热5min后进行16.5%聚丙烯凝胶电泳,经考马斯亮蓝染色,结果如图3所示。并且本实施例中,PBS缓冲液或TE缓冲液等洗涤均可再次使用,因此适应缓冲液范围广。

[0055] 上述结果表明,其中丝氨酸(Ser)突变为苏氨酸(Thr)(S27T),丙氨酸(Ala)(S27A),缬氨酸(Val)(S27V),天冬酰胺(Asn)(S27N),甲硫氨酸(Met)(S27M),脯氨酸(Pro)(S27P),赖氨酸(Lys)(S27K),半胱氨酸(Cys)(S27C),异亮氨酸(Ile)(S27I)等对Twinstrep的结富集能力相当;将27位突变为甘氨酸(Gly)(S27G)时,首次用于富集Twinstrep标签的蛋白载量较高,重生后载量降低但重复性仍然优于StrepTactin。将27位突变为苯丙氨酸(Phe)(S27F),谷氨酰胺(Gln)(S27Q),组氨酸(His)(S27H),精氨酸(Arg)(S27R),亮氨酸(Leu)(S27L)时,富集Twinstrep的载量相对降低,但是相对重生利用性好,洗脱后用Tri-HCl缓冲液、PBS缓冲液或TE对突变体进行洗涤再生即可再次利用,而StrepTactin如果只简单用Tri-HCl(pH 7.4)缓冲液重生后对Twinstrep融合蛋白几乎无富集能力。

[0056] 将27位突变为酸性氨基酸天冬氨酸(Asp)(S27D)和谷氨酸(Glu)(S27E)以及酪氨酸(Tyr)(S27Y),色氨酸(Trp)(S27W),对Twinstrep与Biotin的结合效果明显减弱,特别是突变为D和E对Twinstrep则削弱为无富集效果。

[0057] 实施例5、27位相关突变体富集Strep修饰的蛋白的筛选

[0058] 将以上筛选出的对带Twinstrep标签的蛋白富集效果较好的几个突变体进行对带Strep标签的蛋白富集测试。Strep标签的蛋白氨基酸序列如SEQ ID NO.41所示,制备方法与TwinStrep-eGFP相同,区别是连入Strep-eGFP如SEQ ID NO.42所示的核苷酸序列。结果如图4所示。结果显示,S27A对带Strep标签的蛋白富集的载量较高,S27T,S27C,S27P,S27H,S27I,S27K,S27V对带Strep标签的蛋白富集效果也相对明显。

[0059] 实施例6、27位相关突变体富集生物素修饰的蛋白的筛选

[0060] 1、生物素修饰的eGFP(Bio-eGFP)的表达及裂解液制备

[0061] 生物素可以在生物素连接酶(BirA)的作用下特异性的修饰于Avi标签中的赖氨酸残基上即生成生物素化的Avi标签。

[0062] 1)取5μL抽提的含CBD-BirA质粒,CBD-BirA基因序列如SEQ ID NO.43所示(含CBD-BirA质粒由限制性内切酶XhoI和NcoI酶切PET28a-CBD,将BirA通过XhoI酶切位点用T4连接酶连接,PET28a-CBD由BSAI酶切位点将CBD与PET28a载体链接)和含Avi-eGFP的质粒,Avi-eGFP基因序列如SEQ ID NO.44所示(含Avi-eGFP的质粒由限制性内切酶BamHI和XhoI酶切PET22b-avi质粒,将eGFP通过BamHI和XhoI酶切位点用T4连接酶连接,PET22b-avi质粒通过

重组将avi与PET22b连接)，然后加入至100μL的BL21 codon plus(DE3)感受态细胞中，冰浴30min后42℃热激90s，再在冰上静置2min，加入900μL的LB培养基在37℃、200rpm的摇床内复苏1h，涂布于含100μg/mL的氨苄和50μg/mL的卡那霉素抗性的平板上，37℃恒温过夜培养；

[0063] 2)第二天从过夜培养的平板上挑取一个单菌落至10mL含100μg/mL氨苄青霉素和50μg/mL的卡那霉素的LB培养基中，在37℃、200rpm的摇床中培养12h，从培养后的10mL转接至1L含100μg/mL氨苄青霉素和50μg/mL的卡那霉素的LB培养基中，在37℃、200rpm的摇床中培养，当OD_{600}达到0.6时，将菌液冷却至25℃；冷却下来的菌液加入终浓度1mM的IPTG和1mM的Biotin后于25℃、220rpm培养10h；培养结束后于大容量低温离心机内4℃、3500rpm离心20min收集所有大肠杆菌，倒净所有上清，用30mL 50mM Tris-HCl(pH7.4)、150mM NaCl缓冲液重悬大肠杆菌，加入终浓度1mM的PMSF；

[0064] 3)将重悬的大肠杆菌使用超声破碎仪破碎，低温条件下以40％的功率，超声3s，停7s，超声20min，超声后的菌液在4℃、15000rpm、离心20min，取上清，并将上清液用0.45μm滤膜抽滤到一个干净50mL离心管内，放置于冰上，即得Bio-eGFP裂解液。

[0065] 制备Bio-BSA，称取664.5mgBSA蛋白用缓冲液溶解，称取34.1mgBiotin-NHS至溶解后的BSA中，25℃过夜反应后透析到200mM$NaHCO_3$、500mMNaCl缓冲液。将以上筛选出的突变体对Bio-BSA和Bio-eGFP进行了富集测试，结果如图5所示。结果显示，S27T、S27M、S27C、S27K、S27A、S27V、S27I、S27P、S27H、S27G的富集效果较为明显。表明这些突变体不仅能用于Strep Tag II的富集纯化，也可用于biotin修饰蛋白的富集纯化。

[0066] 以上所述实施例仅是为充分说明本发明而所举的较佳的实施例，本发明的保护范围不限于此。本技术领域的技术人员在本发明基础上所作的等同替代或变换，均在本发明的保护范围之内。本发明的保护范围以权利要求书为准。

[0001] 序列表

[0002] <110> 西南大学

[0003] <120> 链霉亲和素第27位丝氨酸突变的突变蛋白及其应用

[0004] <160> 45

[0005] <170> SIPOSequenceListing 1.0

[0006] <210> 1

[0007] <211> 84

[0008] <212> DNA

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[0012] tggagtcacc ctcaattcga gaaa 84

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[0014] <211> 28

[0015] <212> PRT

[0016] <213> 人工序列(Artificial Sequence)

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[0019] 1 5 10 15

[0020] Gly Gly Gly Ser Trp Ser His Pro Gln Phe Glu Lys

[0021] 20 25

[0022] <210> 3

[0023] <211> 381

[0024] <212> DNA

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[0029] gtcctgaccg gtcgttacga cagcgccccg gccaccgacg gcagcggcac cgccctcggt 180

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[0035] <211> 126

[0036] <212> PRT

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[0040] 1 5 10 15

[0041] Val Thr Ala Gly Ala Asp Gly Ala Leu Thr Gly Thr Tyr Val Thr Ala

[0042] 20 25 30

[0043] Arg Gly Asn Ala Glu Ser Arg Tyr Val Leu Thr Gly Arg Tyr Asp Ser

[0044] 35 40 45

[0045] Ala Pro Ala Thr Asp Gly Ser Gly Thr Ala Leu Gly Trp Thr Val Ala

[0046] 50 55 60

[0047] Trp Lys Asn Asn Tyr Arg Asn Ala His Ser Ala Thr Thr Trp Ser Gly

[0048] 65 70 75 80

[0049] Gln Tyr Val Gly Gly Ala Glu Ala Arg Ile Asn Thr Gln Trp Leu Leu

[0050] 85 90 95

[0051] Thr Ser Gly Thr Thr Glu Ala Asn Ala Trp Lys Ser Thr Leu Val Gly

[0052] 100 105 110

[0053] His Asp Thr Phe Thr Lys Val Lys Pro Ser Ala Ala Lys Ser

[0054] 115 120 125

[0055] <210> 5

[0056] <211> 381

[0057] <212> DNA

[0058] <213> 人工序列(Artificial Sequence)

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[0069] <212> PRT

[0070] <213> 人工序列(Artificial Sequence)

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[0073] 1 5 10 15

[0074] Val Thr Ala Gly Ala Asp Gly Ala Leu Thr Gly Thr Tyr Val Thr Ala

[0075] 20 25 30

[0076] Arg Gly Asn Ala Glu Ser Arg Tyr Val Leu Thr Gly Arg Tyr Asp Ser

[0077] 35 40 45

[0078] Ala Pro Ala Thr Asp Gly Ser Gly Thr Ala Leu Gly Trp Thr Val Ala

[0079] 50 55 60

[0080] Trp Lys Asn Asn Tyr Arg Asn Ala His Ser Ala Thr Thr Trp Ser Gly

[0081] 65 70 75 80

[0082] Gln Tyr Val Gly Gly Ala Glu Ala Arg Ile Asn Thr Gln Trp Leu Leu

[0083] 85 90 95

[0084] Thr Ser Gly Thr Thr Glu Ala Asn Ala Trp Lys Ser Thr Leu Val Gly
[0085] 100 105 110
[0086] His Asp Thr Phe Thr Lys Val Lys Pro Ser Ala Ala Lys Ser
[0087] 115 120 125
[0088] <210> 7
[0089] <211> 381
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[0094] gccgacggcg ccctgaccgg tacctacgtc acggcccgtg gcaacgccga gagccgctac 120
[0095] gtcctgaccg gtcgttacga cagcgccccg gccaccgacg gcagcggcac cgccctcggt 180
[0096] tggacggtgg cctggaagaa taactaccgc aacgcccact ccgcgaccac gtggagcggc 240
[0097] cagtacgtcg gcggcgccga ggcgaggatc aacacccagt ggctgctgac ctccggcacc 300
[0098] accgaggcca acgcctggaa gtccacgctg gtcggccacg acacgttcac caaggtgaag 360
[0099] ccgtccgccg ccaagtccta a 381
[0100] <210> 8
[0101] <211> 126
[0102] <212> PRT
[0103] <213> 人工序列(Artificial Sequence)
[0104] <400> 8
[0105] Met Gly Ile Thr Gly Thr Trp Tyr Asn Gln Leu Gly Ala Thr Phe Ile
[0106] 1 5 10 15
[0107] Val Thr Ala Gly Ala Asp Gly Ala Leu Thr Gly Thr Tyr Val Thr Ala
[0108] 20 25 30
[0109] Arg Gly Asn Ala Glu Ser Arg Tyr Val Leu Thr Gly Arg Tyr Asp Ser
[0110] 35 40 45
[0111] Ala Pro Ala Thr Asp Gly Ser Gly Thr Ala Leu Gly Trp Thr Val Ala
[0112] 50 55 60
[0113] Trp Lys Asn Asn Tyr Arg Asn Ala His Ser Ala Thr Thr Trp Ser Gly
[0114] 65 70 75 80
[0115] Gln Tyr Val Gly Gly Ala Glu Ala Arg Ile Asn Thr Gln Trp Leu Leu
[0116] 85 90 95
[0117] Thr Ser Gly Thr Thr Glu Ala Asn Ala Trp Lys Ser Thr Leu Val Gly
[0118] 100 105 110
[0119] His Asp Thr Phe Thr Lys Val Lys Pro Ser Ala Ala Lys Ser
[0120] 115 120 125
[0121] <210> 9
[0122] <211> 381
[0123] <212> DNA
[0124] <213> 人工序列(Artificial Sequence)
[0125] <400> 9

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[0127] gccgacggcg ccctgaccgg tacctacgtc acggcccgtg gcaacgccga gagccgctac 120
[0128] gtcctgaccg gtcgttacga cagcgccccg gccaccgacg gcagcggcac cgccctcggt 180
[0129] tggacggtgg cctggaagaa taactaccgc aacgcccact ccgcgaccac gtggagcggc 240
[0130] cagtacgtcg gcggcgccga ggcgaggatc aacacccagt ggctgctgac ctccggcacc 300
[0131] accgaggcca acgcctggaa gtccacgctg gtcggccacg acacgttcac caaggtgaag 360
[0132] ccgtccgccg ccaagtccta a 381
[0133] <210> 10
[0134] <211> 126
[0135] <212> PRT
[0136] <213> 人工序列(Artificial Sequence)
[0137] <400> 10
[0138] Met Gly Ile Thr Gly Thr Trp Tyr Asn Gln Leu Gly Cys Thr Phe Ile
[0139] 1 5 10 15
[0140] Val Thr Ala Gly Ala Asp Gly Ala Leu Thr Gly Thr Tyr Val Thr Ala
[0141] 20 25 30
[0142] Arg Gly Asn Ala Glu Ser Arg Tyr Val Leu Thr Gly Arg Tyr Asp Ser
[0143] 35 40 45
[0144] Ala Pro Ala Thr Asp Gly Ser Gly Thr Ala Leu Gly Trp Thr Val Ala
[0145] 50 55 60
[0146] Trp Lys Asn Asn Tyr Arg Asn Ala His Ser Ala Thr Thr Trp Ser Gly
[0147] 65 70 75 80
[0148] Gln Tyr Val Gly Gly Ala Glu Ala Arg Ile Asn Thr Gln Trp Leu Leu
[0149] 85 90 95
[0150] Thr Ser Gly Thr Thr Glu Ala Asn Ala Trp Lys Ser Thr Leu Val Gly
[0151] 100 105 110
[0152] His Asp Thr Phe Thr Lys Val Lys Pro Ser Ala Ala Lys Ser
[0153] 115 120 125
[0154] <210> 11
[0155] <211> 0
[0156] <212> DNA
[0157] <213> 人工序列(Artificial Sequence)
[0158] <400> 11
[0159] <210> 12
[0160] <211> 126
[0161] <212> PRT
[0162] <213> 人工序列(Artificial Sequence)
[0163] <400> 12
[0164] Met Gly Ile Thr Gly Thr Trp Tyr Asn Gln Leu Gly Asp Thr Phe Ile
[0165] 1 5 10 15
[0166] Val Thr Ala Gly Ala Asp Gly Ala Leu Thr Gly Thr Tyr Val Thr Ala
[0167] 20 25 30

[0168] Arg Gly Asn Ala Glu Ser Arg Tyr Val Leu Thr Gly Arg Tyr Asp Ser
[0169] 35 40 45
[0170] Ala Pro Ala Thr Asp Gly Ser Gly Thr Ala Leu Gly Trp Thr Val Ala
[0171] 50 55 60
[0172] Trp Lys Asn Asn Tyr Arg Asn Ala His Ser Ala Thr Thr Trp Ser Gly
[0173] 65 70 75 80
[0174] Gln Tyr Val Gly Gly Ala Glu Ala Arg Ile Asn Thr Gln Trp Leu Leu
[0175] 85 90 95
[0176] Thr Ser Gly Thr Thr Glu Ala Asn Ala Trp Lys Ser Thr Leu Val Gly
[0177] 100 105 110
[0178] His Asp Thr Phe Thr Lys Val Lys Pro Ser Ala Ala Lys Ser
[0179] 115 120 125
[0180] <210> 13
[0181] <211> 381
[0182] <212> DNA
[0183] <213> 人工序列(Artificial Sequence)
[0184] <400> 13
[0185] atgggcatca ccggcacctg gtacaaccag ctcggcgaga ccttcatcgt gaccgcgggc 60
[0186] gccgacggcg ccctgaccgg tacctacgtc acggcccgtg gcaacgccga gagccgctac 120
[0187] gtcctgaccg gtcgttacga cagcgccccg gccaccgacg gcagcggcac cgccctcggt 180
[0188] tggacggtgg cctggaagaa taactaccgc aacgcccact ccgcgaccac gtggagcggc 240
[0189] cagtacgtcg gcggcgccga ggcgaggatc aacacccagt ggctgctgac ctccggcacc 300
[0190] accgaggcca acgcctggaa gtccacgctg gtcggccacg acacgttcac caaggtgaag 360
[0191] ccgtccgccg ccaagtccta a 381
[0192] <210> 14
[0193] <211> 126
[0194] <212> PRT
[0195] <213> 人工序列(Artificial Sequence)
[0196] <400> 14
[0197] Met Gly Ile Thr Gly Thr Trp Tyr Asn Gln Leu Gly Glu Thr Phe Ile
[0198] 1 5 10 15
[0199] Val Thr Ala Gly Ala Asp Gly Ala Leu Thr Gly Thr Tyr Val Thr Ala
[0200] 20 25 30
[0201] Arg Gly Asn Ala Glu Ser Arg Tyr Val Leu Thr Gly Arg Tyr Asp Ser
[0202] 35 40 45
[0203] Ala Pro Ala Thr Asp Gly Ser Gly Thr Ala Leu Gly Trp Thr Val Ala
[0204] 50 55 60
[0205] Trp Lys Asn Asn Tyr Arg Asn Ala His Ser Ala Thr Thr Trp Ser Gly
[0206] 65 70 75 80
[0207] Gln Tyr Val Gly Gly Ala Glu Ala Arg Ile Asn Thr Gln Trp Leu Leu
[0208] 85 90 95
[0209] Thr Ser Gly Thr Thr Glu Ala Asn Ala Trp Lys Ser Thr Leu Val Gly

[0210] 100 105 110

[0211] His Asp Thr Phe Thr Lys Val Lys Pro Ser Ala Ala Lys Ser

[0212] 115 120 125

[0213] <210> 15

[0214] <211> 381

[0215] <212> DNA

[0216] <213> 人工序列(Artificial Sequence)

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[0219] gccgacggcg ccctgaccgg tacctacgtc acggcccgtg gcaacgccga gagccgctac 120

[0220] gtcctgaccg gtcgttacga cagcgccccg gccaccgacg gcagcggcac cgccctcggt 180

[0221] tggacggtgg cctggaagaa taactaccgc aacgcccact ccgcgaccac gtggagcggc 240

[0222] cagtacgtcg gcggcgccga ggcgaggatc aacacccagt ggctgctgac ctccggcacc 300

[0223] accgaggcca acgcctggaa gtccacgctg gtcggccacg acacgttcac caaggtgaag 360

[0224] ccgtccgccg ccaagtccta a 381

[0225] <210> 16

[0226] <211> 126

[0227] <212> PRT

[0228] <213> 人工序列(Artificial Sequence)

[0229] <400> 16

[0230] Met Gly Ile Thr Gly Thr Trp Tyr Asn Gln Leu Gly Phe Thr Phe Ile

[0231] 1 5 10 15

[0232] Val Thr Ala Gly Ala Asp Gly Ala Leu Thr Gly Thr Tyr Val Thr Ala

[0233] 20 25 30

[0234] Arg Gly Asn Ala Glu Ser Arg Tyr Val Leu Thr Gly Arg Tyr Asp Ser

[0235] 35 40 45

[0236] Ala Pro Ala Thr Asp Gly Ser Gly Thr Ala Leu Gly Trp Thr Val Ala

[0237] 50 55 60

[0238] Trp Lys Asn Asn Tyr Arg Asn Ala His Ser Ala Thr Thr Trp Ser Gly

[0239] 65 70 75 80

[0240] Gln Tyr Val Gly Gly Ala Glu Ala Arg Ile Asn Thr Gln Trp Leu Leu

[0241] 85 90 95

[0242] Thr Ser Gly Thr Thr Glu Ala Asn Ala Trp Lys Ser Thr Leu Val Gly

[0243] 100 105 110

[0244] His Asp Thr Phe Thr Lys Val Lys Pro Ser Ala Ala Lys Ser

[0245] 115 120 125

[0246] <210> 17

[0247] <211> 381

[0248] <212> DNA

[0249] <213> 人工序列(Artificial Sequence)

[0250] <400> 17

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[0252] gccgacggcg ccctgaccgg tacctacgtc acggcccgtg gcaacgccga gagccgctac 120
[0253] gtcctgaccg gtcgttacga cagcgccccg gccaccgacg gcagcggcac cgccctcggt 180
[0254] tggacggtgg cctggaagaa taactaccgc aacgcccact ccgcgaccac gtggagcggc 240
[0255] cagtacgtcg gcggcgccga ggcgaggatc aacacccagt ggctgctgac ctccggcacc 300
[0256] accgaggcca acgcctggaa gtccacgctg gtcggccacg acacgttcac caaggtgaag 360
[0257] ccgtccgccg ccaagtccta a 381
[0258] <210> 18
[0259] <211> 126
[0260] <212> PRT
[0261] <213> 人工序列(Artificial Sequence)
[0262] <400> 18
[0263] Met Gly Ile Thr Gly Thr Trp Tyr Asn Gln Leu Gly His Thr Phe Ile
[0264] 1 5 10 15
[0265] Val Thr Ala Gly Ala Asp Gly Ala Leu Thr Gly Thr Tyr Val Thr Ala
[0266] 20 25 30
[0267] Arg Gly Asn Ala Glu Ser Arg Tyr Val Leu Thr Gly Arg Tyr Asp Ser
[0268] 35 40 45
[0269] Ala Pro Ala Thr Asp Gly Ser Gly Thr Ala Leu Gly Trp Thr Val Ala
[0270] 50 55 60
[0271] Trp Lys Asn Asn Tyr Arg Asn Ala His Ser Ala Thr Thr Trp Ser Gly
[0272] 65 70 75 80
[0273] Gln Tyr Val Gly Gly Ala Glu Ala Arg Ile Asn Thr Gln Trp Leu Leu
[0274] 85 90 95
[0275] Thr Ser Gly Thr Thr Glu Ala Asn Ala Trp Lys Ser Thr Leu Val Gly
[0276] 100 105 110
[0277] His Asp Thr Phe Thr Lys Val Lys Pro Ser Ala Ala Lys Ser
[0278] 115 120 125
[0279] <210> 19
[0280] <211> 381
[0281] <212> DNA
[0282] <213> 人工序列(Artificial Sequence)
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[0285] gccgacggcg ccctgaccgg tacctacgtc acggcccgtg gcaacgccga gagccgctac 120
[0286] gtcctgaccg gtcgttacga cagcgccccg gccaccgacg gcagcggcac cgccctcggt 180
[0287] tggacggtgg cctggaagaa taactaccgc aacgcccact ccgcgaccac gtggagcggc 240
[0288] cagtacgtcg gcggcgccga ggcgaggatc aacacccagt ggctgctgac ctccggcacc 300
[0289] accgaggcca acgcctggaa gtccacgctg gtcggccacg acacgttcac caaggtgaag 360
[0290] ccgtccgccg ccaagtccta a 381
[0291] <210> 20
[0292] <211> 126
[0293] <212> PRT

[0294] <213> 人工序列(Artificial Sequence)

[0295] <400> 20

[0296] Met Gly Ile Thr Gly Thr Trp Tyr Asn Gln Leu Gly Ile Thr Phe Ile

[0297] 1 5 10 15

[0298] Val Thr Ala Gly Ala Asp Gly Ala Leu Thr Gly Thr Tyr Val Thr Ala

[0299] 20 25 30

[0300] Arg Gly Asn Ala Glu Ser Arg Tyr Val Leu Thr Gly Arg Tyr Asp Ser

[0301] 35 40 45

[0302] Ala Pro Ala Thr Asp Gly Ser Gly Thr Ala Leu Gly Trp Thr Val Ala

[0303] 50 55 60

[0304] Trp Lys Asn Asn Tyr Arg Asn Ala His Ser Ala Thr Thr Trp Ser Gly

[0305] 65 70 75 80

[0306] Gln Tyr Val Gly Gly Ala Glu Ala Arg Ile Asn Thr Gln Trp Leu Leu

[0307] 85 90 95

[0308] Thr Ser Gly Thr Thr Glu Ala Asn Ala Trp Lys Ser Thr Leu Val Gly

[0309] 100 105 110

[0310] His Asp Thr Phe Thr Lys Val Lys Pro Ser Ala Ala Lys Ser

[0311] 115 120 125

[0312] <210> 21

[0313] <211> 381

[0314] <212> DNA

[0315] <213> 人工序列(Artificial Sequence)

[0316] <400> 21

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[0318] gccgacggcg ccctgaccgg tacctacgtc acggcccgtg gcaacgccga gagccgctac 120

[0319] gtcctgaccg gtcgttacga cagcgccccg gccaccgacg gcagcggcac cgccctcggt 180

[0320] tggacggtgg cctggaagaa taactaccgc aacgcccact ccgcgaccac gtggagcggc 240

[0321] cagtacgtcg gcggcgccga ggcgaggatc aacacccagt ggctgctgac ctccggcacc 300

[0322] accgaggcca acgcctggaa gtccacgctg gtcggccacg acacgttcac caaggtgaag 360

[0323] ccgtccgccg ccaagtccta a 381

[0324] <210> 22

[0325] <211> 126

[0326] <212> PRT

[0327] <213> 人工序列(Artificial Sequence)

[0328] <400> 22

[0329] Met Gly Ile Thr Gly Thr Trp Tyr Asn Gln Leu Gly Lys Thr Phe Ile

[0330] 1 5 10 15

[0331] Val Thr Ala Gly Ala Asp Gly Ala Leu Thr Gly Thr Tyr Val Thr Ala

[0332] 20 25 30

[0333] Arg Gly Asn Ala Glu Ser Arg Tyr Val Leu Thr Gly Arg Tyr Asp Ser

[0334] 35 40 45

[0335] Ala Pro Ala Thr Asp Gly Ser Gly Thr Ala Leu Gly Trp Thr Val Ala

[0336] 50 55 60

[0337] Trp Lys Asn Asn Tyr Arg Asn Ala His Ser Ala Thr Thr Trp Ser Gly

[0338] 65 70 75 80

[0339] Gln Tyr Val Gly Gly Ala Glu Ala Arg Ile Asn Thr Gln Trp Leu Leu

[0340] 85 90 95

[0341] Thr Ser Gly Thr Thr Glu Ala Asn Ala Trp Lys Ser Thr Leu Val Gly

[0342] 100 105 110

[0343] His Asp Thr Phe Thr Lys Val Lys Pro Ser Ala Ala Lys Ser

[0344] 115 120 125

[0345] <210> 23

[0346] <211> 381

[0347] <212> DNA

[0348] <213> 人工序列(Artificial Sequence)

[0349] <400> 23

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[0351] gccgacggcg ccctgaccgg tacctacgtc acggcccgtg gcaacgccga gagccgctac 120

[0352] gtcctgaccg gtcgttacga cagcgccccg gccaccgacg gcagcggcac cgccctcggt 180

[0353] tggacggtgg cctggaagaa taactaccgc aacgcccact ccgcgaccac gtggagcggc 240

[0354] cagtacgtcg gcggcgccga ggcgaggatc aacacccagt ggctgctgac ctccggcacc 300

[0355] accgaggcca acgcctggaa gtccacgctg gtcggccacg acacgttcac caaggtgaag 360

[0356] ccgtccgccg ccaagtccta a 381

[0357] <210> 24

[0358] <211> 126

[0359] <212> PRT

[0360] <213> 人工序列(Artificial Sequence)

[0361] <400> 24

[0362] Met Gly Ile Thr Gly Thr Trp Tyr Asn Gln Leu Gly Leu Thr Phe Ile

[0363] 1 5 10 15

[0364] Val Thr Ala Gly Ala Asp Gly Ala Leu Thr Gly Thr Tyr Val Thr Ala

[0365] 20 25 30

[0366] Arg Gly Asn Ala Glu Ser Arg Tyr Val Leu Thr Gly Arg Tyr Asp Ser

[0367] 35 40 45

[0368] Ala Pro Ala Thr Asp Gly Ser Gly Thr Ala Leu Gly Trp Thr Val Ala

[0369] 50 55 60

[0370] Trp Lys Asn Asn Tyr Arg Asn Ala His Ser Ala Thr Thr Trp Ser Gly

[0371] 65 70 75 80

[0372] Gln Tyr Val Gly Gly Ala Glu Ala Arg Ile Asn Thr Gln Trp Leu Leu

[0373] 85 90 95

[0374] Thr Ser Gly Thr Thr Glu Ala Asn Ala Trp Lys Ser Thr Leu Val Gly

[0375] 100 105 110

[0376] His Asp Thr Phe Thr Lys Val Lys Pro Ser Ala Ala Lys Ser

[0377] 115 120 125

[0378] <210> 25
[0379] <211> 381
[0380] <212> DNA
[0381] <213> 人工序列(Artificial Sequence)
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[0384] gccgacggcg ccctgaccgg tacctacgtc acggcccgtg gcaacgccga gagccgctac 120
[0385] gtcctgaccg gtcgttacga cagcgccccg gccaccgacg gcagcggcac cgccctcggt 180
[0386] tggacggtgg cctggaagaa taactaccgc aacgcccact ccgcgaccac gtggagcggc 240
[0387] cagtacgtcg gcggcgccga ggcgaggatc aacacccagt ggctgctgac ctccggcacc 300
[0388] accgaggcca acgcctggaa gtccacgctg gtcggccacg acacgttcac caaggtgaag 360
[0389] ccgtccgccg ccaagtccta a 381
[0390] <210> 26
[0391] <211> 126
[0392] <212> PRT
[0393] <213> 人工序列(Artificial Sequence)
[0394] <400> 26
[0395] Met Gly Ile Thr Gly Thr Trp Tyr Asn Gln Leu Gly Met Thr Phe Ile
[0396] 1 5 10 15
[0397] Val Thr Ala Gly Ala Asp Gly Ala Leu Thr Gly Thr Tyr Val Thr Ala
[0398] 20 25 30
[0399] Arg Gly Asn Ala Glu Ser Arg Tyr Val Leu Thr Gly Arg Tyr Asp Ser
[0400] 35 40 45
[0401] Ala Pro Ala Thr Asp Gly Ser Gly Thr Ala Leu Gly Trp Thr Val Ala
[0402] 50 55 60
[0403] Trp Lys Asn Asn Tyr Arg Asn Ala His Ser Ala Thr Thr Trp Ser Gly
[0404] 65 70 75 80
[0405] Gln Tyr Val Gly Gly Ala Glu Ala Arg Ile Asn Thr Gln Trp Leu Leu
[0406] 85 90 95
[0407] Thr Ser Gly Thr Thr Glu Ala Asn Ala Trp Lys Ser Thr Leu Val Gly
[0408] 100 105 110
[0409] His Asp Thr Phe Thr Lys Val Lys Pro Ser Ala Ala Lys Ser
[0410] 115 120 125
[0411] <210> 27
[0412] <211> 381
[0413] <212> DNA
[0414] <213> 人工序列(Artificial Sequence)
[0415] <400> 27
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[0417] gccgacggcg ccctgaccgg tacctacgtc acggcccgtg gcaacgccga gagccgctac 120
[0418] gtcctgaccg gtcgttacga cagcgccccg gccaccgacg gcagcggcac cgccctcggt 180
[0419] tggacggtgg cctggaagaa taactaccgc aacgcccact ccgcgaccac gtggagcggc 240

[0420] cagtacgtcg gcggcgccga ggcgaggatc aacacccagt ggctgctgac ctccggcacc 300
[0421] accgaggcca acgcctggaa gtccacgctg gtcggccacg acacgttcac caaggtgaag 360
[0422] ccgtccgccg ccaagtccta a 381
[0423] <210> 28
[0424] <211> 126
[0425] <212> PRT
[0426] <213> 人工序列(Artificial Sequence)
[0427] <400> 28
[0428] Met Gly Ile Thr Gly Thr Trp Tyr Asn Gln Leu Gly Asn Thr Phe Ile
[0429] 1 5 10 15
[0430] Val Thr Ala Gly Ala Asp Gly Ala Leu Thr Gly Thr Tyr Val Thr Ala
[0431] 20 25 30
[0432] Arg Gly Asn Ala Glu Ser Arg Tyr Val Leu Thr Gly Arg Tyr Asp Ser
[0433] 35 40 45
[0434] Ala Pro Ala Thr Asp Gly Ser Gly Thr Ala Leu Gly Trp Thr Val Ala
[0435] 50 55 60
[0436] Trp Lys Asn Asn Tyr Arg Asn Ala His Ser Ala Thr Thr Trp Ser Gly
[0437] 65 70 75 80
[0438] Gln Tyr Val Gly Gly Ala Glu Ala Arg Ile Asn Thr Gln Trp Leu Leu
[0439] 85 90 95
[0440] Thr Ser Gly Thr Thr Glu Ala Asn Ala Trp Lys Ser Thr Leu Val Gly
[0441] 100 105 110
[0442] His Asp Thr Phe Thr Lys Val Lys Pro Ser Ala Ala Lys Ser
[0443] 115 120 125
[0444] <210> 29
[0445] <211> 381
[0446] <212> DNA
[0447] <213> 人工序列(Artificial Sequence)
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[0449] atgggcatca ccggcacctg gtacaaccag ctcggccaga ccttcatcgt gaccgcgggc 60
[0450] gccgacggcg ccctgaccgg tacctacgtc acggcccgtg gcaacgccga gagccgctac 120
[0451] gtcctgaccg gtcgttacga cagcgccccg gccaccgacg gcagcggcac cgccctcggt 180
[0452] tggacggtgg cctggaagaa taactaccgc aacgcccact ccgcgaccac gtggagcggc 240
[0453] cagtacgtcg gcggcgccga ggcgaggatc aacacccagt ggctgctgac ctccggcacc 300
[0454] accgaggcca acgcctggaa gtccacgctg gtcggccacg acacgttcac caaggtgaag 360
[0455] ccgtccgccg ccaagtccta a 381
[0456] <210> 30
[0457] <211> 126
[0458] <212> PRT
[0459] <213> 人工序列(Artificial Sequence)
[0460] <400> 30
[0461] Met Gly Ile Thr Gly Thr Trp Tyr Asn Gln Leu Gly Gln Thr Phe Ile

[0462] 1 5 10 15
[0463] Val Thr Ala Gly Ala Asp Gly Ala Leu Thr Gly Thr Tyr Val Thr Ala
[0464] 20 25 30
[0465] Arg Gly Asn Ala Glu Ser Arg Tyr Val Leu Thr Gly Arg Tyr Asp Ser
[0466] 35 40 45
[0467] Ala Pro Ala Thr Asp Gly Ser Gly Thr Ala Leu Gly Trp Thr Val Ala
[0468] 50 55 60
[0469] Trp Lys Asn Asn Tyr Arg Asn Ala His Ser Ala Thr Thr Trp Ser Gly
[0470] 65 70 75 80
[0471] Gln Tyr Val Gly Gly Ala Glu Ala Arg Ile Asn Thr Gln Trp Leu Leu
[0472] 85 90 95
[0473] Thr Ser Gly Thr Thr Glu Ala Asn Ala Trp Lys Ser Thr Leu Val Gly
[0474] 100 105 110
[0475] His Asp Thr Phe Thr Lys Val Lys Pro Ser Ala Ala Lys Ser
[0476] 115 120 125
[0477] <210> 31
[0478] <211> 381
[0479] <212> DNA
[0480] <213> 人工序列(Artificial Sequence)
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[0482] atgggcatca ccggcacctg gtacaaccag ctcggccgta ccttcatcgt gaccgcgggc 60
[0483] gccgacggcg ccctgaccgg tacctacgtc acggcccgtg gcaacgccga gagccgctac 120
[0484] gtcctgaccg gtcgttacga cagcgccccg gccaccgacg gcagcggcac cgccctcggt 180
[0485] tggacggtgg cctggaagaa taactaccgc aacgcccact ccgcgaccac gtggagcggc 240
[0486] cagtacgtcg gcggcgccga ggcgaggatc aacacccagt ggctgctgac ctccggcacc 300
[0487] accgaggcca acgcctggaa gtccacgctg gtcggccacg acacgttcac caaggtgaag 360
[0488] ccgtccgccg ccaagtccta a 381
[0489] <210> 32
[0490] <211> 126
[0491] <212> PRT
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[0495] 1 5 10 15
[0496] Val Thr Ala Gly Ala Asp Gly Ala Leu Thr Gly Thr Tyr Val Thr Ala
[0497] 20 25 30
[0498] Arg Gly Asn Ala Glu Ser Arg Tyr Val Leu Thr Gly Arg Tyr Asp Ser
[0499] 35 40 45
[0500] Ala Pro Ala Thr Asp Gly Ser Gly Thr Ala Leu Gly Trp Thr Val Ala
[0501] 50 55 60
[0502] Trp Lys Asn Asn Tyr Arg Asn Ala His Ser Ala Thr Thr Trp Ser Gly
[0503] 65 70 75 80

[0504] Gln Tyr Val Gly Gly Ala Glu Ala Arg Ile Asn Thr Gln Trp Leu Leu
[0505] 85 90 95
[0506] Thr Ser Gly Thr Thr Glu Ala Asn Ala Trp Lys Ser Thr Leu Val Gly
[0507] 100 105 110
[0508] His Asp Thr Phe Thr Lys Val Lys Pro Ser Ala Ala Lys Ser
[0509] 115 120 125
[0510] <210> 33
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[0517] gtcctgaccg gtcgttacga cagcgccccg gccaccgacg gcagcggcac cgccctcggt 180
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[0519] cagtacgtcg gcggcgccga ggcgaggatc aacacccagt ggctgctgac ctccggcacc 300
[0520] accgaggcca acgcctggaa gtccacgctg gtcggccacg acacgttcac caaggtgaag 360
[0521] ccgtccgccg ccaagtccta a 381
[0522] <210> 34
[0523] <211> 126
[0524] <212> PRT
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[0528] 1 5 10 15
[0529] Val Thr Ala Gly Ala Asp Gly Ala Leu Thr Gly Thr Tyr Val Thr Ala
[0530] 20 25 30
[0531] Arg Gly Asn Ala Glu Ser Arg Tyr Val Leu Thr Gly Arg Tyr Asp Ser
[0532] 35 40 45
[0533] Ala Pro Ala Thr Asp Gly Ser Gly Thr Ala Leu Gly Trp Thr Val Ala
[0534] 50 55 60
[0535] Trp Lys Asn Asn Tyr Arg Asn Ala His Ser Ala Thr Thr Trp Ser Gly
[0536] 65 70 75 80
[0537] Gln Tyr Val Gly Gly Ala Glu Ala Arg Ile Asn Thr Gln Trp Leu Leu
[0538] 85 90 95
[0539] Thr Ser Gly Thr Thr Glu Ala Asn Ala Trp Lys Ser Thr Leu Val Gly
[0540] 100 105 110
[0541] His Asp Thr Phe Thr Lys Val Lys Pro Ser Ala Ala Lys Ser
[0542] 115 120 125
[0543] <210> 35
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[0546] <213> 人工序列(Artificial Sequence)

[0547] <400> 35

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[0550] gtcctgaccg gtcgttacga cagcgccccg gccaccgacg gcagcggcac cgccctcggt 180

[0551] tggacggtgg cctggaagaa taactaccgc aacgcccact ccgcgaccac gtggagcggc 240

[0552] cagtacgtcg gcggcgccga ggcgaggatc aacacccagt ggctgctgac ctccggcacc 300

[0553] accgaggcca acgcctggaa gtccacgctg gtcggccacg acacgttcac caaggtgaag 360

[0554] ccgtccgccg ccaagtccta a 381

[0555] <210> 36

[0556] <211> 126

[0557] <212> PRT

[0558] <213> 人工序列(Artificial Sequence)

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[0563] 20 25 30

[0564] Arg Gly Asn Ala Glu Ser Arg Tyr Val Leu Thr Gly Arg Tyr Asp Ser

[0565] 35 40 45

[0566] Ala Pro Ala Thr Asp Gly Ser Gly Thr Ala Leu Gly Trp Thr Val Ala

[0567] 50 55 60

[0568] Trp Lys Asn Asn Tyr Arg Asn Ala His Ser Ala Thr Thr Trp Ser Gly

[0569] 65 70 75 80

[0570] Gln Tyr Val Gly Gly Ala Glu Ala Arg Ile Asn Thr Gln Trp Leu Leu

[0571] 85 90 95

[0572] Thr Ser Gly Thr Thr Glu Ala Asn Ala Trp Lys Ser Thr Leu Val Gly

[0573] 100 105 110

[0574] His Asp Thr Phe Thr Lys Val Lys Pro Ser Ala Ala Lys Ser

[0575] 115 120 125

[0576] <210> 37

[0577] <211> 381

[0578] <212> DNA

[0579] <213> 人工序列(Artificial Sequence)

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[0583] gtcctgaccg gtcgttacga cagcgccccg gccaccgacg gcagcggcac cgccctcggt 180

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[0585] cagtacgtcg gcggcgccga ggcgaggatc aacacccagt ggctgctgac ctccggcacc 300

[0586] accgaggcca acgcctggaa gtccacgctg gtcggccacg acacgttcac caaggtgaag 360

[0587] ccgtccgccg ccaagtccta a 381

[0588] <210> 38
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[0590] <212> PRT
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[0594] 1 5 10 15
[0595] Val Thr Ala Gly Ala Asp Gly Ala Leu Thr Gly Thr Tyr Val Thr Ala
[0596] 20 25 30
[0597] Arg Gly Asn Ala Glu Ser Arg Tyr Val Leu Thr Gly Arg Tyr Asp Ser
[0598] 35 40 45
[0599] Ala Pro Ala Thr Asp Gly Ser Gly Thr Ala Leu Gly Trp Thr Val Ala
[0600] 50 55 60
[0601] Trp Lys Asn Asn Tyr Arg Asn Ala His Ser Ala Thr Thr Trp Ser Gly
[0602] 65 70 75 80
[0603] Gln Tyr Val Gly Gly Ala Glu Ala Arg Ile Asn Thr Gln Trp Leu Leu
[0604] 85 90 95
[0605] Thr Ser Gly Thr Thr Glu Ala Asn Ala Trp Lys Ser Thr Leu Val Gly
[0606] 100 105 110
[0607] His Asp Thr Phe Thr Lys Val Lys Pro Ser Ala Ala Lys Ser
[0608] 115 120 125
[0609] <210> 39
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[0611] <212> DNA
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[0615] gccgacggcg ccctgaccgg tacctacgtc acggcccgtg gcaacgccga gagccgctac 120
[0616] gtcctgaccg gtcgttacga cagcgccccg gccaccgacg gcagcggcac cgccctcggt 180
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[0618] cagtacgtcg gcggcgccga ggcgaggatc aacacccagt ggctgctgac ctccggcacc 300
[0619] accgaggcca acgcctggaa gtccacgctg gtcggccacg acacgttcac caaggtgaag 360
[0620] ccgtccgccg ccaagtccta a 381
[0621] <210> 40
[0622] <211> 126
[0623] <212> PRT
[0624] <213> 人工序列(Artificial Sequence)
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[0626] Met Gly Ile Thr Gly Thr Trp Tyr Asn Gln Leu Gly Trp Thr Phe Ile
[0627] 1 5 10 15
[0628] Val Thr Ala Gly Ala Asp Gly Ala Leu Thr Gly Thr Tyr Val Thr Ala
[0629] 20 25 30

[0630] Arg Gly Asn Ala Glu Ser Arg Tyr Val Leu Thr Gly Arg Tyr Asp Ser
[0631] 35 40 45
[0632] Ala Pro Ala Thr Asp Gly Ser Gly Thr Ala Leu Gly Trp Thr Val Ala
[0633] 50 55 60
[0634] Trp Lys Asn Asn Tyr Arg Asn Ala His Ser Ala Thr Thr Trp Ser Gly
[0635] 65 70 75 80
[0636] Gln Tyr Val Gly Gly Ala Glu Ala Arg Ile Asn Thr Gln Trp Leu Leu
[0637] 85 90 95
[0638] Thr Ser Gly Thr Thr Glu Ala Asn Ala Trp Lys Ser Thr Leu Val Gly
[0639] 100 105 110
[0640] His Asp Thr Phe Thr Lys Val Lys Pro Ser Ala Ala Lys Ser
[0641] 115 120 125
[0642] <210> 41
[0643] <211> 264
[0644] <212> PRT
[0645] <213> 人工序列(Artificial Sequence)
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[0648] 1 5 10 15
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[0650] 20 25 30
[0651] Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val
[0652] 35 40 45
[0653] Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr
[0654] 50 55 60
[0655] Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro
[0656] 65 70 75 80
[0657] Val Pro Trp Pro Thr Leu Val Thr Thr Leu Thr Tyr Gly Val Gln Cys
[0658] 85 90 95
[0659] Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser
[0660] 100 105 110
[0661] Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp
[0662] 115 120 125
[0663] Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr
[0664] 130 135 140
[0665] Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly
[0666] 145 150 155 160
[0667] Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val
[0668] 165 170 175
[0669] Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn Phe Lys
[0670] 180 185 190
[0671] Ile Arg His Asn Ile Glu Asp Gly Gly Val Gln Leu Ala Asp His Tyr

[0672] 195 200 205

[0673] Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn

[0674] 210 215 220

[0675] His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys

[0676] 225 230 235 240

[0677] Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr

[0678] 245 250 255

[0679] Leu Gly Met Asp Glu Leu Tyr Lys

[0680] 260

[0681] <210> 42

[0682] <211> 795

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[0688] ctggtcgagc tggacggcga cgtaaacggc cacaagttca gcgtgtccgg cgagggcgag 180

[0689] ggcgatgcca cctacggcaa gctgaccctg aagttcatct gcaccaccgg caagctgccc 240

[0690] gtgccctggc ccaccctcgt gaccaccctg acctacggcg tgcagtgctt cagccgctac 300

[0691] cccgaccaca tgaagcagca cgacttcttc aagtccgcca tgcccgaagg ctacgtccag 360

[0692] gagcgcacca tcttcttcaa ggacgacggc aactacaaga cccgcgccga ggtgaagttc 420

[0693] gagggcgaca ccctggtgaa ccgcatcgag ctgaagggca tcgacttcaa ggaggacggc 480

[0694] aacatcctgg ggcacaagct ggagtacaac tacaacagcc acaacgtcta tatcatggcc 540

[0695] gacaagcaga agaacggcat caaggccaac ttcaagatcc gccacaacat cgaggacggc 600

[0696] ggcgtgcagc tcgccgacca ctaccagcag aacacccccа tcggcgacgg ccccgtgctg 660

[0697] ctgcccgaca accactacct gagcacccag tccgccctga gcaaagaccc caacgagaag 720

[0698] cgcgatcaca tggtcctgct ggagttcgtg accgccgccg ggatcactct cggcatggac 780

[0699] gagctgtaca agtaa 795

[0700] <210> 43

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[0702] <212> DNA

[0703] <213> 人工序列(Artificial Sequence)

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[0706] gatactacta actcaatcaa tcctcagttt aaggttacta ataccggaag cagtgcaatt 120

[0707] gatttgtcca aactcacatt gagatattat tatacagtag acggacagaa agatcagacc 180

[0708] ttctggtgtg accatgctgc aataatcggc agtaacggca gctacaacgg aattacttca 240

[0709] aatgtaaaag gaacatttgt aaaaatgagt tcctcaacaa ataacgcaga cacctacctt 300

[0710] gaaatcagct ttacaggcgg aactcttgaa ccgggtgcac atgttcagat acaaggtaga 360

[0711] tttgcaaaga atgactggag taactataca cagtcaaatg actactcatt taagtctgct 420

[0712] tcacagtttg ttgaatggga tcaggtaaca gcatacttga acggtgttct tgtatggggt 480

[0713] aaagaacccg gtggcagtgt agtaccatca acacagcctg taacaacacc acctgcaaca 540

[0714] acaaaaccac ctgcaacaac aataccgccg acagatgatc cgaatgcaga aaatctttat 600

[0715] ttccaaggta tgaaggataa caccgtgcca ctgaaattga ttgccctgtt agcgaacggt 660

[0716] gaatttcact ctggcgagca gttgggtgaa acgctgggaa tgagccgggc ggctattaat 720

[0717] aaacacattc agacactgcg tgactggggc gttgatgtct ttaccgttcc gggtaaagga 780

[0718] tacagcctgc ctgagcccat ccagttactt aatgctgaac agatattggg tcagctggat 840

[0719] ggcggtagtg tagccgtgct gccagttatt gactccacga atcagtacct tcttgatcgt 900

[0720] atcggagagc ttaaatcggg cgatgcctgt gttgcagaat accagcaggc tggccgtggt 960

[0721] cgccgggggc ggaaatggtt ttcgcctttt ggcgcaaact tatatttgtc gatgttctgg 1020

[0722] cgtctggaac aaggcccggc ggcggcgatt ggtttaagtc tggttatcgg tatcgtgatg 1080

[0723] gcggaagtat tacgcaagct gggagcagat aaagttcgtg tcaaatggcc taatgacctc 1140

[0724] tatctgcagg atcgcaagct ggcaggcatt cttgtggagc tgactggcaa aactggcgat 1200

[0725] gcggcgcaaa tagtcattgg agccgggatc aacatggcaa tgcgccgtgt tgaagagagt 1260

[0726] gtcgttaatc aggggtggat cacgctgcag gaagcgggga tcaatctcga tcgtaatacg 1320

[0727] ttggcggcca tgctaatacg tgaattacgt gctgcgttgg aactcttcga acaagaagga 1380

[0728] ttggcacctt atctgtcgcg ctgggaaaag ctggataatt ttattaatcg cccagtgaaa 1440

[0729] cttatcattg gtgataaaga aatatttggc atttcacgcg gaatagacaa acaggggget 1500

[0730] ttattacttg agcaggatgg aataataaaa ccctggatgg gcggtgaaat atccctgcgt 1560

[0731] agtgcagaaa aataa 1575

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[0733] <211> 825

[0734] <212> DNA

[0735] <213> 人工序列(Artificial Sequence)

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[0738] cgttccaccc cgccgacccc gagcactcct cctaccggat ccgtgagcaa gggcgaggag 120

[0739] ctgttcaccg gggtggtgcc catcctggtc gagctggacg gcgacgtaaa cggccacaag 180

[0740] ttcagcgtgt ccggcgaggg cgagggcgat gccacctacg gcaagctgac cctgaagttc 240

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[0742] ggcgtgcagt gcttcagccg ctaccccgac cacatgaagc agcacgactt cttcaagtcc 360

[0743] gccatgcccg aaggctacgt ccaggagcgc accatcttct tcaaggacga cggcaactac 420

[0744] aagacccgcg ccgaggtgaa gttcgagggc gacaccctgg tgaaccgcat cgagctgaag 480

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[0746] agccacaacg tctatatcat ggccgacaag cagaagaacg gcatcaaggc caacttcaag 600

[0747] atccgccaca acatcgagga cggcggcgtg cagctcgccg accactacca gcagaacacc 660

[0748] cccatcggcg acggccccgt gctgctgccc gacaaccact acctgagcac ccagtccgcc 720

[0749] ctgagcaaag accccaacga gaagcgcgat cacatggtcc tgctggagtt cgtgaccgcc 780

[0750] gccgggatca ctctcggcat ggacgagctg tacaagctcg agtaa 825

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[0758] tcgtataatg tgtggaattg tgagcggata acaattcccc tctagaaata attttgttta 180
[0759] actttaagaa ggagatatac catgggcagc tcacatcatc atacaacacc aagcacaagc 240
[0760] ggcgggggaa gcggcatcac cggcacctgg tacaaccagc tcggctcgac cttcatcgtg 300
[0761] accgcgggcg ccgacggcgc cctgaccggt acctacgtca cggcccgtgg caacgccgag 360
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[0799] aaacgccagc aacgcggcct ttttacggtt cctggccttt tgctggcctt ttgctcacat 2640

[0800] gttctttcct gcgttatccc ctgattctgt ggataaccgt attaccgcct ttgagtgagc 2700

[0801] tgataccgct cgccgcagcc gaacgaccga gcgcagcgag tcagtgagcg aggaagcgga 2760

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[0803] ttccgacacc atcgaatggt gcaaaacctt tcgcggtatg gcatgatagc gcccggaaga 2880

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[0805] cggtgtctct tatcagaccg tttcccgcgt ggtgaaccag gccagccacg tttctgcgaa 3000

[0806] aacgcgggaa aaagtggaag cggcgatggc ggagctgaat tacattccca accgcgtggc 3060

[0807] acaacaactg gcgggcaaac agtcgttgct gattggcgtt gccacctcca gtctggccct 3120

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[0813] tctggtcgca ttgggtcacc agcaaatcgc gctgttagcg ggcccattaa gttctgtctc 3480

[0814] ggcgcgtctg cgtctggctg gctggcataa atatctcact cgcaatcaaa ttcagccgat 3540

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[0816] gaatgagggc atcgttccca ctgcgatgct ggttgccaac gatcagatgg cgctgggcgc 3660

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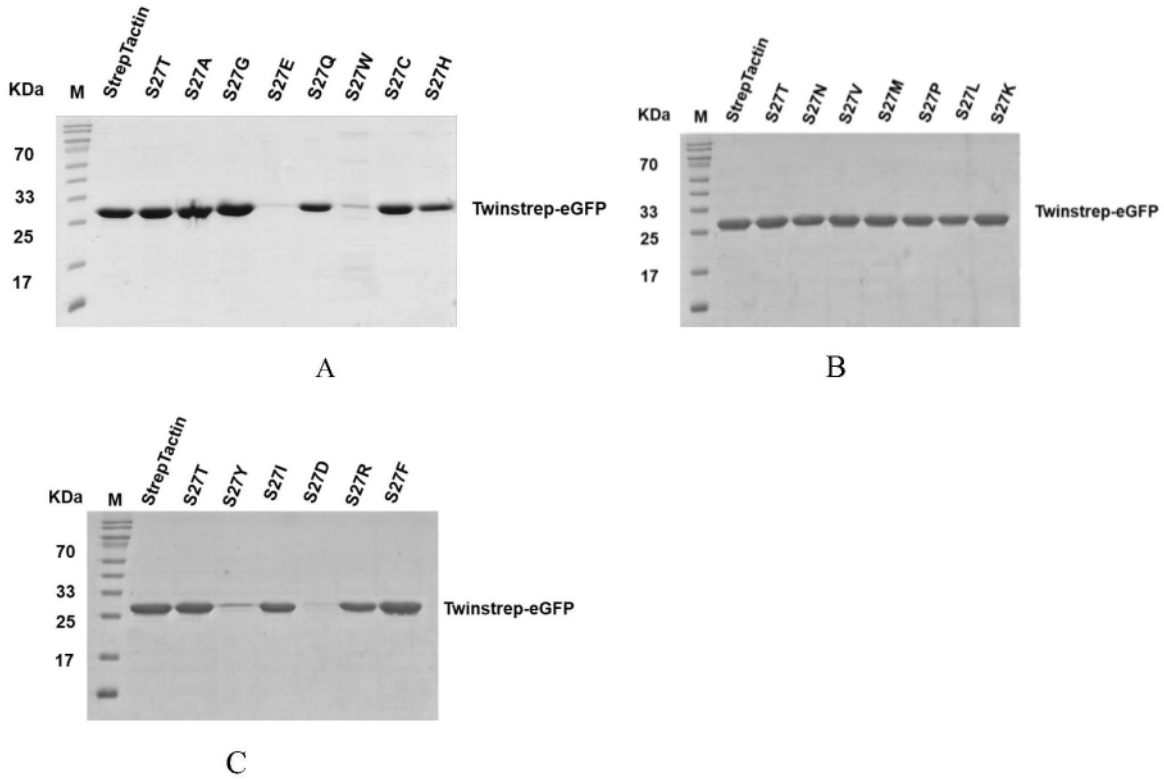
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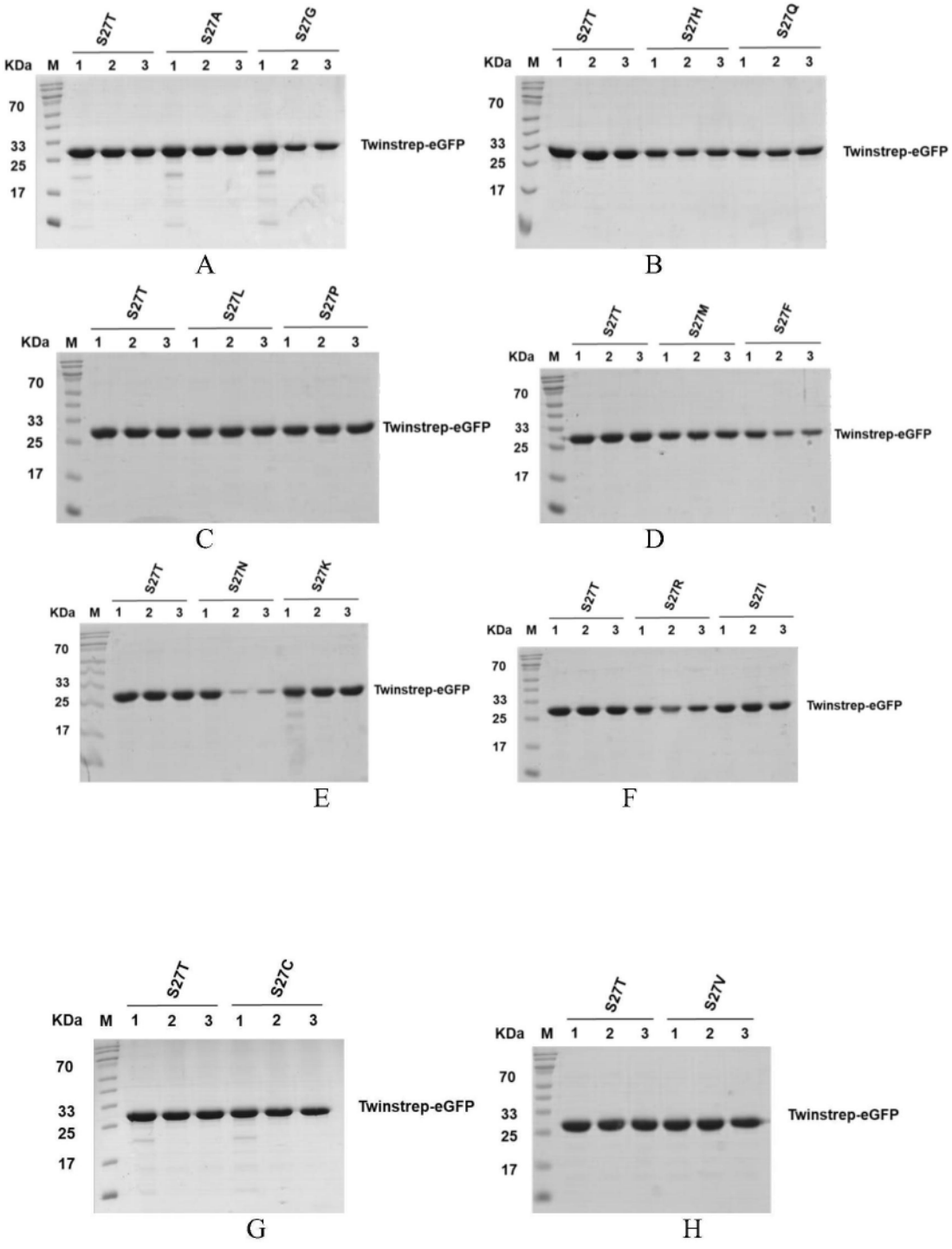
M
StrepTactin
S27T
S27A
S27G
S27E
S27Q
S27W
S27C
S27H
70
33
25
17
Twinstrep-eGFP
A
KDa
M
StrepTactin
S27T
S27N
S27V
S27M
S27P
S27L
S27K
70
33
25
17
Twinstrep-eGFP
B
KDa
M
StrepTactin
S27T
S27Y
S27I
S27D
S27R
S27F
70
33
25
17
Twinstrep-eGFP
C

图1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S27A
S27G
KDa M 1 2 3 1 2 3 1 2 3
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Twinstrep-eGFP
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S27Q
KDa M 1 2 3 1 2 3 1 2 3
70
33
25
17
Twinstrep-eGFP
B
S27T
S27L
S27P
KDa M 1 2 3 1 2 3 1 2 3
70
33
25
17
Twinstrep-eGFP
C
S27T
S27M
S27F
KDa M 1 2 3 1 2 3 1 2 3
70
33
25
17
Twinstrep-eGFP
D
S27T
S27N
S27K
KDa M 1 2 3 1 2 3 1 2 3
70
33
25
17
Twinstrep-eGFP
E
S27T
S27R
S27I
KDa M 1 2 3 1 2 3 1 2 3
70
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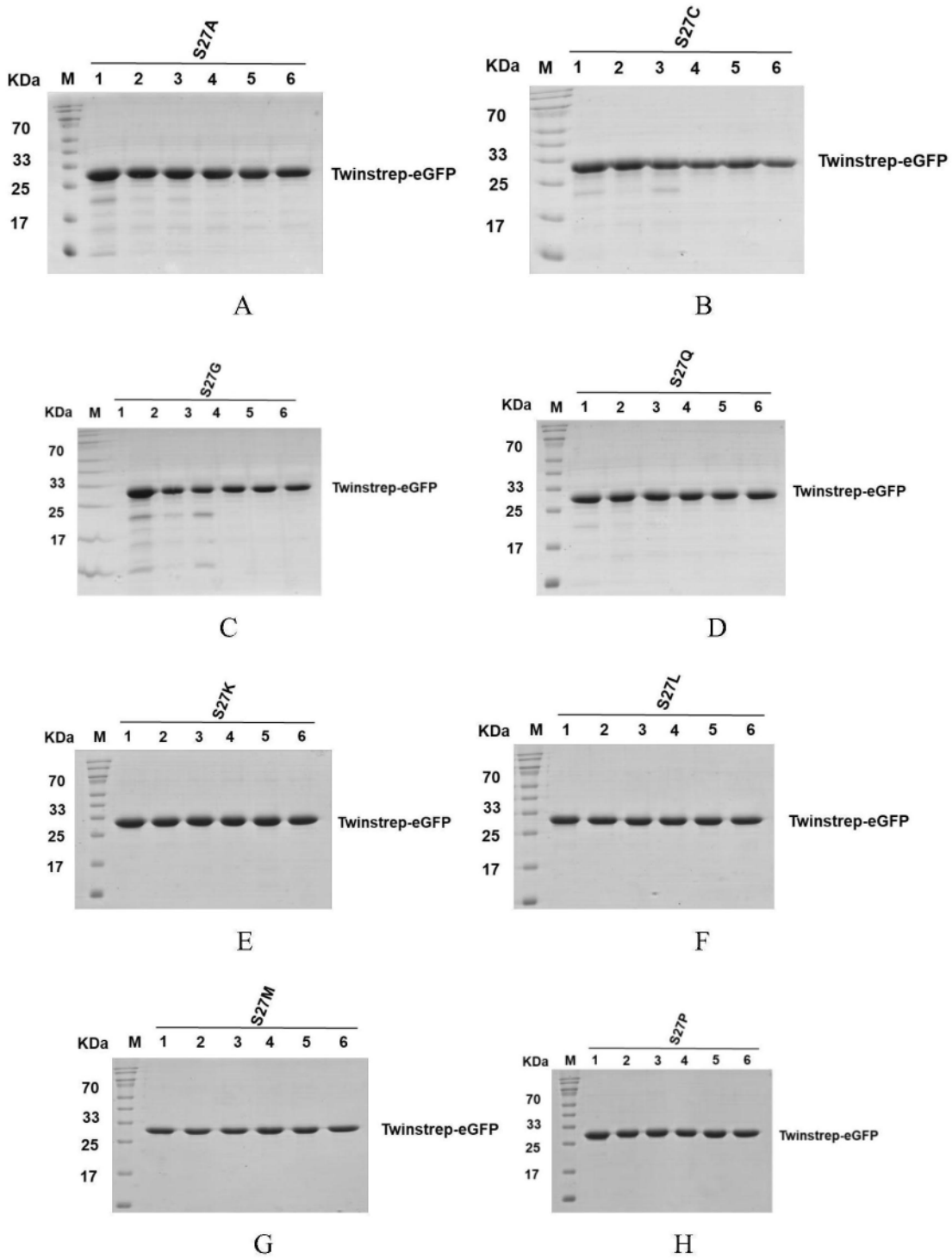
25
17
Twinstrep-eGFP
F
S27T
S27C
KDa M 1 2 3 1 2 3
70
33
25
17
Twinstrep-eGFP
G
S27T
S27V
KDa M 1 2 3 1 2 3
70
33
25
17
Twinstrep-eGFP
H

图2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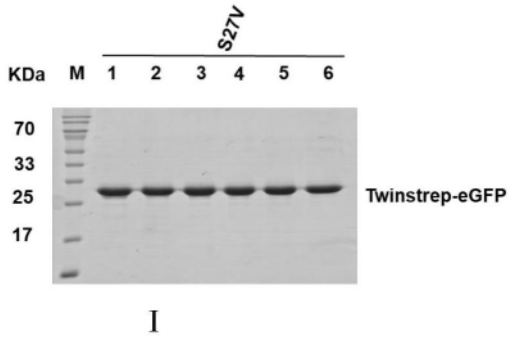
33
25
17
Twinstrep-eGFP
A
S27C
KDa M 1 2 3 4 5 6
70
33
25
17
Twinstrep-eGFP
B
S27G
KDa M 1 2 3 4 5 6
70
33
25
17
Twinstrep-eGFP
C
S27Q
KDa M 1 2 3 4 5 6
70
33
25
17
Twinstrep-eGFP
D
S27K
KDa M 1 2 3 4 5 6
70
33
25
17
Twinstrep-eGFP
E
S27L
KDa M 1 2 3 4 5 6
70
33
25
17
Twinstrep-eGFP
F
S27M
KDa M 1 2 3 4 5 6
70
33
25
17
Twinstrep-eGFP
G
S27P
KDa M 1 2 3 4 5 6
70
33
25
17
Twinstrep-eGFP
H


S27V
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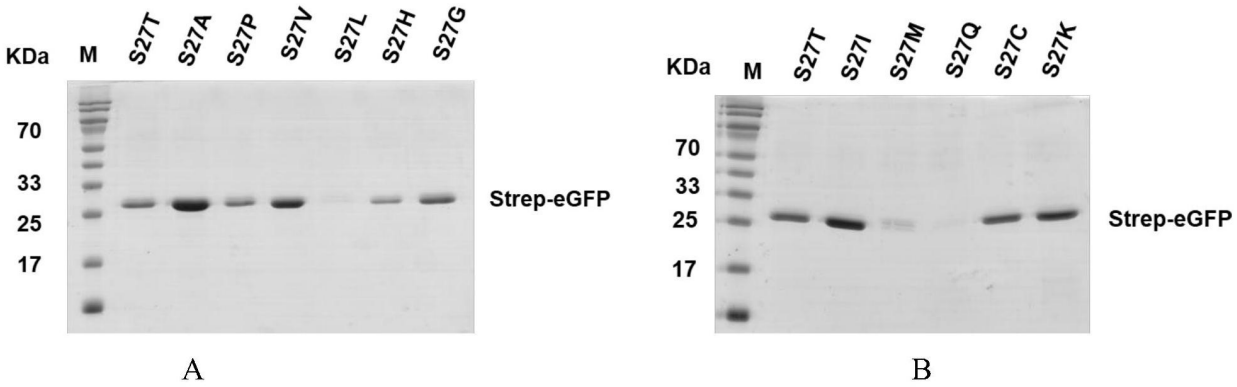
M
1
2
3
4
5
6
70
33
25
17
Twinstrep-eGFP

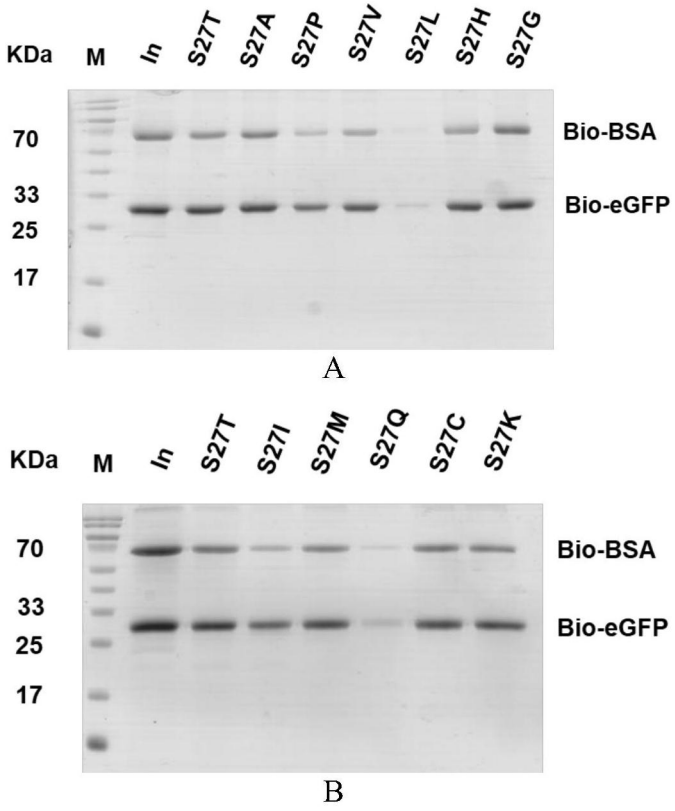
I

图3


KDa
M
S27T
S27A
S27P
S27V
S27L
S27H
S27G
70
33
25
17
Strep-eGFP
KDa
M
S27T
S27I
S27M
S27Q
S27C
S27K
70
33
25
17
Strep-eGFP

A B

图4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M
In
S27T
S27A
S27P
S27V
S27L
S27H
S27G
70
33
25
17
Bio-BSA
Bio-eGFP
A
KDa
M
In
S27T
S27I
S27M
S27Q
S27C
S27K
70
33
25
17
Bio-BSA
Bio-eGFP
B

图5