DRAWINGS

FIG. 1

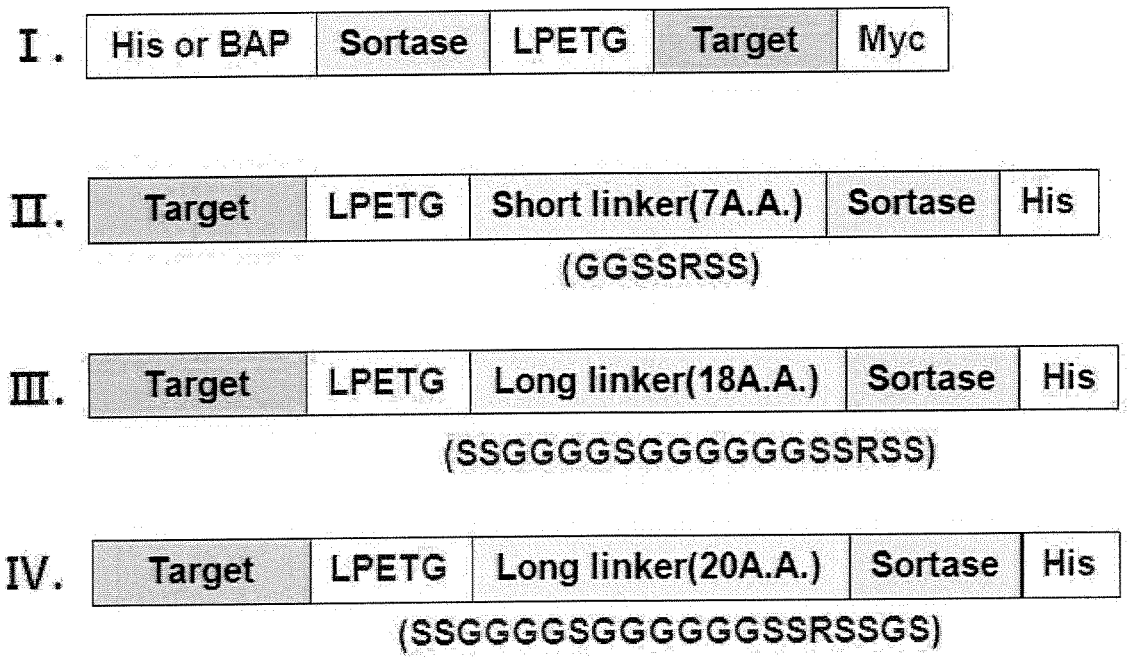


FIG. 2

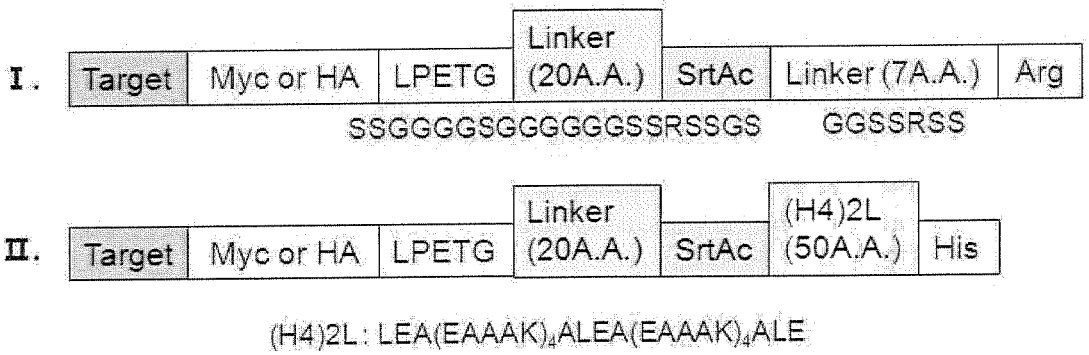


FIG. 3

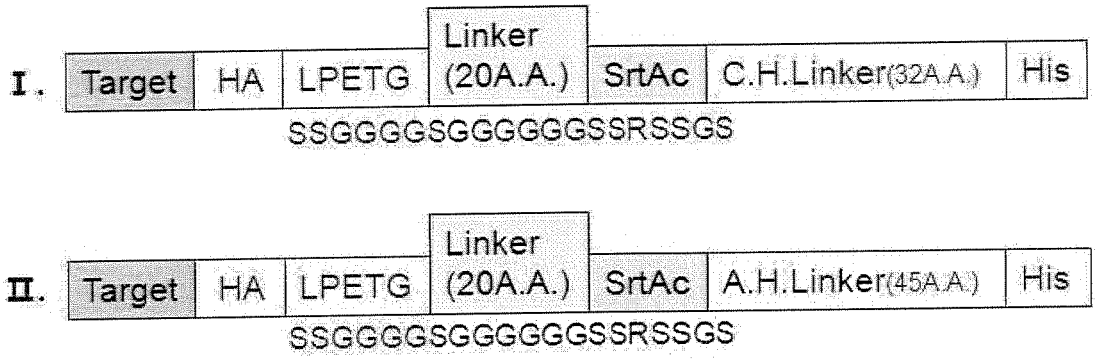

I.
Target
HA
LPETG
Linker (20A.A.)
SrtAc
C.H.Linker(32A.A.)
His
SSGGGGSGGGGGGSSRSSGS
II.
Target
HA
LPETG
Linker (20A.A.)
SrtAc
A.H.Linker(45A.A.)
His
SSGGGGSGGGGGGSSRSSGS

FIG. 4

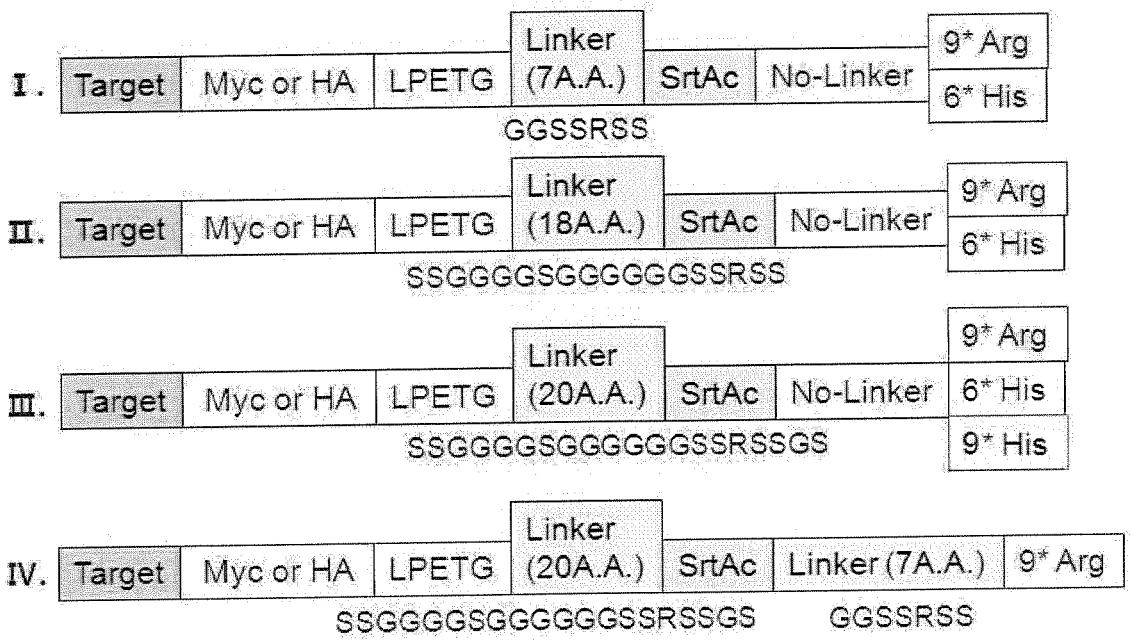

I.
Target
Myc or HA
LPETG
Linker (7A.A.)
SrtAc
No-Linker
9* Arg
6* His
GGSSRSS
II.
Target
Myc or HA
LPETG
Linker (18A.A.)
SrtAc
No-Linker
9* Arg
6* His
SSGGGGSGGGGGGSSRSS
III.
Target
Myc or HA
LPETG
Linker (20A.A.)
SrtAc
No-Linker
9* Arg
6* His
9* His
SSGGGGSGGGGGGSSRSSGS
IV.
Target
Myc or HA
LPETG
Linker (20A.A.)
SrtAc
Linker (7A.A.)
9* Arg
SSGGGGSGGGGGGSSRSSGS
GGSSRSS

FIG. 5

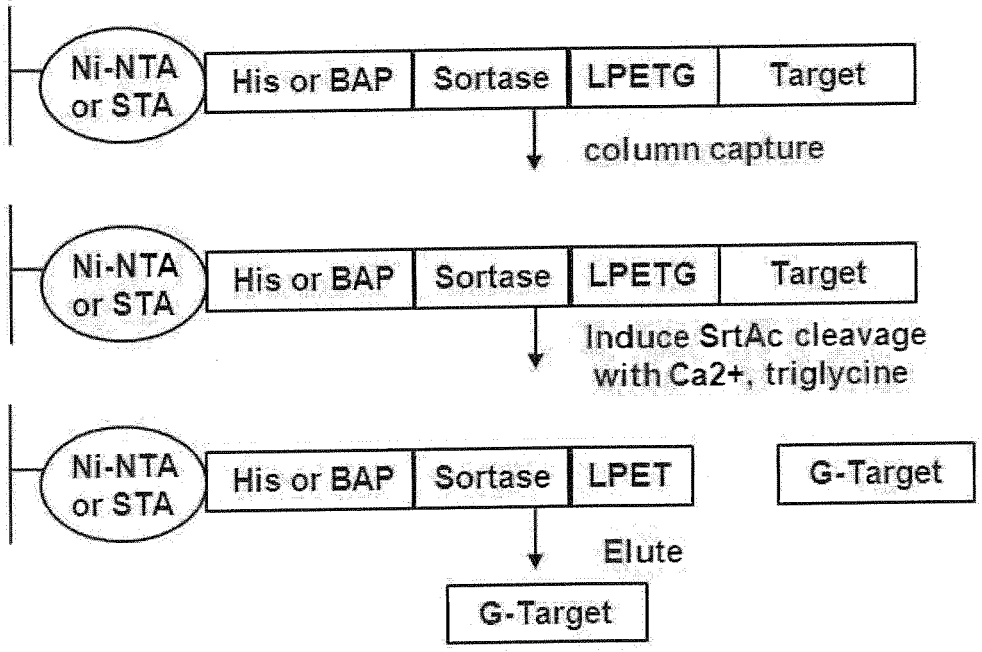

Ni-NTA or STA
His or BAP
Sortase
LPETG
Target
column capture
Ni-NTA or STA
His or BAP
Sortase
LPETG
Target
Induce SrtAc cleavage with Ca2+, triglycine
Ni-NTA or STA
His or BAP
Sortase
LPET
G-Target
Elute
G-Target

FIG. 6

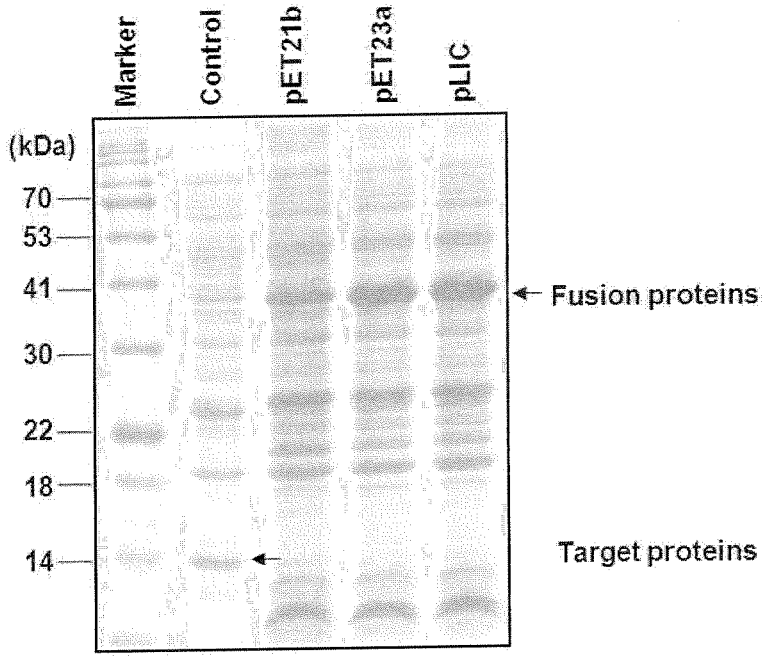

Marker
Control
pET21b
pET23a
pLIC
(kDa)
70
53
41
30
22
18
14
Fusion proteins
Target proteins

FIG. 7

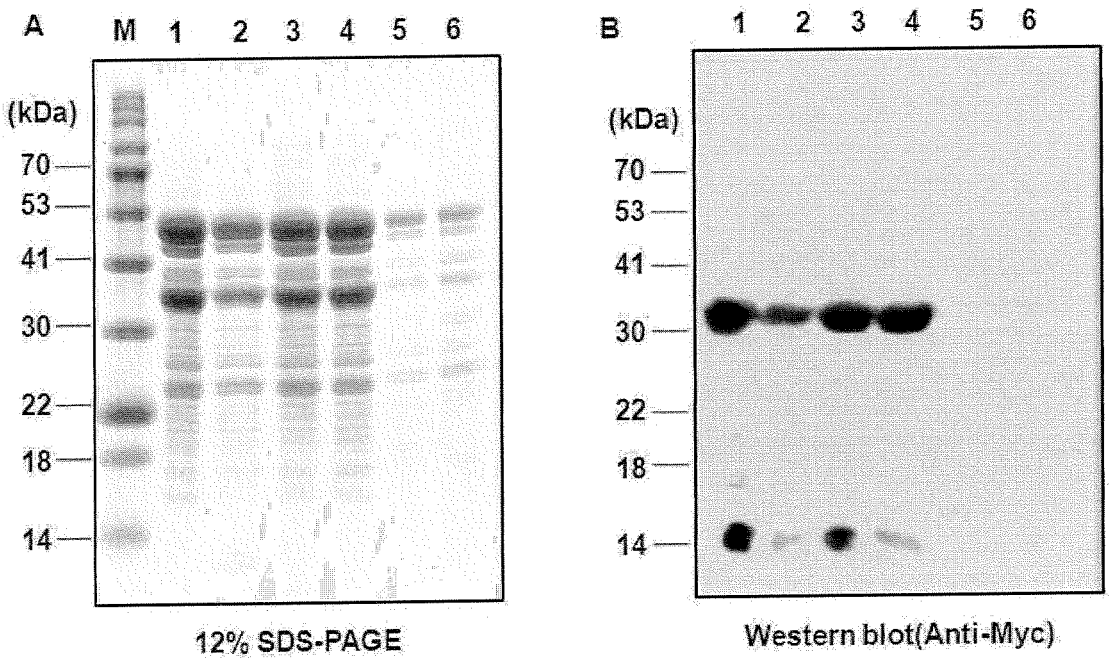

A
M 1 2 3 4 5 6
(kDa)
70
53
41
30
22
18
14
12% SDS-PAGE
B
1 2 3 4 5 6
(kDa)
70
53
41
30
22
18
14
Western blot(Anti-Myc)

FIG. 8

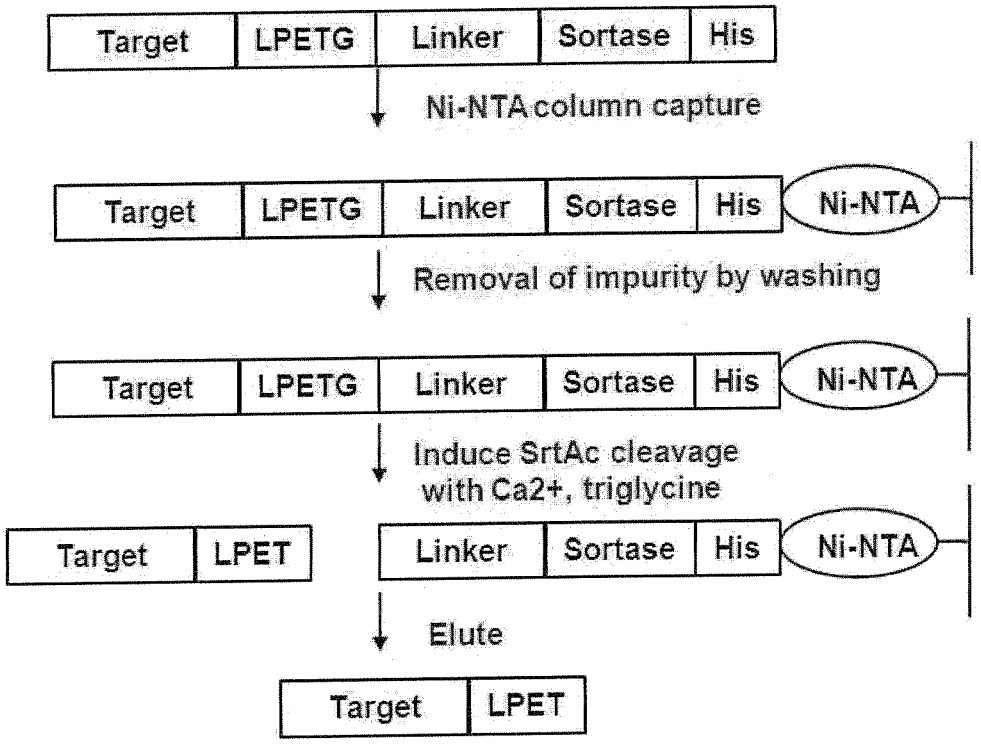

Target
LPETG
Linker
Sortase
His
Ni-NTA column capture
Ni-NTA
Removal of impurity by washing
Induce SrtAc cleavage
with Ca2+, triglycine
LPET
Elute

FIG. 9

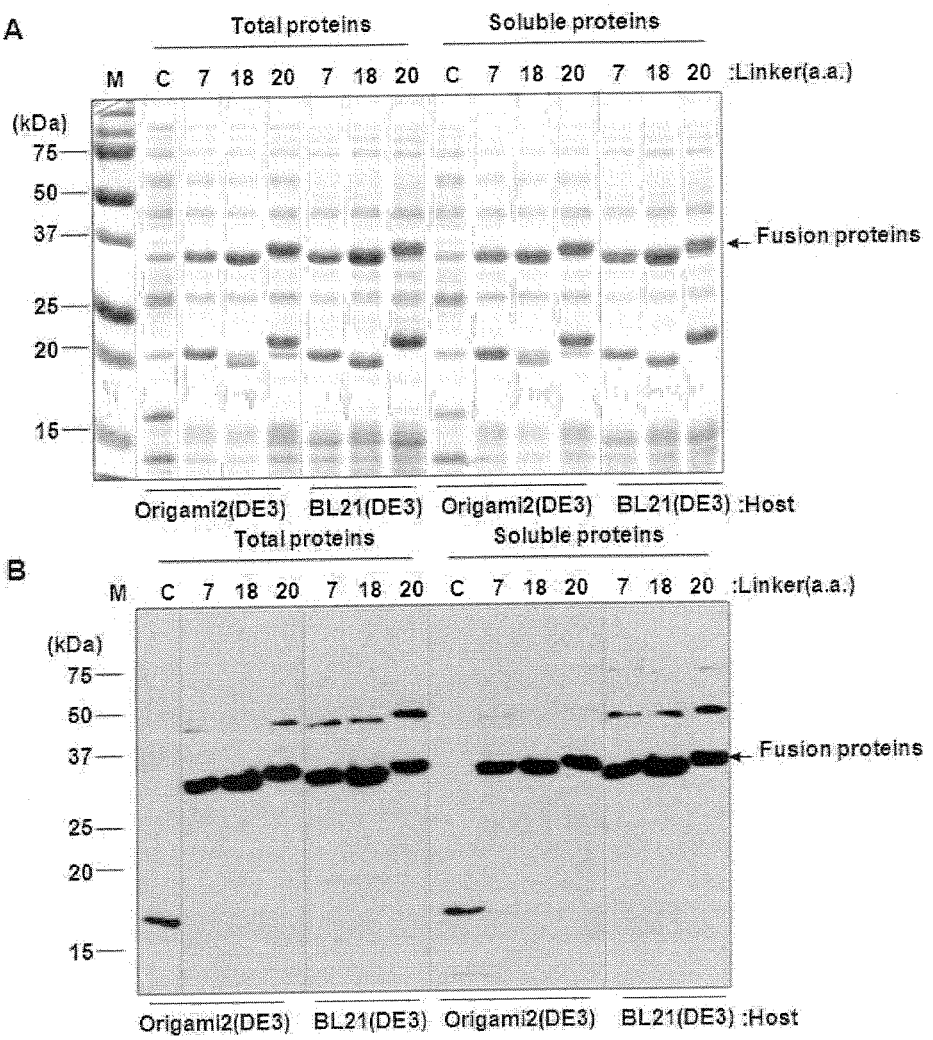

A
Total proteins
Soluble proteins
M C 7 18 20 7 18 20 C 7 18 20 7 18 20 :Linker(a.a.)
(kDa)
75
50
37
25
20
15
Fusion proteins
Origami2(DE3) BL21(DE3) Origami2(DE3) BL21(DE3) :Host
B
Total proteins
Soluble proteins
M C 7 18 20 7 18 20 C 7 18 20 7 18 20 :Linker(a.a.)
(kDa)
75
50
37
25
20
15
Fusion proteins
Origami2(DE3) BL21(DE3) Origami2(DE3) BL21(DE3) :Host

FIG. 10

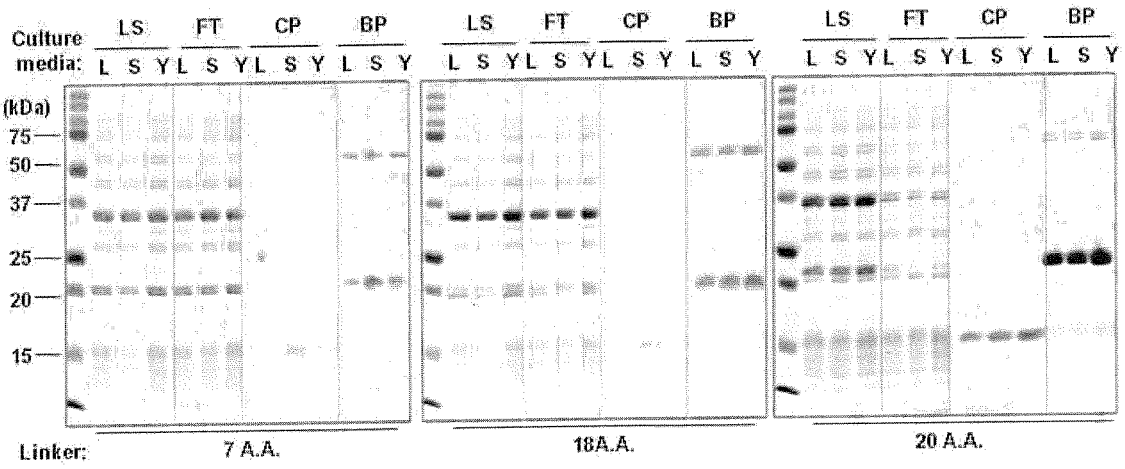

Culture media:
LS FT CP BP
L S Y L S Y L S Y L S Y
(kDa)
75
50
37
25
20
15
Linker:
7 A.A.
18A.A.
20 A.A.

FIG. 11

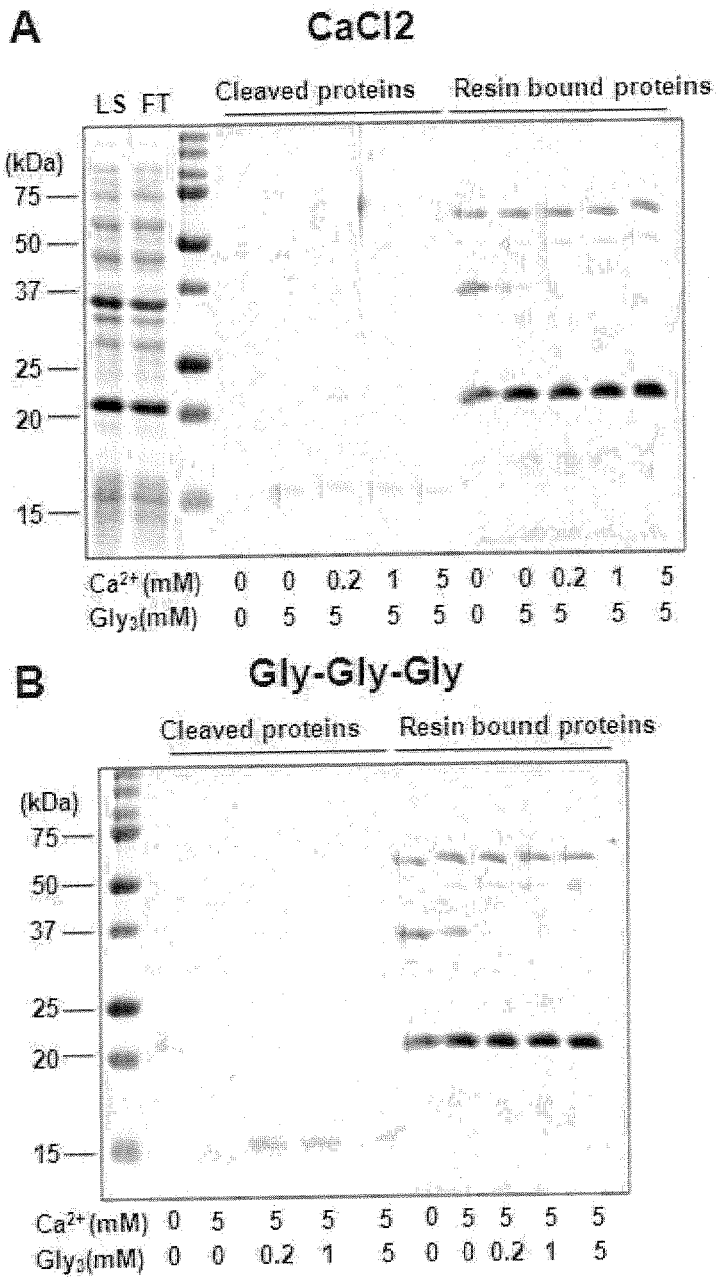

A
CaCl2
LS FT
Cleaved proteins
Resin bound proteins
(kDa)
75
50
37
25
20
15
Ca2+(mM) 0 0 0.2 1 5 0 0 0.2 1 5
Gly3(mM) 0 5 5 5 5 0 5 5 5 5
B
Gly-Gly-Gly
Cleaved proteins
Resin bound proteins
(kDa)
75
50
37
25
20
15
Ca2+(mM) 0 5 5 5 5 0 5 5 5 5
Gly3(mM) 0 0 0.2 1 5 0 0 0.2 1 5

FIG. 12

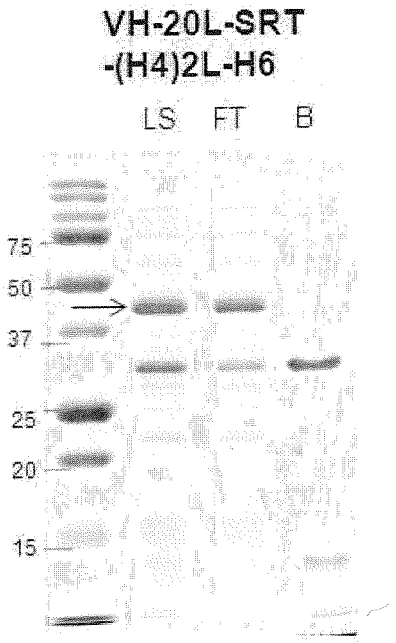

VH-20L-SRT
-(H4)2L-H6
LS
FT
B
75
50
37
25
20
15

FIG. 13

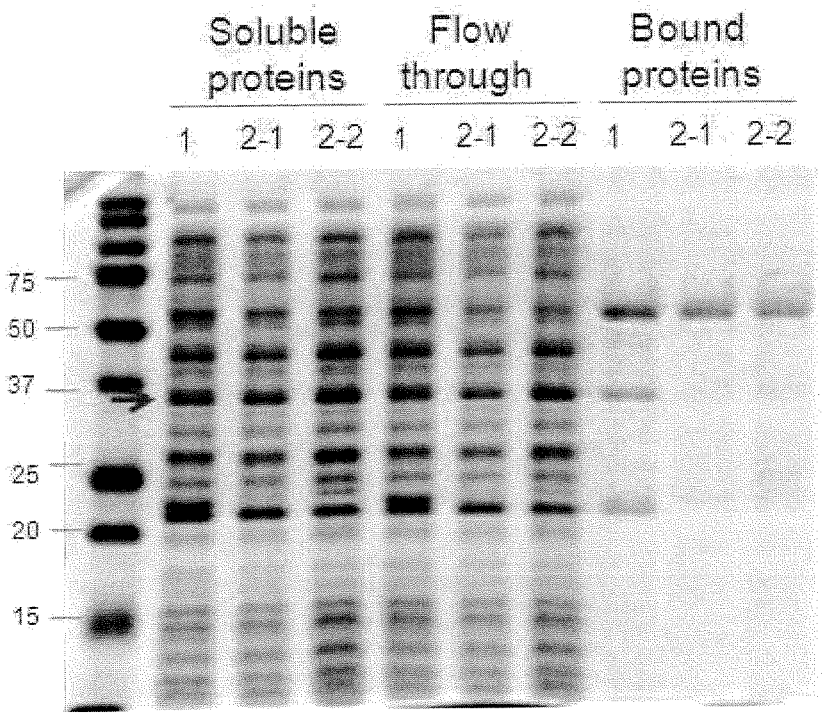

Soluble proteins
Flow through
Bound proteins
1
2-1
2-2
1
2-1
2-2
1
2-1
2-2
75
50
37
25
20
15

FIG. 14

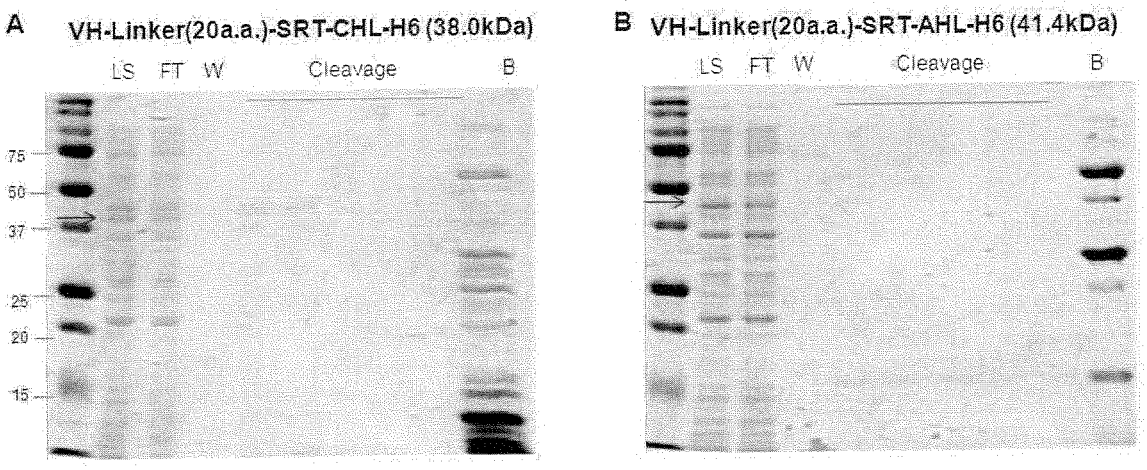

A
VH-Linker(20a.a.)-SRT-CHL-H6 (38.0kDa)
LS FT W Cleavage B
75
50
37
25
20
15
B
VH-Linker(20a.a.)-SRT-AHL-H6 (41.4kDa)
LS FT W Cleavage B

FIG. 15

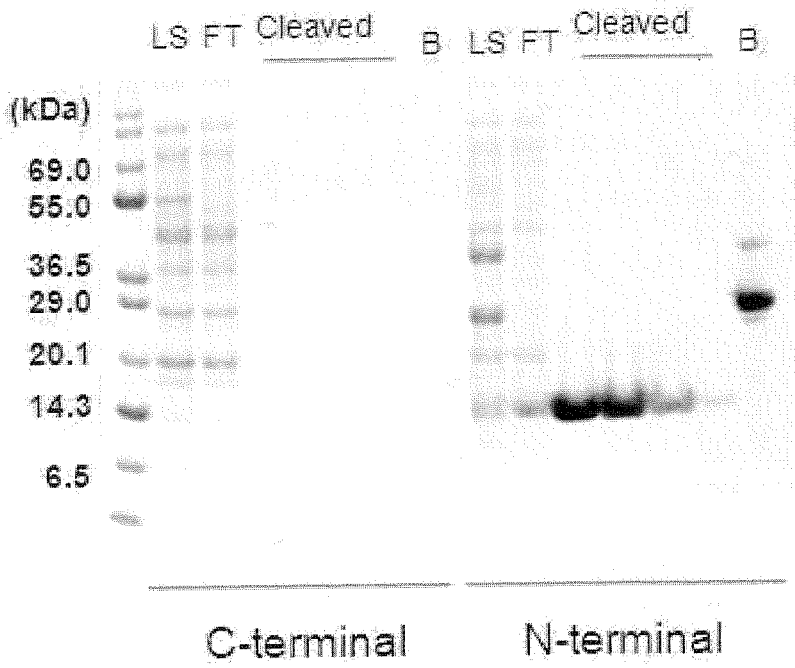
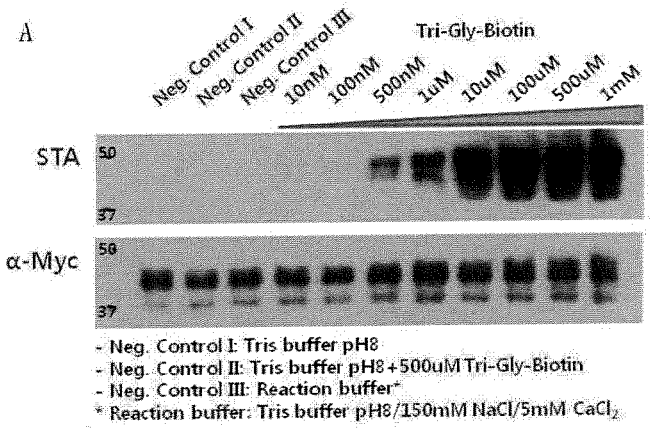

LS FT Cleaved B LS FT Cleaved B
(kDa)
69.0
55.0
36.5
29.0
20.1
14.3
6.5
C-terminal
N-terminal

FIG. 16


A
Neg. Control I
Neg. Control II
Neg. Control III
Tri-Gly-Biotin
10nM
100nM
500nM
1uM
10uM
100uM
500uM
1mM
STA
α-Myc
50
37
- Neg. Control I: Tris buffer pH8
- Neg. Control II: Tris buffer pH8+500uM Tri-Gly-Biotin
- Neg. Control III: Reaction buffer*
* Reaction buffer: Tris buffer pH8/150mM NaCl/5mM CaCl2

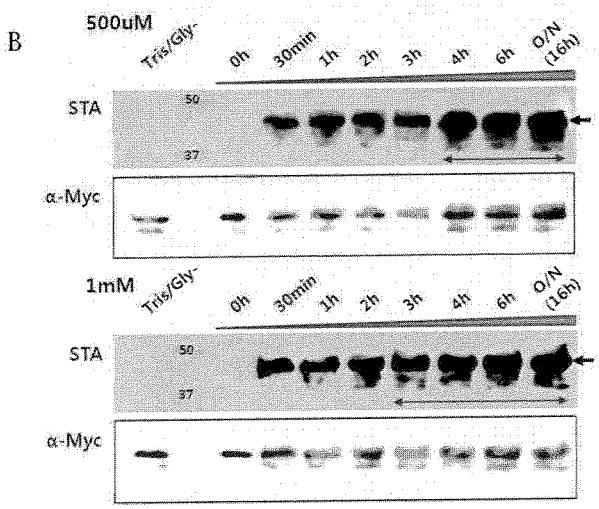
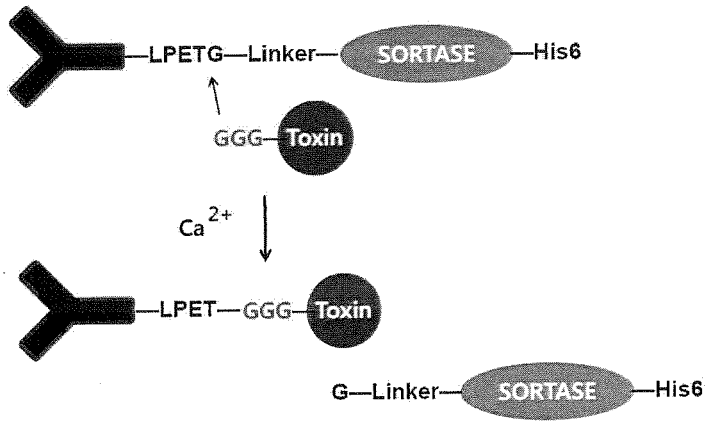

B
500uM
Tris/Gly-
0h
30min
1h
2h
3h
4h
6h
O/N (16h)
STA
α-Myc
50
37
1mM

FIG. 17


LPETG—Linker—
SORTASE
—His6
GGG—
Toxin
Ca2+
LPET—GGG—
Toxin
G—Linker—
SORTASE
—His6

SEQUENCE LISTING

<110> Scripps Korea Antibody Institute

<120> METHOD FOR REFINING PROTEIN INCLUDING SELF-CUTTING CASSETTE AND USE THEREOF

<130> PF-B1593

<140> PCT/KR2014/003639
<141> 2014-04-25

<150> 10-2013-0046322
<151> 2013-04-25

<160> 57

<170> PatentIn version 3.2

<210> 1
<211> 49
<212> PRT
<213> Artificial

<220>
<223> Linker 50 a.a.

<400> 1

Leu Glu Ala Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Glu Ala Ala
1 5 10 15

Ala Lys Glu Ala Ala Ala Lys Ala Leu Glu Ala Glu Ala Ala Ala Lys
20 25 30

Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Ala
35 40 45

Leu

<210> 2
<211> 32
<212> PRT
<213> Artificial

<220>
<223> CH Linker

<400> 2

Thr Arg Ala Arg Leu Ser Lys Glu Leu Gln Ala Ala Gln Ala Arg Leu
1 5 10 15

Gly Ala Asp Met Glu Asp Val Cys Gly Arg Leu Val Gln Tyr Arg Gly
20 25 30

<210> 3
<211> 45
<212> PRT
<213> Artificial

<220>
<223> AH Linker

<400> 3

Lys Glu Gln Gln Asn Ala Phe Tyr Glu Ile Leu His Leu Pro Asn Leu
1 5 10 15

Asn Glu Glu Gln Arg Asn Gly Phe Ile Gln Ser Leu Lys Asp Asp Pro
20 25 30

Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala Lys Lys Leu
35 40 45

<210> 4
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Flexible Linker

<400> 4

Ser Ser Gly Gly Gly Gly Gly Gly Ser Ser Arg Ser Ser Gly Ser
1 5 10 15

<210> 5
<211> 7
<212> PRT
<213> Artificial

<220>
<223> Linker 7 a.a.

<400> 5

Gly Gly Ser Ser Arg Ser Ser
1 5

<210> 6
<211> 18
<212> PRT
<213> Artificial

<220>
<223> Linker 18 a.a.

<400> 6

Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly Ser Ser Arg
1 5 10 15

Ser Ser

<210> 7
<211> 20
<212> PRT
<213> Artificial

<220>
<223> Linker 20 a.a.

<400> 7

Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly Ser Ser Arg
1 5 10 15

Ser Ser Gly Ser
20

<210> 8
<211> 147
<212> PRT
<213> Artificial

<220>
<223> S.aureus Sortase A 60-206

<400> 8

Gln Ala Lys Pro Gln Ile Pro Lys Asp Lys Ser Lys Val Ala Gly Tyr
1 5 10 15

Ile Glu Ile Pro Asp Ala Asp Ile Lys Glu Pro Val Tyr Pro Gly Pro
20 25 30

Ala Thr Pro Glu Gln Leu Asn Arg Gly Val Ser Phe Ala Glu Glu Asn
35 40 45

Glu Ser Leu Asp Asp Gln Asn Ile Ser Ile Ala Gly His Thr Phe Ile
50 55 60

Asp Arg Pro Asn Tyr Gln Phe Thr Asn Leu Lys Ala Ala Lys Lys Gly
65 70 75 80

Ser Met Val Tyr Phe Lys Val Gly Asn Glu Thr Arg Lys Tyr Lys Met
85 90 95

Thr Ser Ile Arg Asp Val Lys Pro Thr Asp Val Gly Val Leu Asp Glu
100 105 110

Gln Lys Gly Lys Asp Lys Gln Leu Thr Leu Ile Thr Cys Asp Asp Tyr
115 120 125

Asn Glu Lys Thr Gly Val Trp Glu Lys Arg Lys Ile Phe Val Ala Thr
130 135 140

Glu Val Lys
145

<210> 9
<211> 39
<212> PRT
<213> Artificial

<220>
<223> H1.winzipA1 coiled coil

<400> 9

Thr Val Ala Gln Leu Glu Glu Lys Val Lys Thr Leu Arg Ala Gln Asn
1 5 10 15

Tyr Glu Leu Lys Ser Arg Val Gln Arg Leu Arg Glu Gln Val Ala Gln
20 25 30

Leu Ala Ser Glu Phe Glu Leu
35

<210> 10
<211> 39
<212> PRT
<213> H2. winzipA2 coiled coil linker

<400> 10

Thr Val Ala Gln Leu Arg Glu Arg Val Lys Thr Leu Arg Ala Gln Asn
1 5 10 15

Tyr Glu Leu Glu Ser Glu Val Gln Arg Leu Arg Glu Gln Val Ala Gln
20 25 30

Leu Ala Ser Glu Phe Glu Leu
35

<210> 11
<211> 39
<212> PRT
<213> H3. Vel A1 coiled coil linker

<400> 11

Thr Val Ala Gln Leu Glu Glu Lys Val Lys Thr Leu Arg Ala Glu Asn
1 5 10 15

Tyr Glu Leu Lys Ser Glu Val Gln Arg Leu Glu Glu Gln Val Ala Gln
20 25 30

Leu Ala Ser Glu Phe Glu Leu
35

<210> 12
<211> 36
<212> PRT
<213> H4.Max coiled coil linker

<400> 12

Thr Met Arg Arg Lys Asn Asp Thr His Gln Gln Asp Ile Asp Asp Leu
1 5 10 15

Lys Arg Gln Asn Ala Leu Leu Glu Gln Gln Val Arg Ala Leu Ala Ser
20 25 30

Glu Phe Glu Leu
35

<210> 13
<211> 52
<212> PRT
<213> H5. EE1234L coiled coil linker

<400> 13

Thr Leu Glu Ile Glu Ala Ala Phe Leu Glu Gln Glu Asn Thr Ala Leu
1 5 10 15

Glu Thr Glu Val Ala Glu Leu Glu Gln Glu Val Gln Arg Leu Glu Asn
20 25 30

Ile Val Ser Gln Tyr Glu Thr Arg Tyr Gly Pro Leu Gly Gly Ala Ser
35 40 45

Glu Phe Glu Leu
50

<210> 14
<211> 44
<212> PRT
<213> H6.VSAL E5 coiled coil linker

<400> 14

Thr Glu Val Ser Ala Leu Lys Glu Lys Val Ser Ala Leu Glu Lys Glu
1 5 10 15

Val Ser Ala Leu Lys Glu Lys Val Ser Ala Leu Glu Lys Glu Val Ser
20 25 30

Ala Leu Glu Lys Gly Gly Ala Ser Glu Phe Glu Leu
35 40

<210> 15
<211> 31
<212> PRT
<213> H7.VSAL E3ox coiled coil linker

<400> 15

Thr Cys Gly Gly Glu Val Ser Ala Leu Glu Lys Glu Val Ser Ala Leu
1 5 10 15

Glu Lys Glu Val Ser Ala Leu Glu Lys Ala Ser Glu Phe Glu Leu
20 25 30

<210> 16
<211> 28
<212> PRT
<213> H8. IAALE3 coiled coil linker

<400> 16

Thr Glu Ile Ala Ala Leu Glu Lys Glu Ile Ala Ala Leu Glu Lys Glu
1 5 10 15

Ile Ala Ala Leu Glu Lys Ala Ser Glu Phe Glu Leu
20 25

<210> 17
<211> 374
<212> PRT
<213> Artificial

<220>
<223> Flag-VH-linker- VSAL E3 ox coiled coil- HA-Flag-LPETG-linker 20-SrtA-His9

<400> 17

Asp Tyr Lys Asp Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val
1 5 10 15

Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn
20 25 30

Ile Lys Asp Thr Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly
35 40 45

Leu Glu Trp Val Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr
50 55 60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys
65 70 75 80

Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala
85 90 95

Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp
100 105 110

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ser Leu Glu Gly
115 120 125

Thr Gly Gly Thr Ser Gly Ser Thr Ser Gly Thr Gly Gly Ser Ser Arg
130 135 140

Ser Ser Ser Thr Thr Cys Gly Gly Glu Val Ser Ala Leu Glu Lys Glu
145 150 155 160

Val Ser Ala Leu Glu Lys Glu Val Ser Ala Leu Glu Lys Ala Ser Glu
165 170 175

Phe Glu Leu Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Lys Asp Tyr Lys
180 185 190

```
Asp Leu Pro Glu Thr Gly Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
        195                 200                 205

Gly Gly Gly Ser Ser Arg Ser Ser Gly Ser Gln Ala Lys Pro Gln Ile
    210                 215                 220

Pro Lys Asp Lys Ser Lys Val Ala Gly Tyr Ile Glu Ile Pro Asp Ala
225                 230                 235                 240

Asp Ile Lys Glu Pro Val Tyr Pro Gly Pro Ala Thr Pro Glu Gln Leu
                245                 250                 255

Asn Arg Gly Val Ser Phe Ala Glu Glu Asn Glu Ser Leu Asp Asp Gln
            260                 265                 270

Asn Ile Ser Ile Ala Gly His Thr Phe Ile Asp Arg Pro Asn Tyr Gln
        275                 280                 285

Phe Thr Asn Leu Lys Ala Ala Lys Lys Gly Ser Met Val Tyr Phe Lys
    290                 295                 300

Val Gly Asn Glu Thr Arg Lys Tyr Lys Met Thr Ser Ile Arg Asp Val
305                 310                 315                 320

Lys Pro Thr Asp Val Gly Val Leu Asp Glu Gln Lys Gly Lys Asp Lys
                325                 330                 335

Gln Leu Thr Leu Ile Thr Cys Asp Asp Tyr Asn Glu Lys Thr Gly Val
            340                 345                 350

Trp Glu Lys Arg Lys Ile Phe Val Ala Thr Glu Val Lys His His His
        355                 360                 365

His His His His His His
    370
```

<210> 18
<211> 377
<212> PRT
<213> Artificial

<220>
<223> VL-linker-VelB1 coiled coil- myc-LPETG-linker 20- SrtA-His9

<400> 18

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn Thr Ala
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
```

```
        35                  40                  45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Ala Leu Glu Gly Thr
            100                 105                 110

Gly Ser Ser Thr Gly Ser Ser Thr Gly Pro Gly Gly Ser Ser Arg Ser
        115                 120                 125

Ser Ser Thr Gly Pro Gly Gly Ser Ser Arg Ser Ser Ser Thr Ser Val
    130                 135                 140

Asp Glu Leu Gln Ala Glu Val Asp Gln Leu Glu Asp Glu Asn Tyr Ala
145                 150                 155                 160

Leu Lys Thr Lys Val Ala Gln Leu Arg Lys Lys Val Glu Lys Leu Ala
                165                 170                 175

Ser Glu Phe Glu Leu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Lys
            180                 185                 190

Leu Pro Glu Thr Leu Pro Glu Thr Gly Ser Ser Gly Gly Gly Gly Ser
        195                 200                 205

Gly Gly Gly Gly Gly Gly Ser Ser Arg Ser Ser Gly Ser Gln Ala Lys
    210                 215                 220

Pro Gln Ile Pro Lys Asp Lys Ser Lys Val Ala Gly Tyr Ile Glu Ile
225                 230                 235                 240

Pro Asp Ala Asp Ile Lys Glu Pro Val Tyr Pro Gly Pro Ala Thr Pro
                245                 250                 255

Glu Gln Leu Asn Arg Gly Val Ser Phe Ala Glu Glu Asn Glu Ser Leu
            260                 265                 270

Asp Asp Gln Asn Ile Ser Ile Ala Gly His Thr Phe Ile Asp Arg Pro
        275                 280                 285

Asn Tyr Gln Phe Thr Asn Leu Lys Ala Ala Lys Lys Gly Ser Met Val
    290                 295                 300

Tyr Phe Lys Val Gly Asn Glu Thr Arg Lys Tyr Lys Met Thr Ser Ile
```

305 310 315 320

Arg Asp Val Lys Pro Thr Asp Val Gly Val Leu Asp Glu Gln Lys Gly
325 330 335

Lys Asp Lys Gln Leu Thr Leu Ile Thr Cys Asp Asp Tyr Asn Glu Lys
340 345 350

Thr Gly Val Trp Glu Lys Arg Lys Ile Phe Val Ala Thr Glu Val Lys
355 360 365

His His His His His His His His His
370 375

<210> 19
<211> 50
<212> DNA
<213> Artificial

<220>
<223> primer 1_sfi

<400> 19
ccgtggccca ggcggccgca agcagcggcc tgaacgacat cttcgaggcc 50

<210> 20
<211> 43
<212> DNA
<213> Artificial

<220>
<223> primer 1

<400> 20
atgtcatatg gcaagcagcg gcctgaacga catcttcgag gcc 43

<210> 21
<211> 45
<212> DNA
<213> Artificial

<220>
<223> primer 2

<400> 21
ctgcatttcg tgccactcga tcttctgggc ctcgaagatg tcgtt 45

<210> 22
<211> 39
<212> DNA
<213> Artificial

<220>
<223> primer 3

<400> 22
atcgagtggc acgaaatgca ggctaagccg cagattccg 39

<210> 23

<211> 39
<212> DNA
<213> Artificial

<220>
<223> primer 4

<400> 23
gccggtctcg ggaagcttct tgacctcggt agcgacaaa 39

<210> 24
<211> 43
<212> DNA
<213> Artificial

<220>
<223> primer 5

<400> 24
cagtaagctt cccgagaccg gcgatatcca gatgactcag agc 43

<210> 25
<211> 36
<212> DNA
<213> Artificial

<220>
<223> primer 6

<400> 25
actcgaaccc gccgtacgtt ttatctctac ctttgt 36

<210> 26
<211> 52
<212> DNA
<213> Artificial

<220>
<223> primer 7

<400> 26
taatggccgg cctggccgcg gccgcttaaa gatcttcttc actaattaac tt 52

<210> 27
<211> 31
<212> DNA
<213> Artificial

<220>
<223> primer 8

<400> 27
atgtcatatg gacattcaga tgacacagag t 31

<210> 28
<211> 46
<212> DNA
<213> Artificial

<220>
<223> primer 9

<400> 28

ggaaccaccg ccggtctcgg gaagaagatc ttcttcacta attaac 46

<210> 29
<211> 74
<212> DNA
<213> Artificial

<220>
<223> primer 10

<400> 29
ggaagatcta gaggaaccac ccccaccacc gcccgagcca ccgccaccgg atgagccggt 60
ctcgggaaga agat 74

<210> 30
<211> 48
<212> DNA
<213> Artificial

<220>
<223> primer 11

<400> 30
gagaccggcg gtggttcctc tagatcttcc caggctaagc cgcagatt 48

<210> 31
<211> 44
<212> DNA
<213> Artificial

<220>
<223> primer 12

<400> 31
taatgcggcc gcttaatgat ggtgatggtg atgatgatga tggc 44

<210> 32
<211> 41
<212> DNA
<213> Artificial

<220>
<223> primer 13

<400> 32
gtggttcctc tagatcttcc tcgaaggtcg cgggatatat t 41

<210> 33
<211> 49
<212> DNA
<213> Artificial

<220>
<223> primer 14

<400> 33
taatggccgg cctggcctta atgatggtga tggtgatgat gatgatggc 49

<210> 34
<211> 42
<212> DNA

<213> Artificial

<220>
<223> primer 15

<400> 34
ggttcctcta gatcttccgg aagccaggct aagccgcaga tt 42

<210> 35
<211> 56
<212> DNA
<213> Artificial

<220>
<223> primer 16

<400> 35
atgatgatgg cgagagctac ggctgctgcc gcccttgacc tcggtagcga caaaga 56

<210> 36
<211> 57
<212> DNA
<213> Artificial

<220>
<223> primer 17

<400> 36
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<210> 37
<211> 100
<212> DNA
<213> Artificial

<220>
<223> primer 18

<400> 37
acgacgacga cggcgctcca gtgccttagc agcggcttcc ttagcagcag cctccttagc 60

agctgcttct ttcgctgcgg cttccgcttc caacgctttc 100

<210> 38
<211> 52
<212> DNA
<213> Artificial

<220>
<223> primer 19

<400> 38
taatgcggcc gcttaacggc gacgacggcg acgacgacga cggcgctcca gt 52

<210> 39
<211> 37
<212> DNA
<213> Artificial

<220>
<223> primer 20

<400> 39

gtgcccgcgt cttgacctcg gtagcgacaa agatctt 37

<210> 40
<211> 49
<212> DNA
<213> Artificial

<220>
<223> primer 21

<400> 40
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<210> 41
<211> 52
<212> DNA
<213> Artificial

<220>
<223> primer 22

<400> 41
gcggtactgc accaggcggc cgcacacgtc ctccatgtcc gcgcccagcc gg 52

<210> 42
<211> 39
<212> DNA
<213> Artificial

<220>
<223> primer 23

<400> 42
gaggtcaaga cgcgggcacg gctgtccaag gagctgcag 39

<210> 43
<211> 53
<212> DNA
<213> Artificial

<220>
<223> primer 24

<400> 43
taatgcggcc gcttaatgat gctgatggtg atggccgcgg tactgcacca ggc 53

<210> 44
<211> 36
<212> DNA
<213> Artificial

<220>
<223> primer 25

<400> 44
cggatcaccc ttgacctcgg tagcgacaaa gatctt 36

<210> 45
<211> 36
<212> DNA
<213> Artificial

<220>
<223> primer 26

<400> 45
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<210> 46
<211> 41
<212> DNA
<213> Artificial

<220>
<223> primer 27

<400> 46
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<210> 47
<211> 54
<212> DNA
<213> Artificial

<220>
<223> primer 28

<400> 47
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<210> 48
<211> 39
<212> PRT
<213> L1.wizipB1 coiled coil linker

<400> 48

Ser Val Asp Glu Leu Gln Ala Glu Val Asp Gln Leu Gln Asp Glu Asn
1 5 10 15

Tyr Ala Leu Lys Thr Lys Val Ala Gln Leu Arg Lys Lys Val Glu Lys
20 25 30

Leu Ala Ser Glu Phe Glu Leu
35

<210> 49
<211> 50
<212> PRT
<213> L2.winzipB2 coiled coil linker

<400> 49

Gly Pro Gly Gly Ser Ser Arg Ser Ser Ser Thr Ser Val Asp Glu Leu
1 5 10 15

Lys Ala Glu Val Asp Gln Leu Gln Asp Gln Asn Tyr Ala Leu Arg Thr
20 25 30

Lys Val Ala Gln Leu Arg Lys Glu Val Glu Lys Leu Ser Glu Glu Phe
35 40 45

Glu Leu
50

<210> 50
<211> 50
<212> PRT
<213> L3. Vel B1 coiled coil linker

<400> 50

Gly Pro Gly Gly Ser Ser Arg Ser Ser Ser Thr Ser Val Asp Glu Leu
1 5 10 15

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

Lys Val Ala Gln Leu Arg Lys Lys Val Glu Lys Leu Ala Ser Glu Phe
35 40 45

Glu Leu
50

<210> 51
<211> 47
<212> PRT
<213> L4. myc coiled coil linker

<400> 51

Gly Pro Gly Gly Ser Ser Arg Ser Ser Ser Thr Ser Val Gln Ala Glu
1 5 10 15

Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Leu Arg Lys Arg Arg Glu
20 25 30

Gln Leu Lys His Lys Leu Glu Gln Leu Ala Ser Glu Phe Glu Leu
35 40 45

<210> 52
<211> 64
<212> PRT
<213> L5. RR1234L coiled coil

<400> 52

Gly Pro Gly Gly Ser Ser Arg Ser Ser Ser Thr Ser Lys Gly Gly Gly
1 5 10 15

Leu Glu Ile Arg Ala Ala Phe Leu Arg Arg Arg Asn Thr Ala Leu Arg
20 25 30

Thr Arg Val Ala Glu Leu Arg Gln Arg Val Gln Arg Leu Arg Asn Ile
35 40 45

Val Ser Gln Tyr Glu Thr Arg Tyr Gly Pro Ala Ser Phe Glu Glu Leu
50 55 60

<210> 53
<211> 52
<212> PRT
<213> L6. VSAL K5 coiled coil linker

<400> 53

Gly Pro Gly Gly Ser Ser Arg Ser Ser Ser Thr Lys Val Ser Ala Leu
1 5 10 15

Lys Glu Lys Val Ser Ala Leu Lys Glu Lys Val Ser Ala Leu Lys Glu
20 25 30

Lys Val Ser Ala Leu Lys Glu Lys Val Ser Ala Leu Lys Glu Gly Gly
35 40 45

Glu Phe Glu Leu
50

<210> 54
<211> 41
<212> PRT
<213> L7. VSAL k3ox coiled coil linker

<400> 54

Gly Pro Gly Gly Ser Ser Arg Ser Ser Ser Thr Cys Gly Gly Lys Val
1 5 10 15

Ser Ala Leu Lys Glu Lys Val Ser Ala Leu Lys Glu Lys Val Ser Ala
20 25 30

Leu Lys Glu Gly Gly Glu Phe Glu Leu
35 40

<210> 55
<211> 39
<212> PRT
<213> L8.IAAL K3 coiled coil linker

<400> 55

Gly Pro Gly Gly Ser Ser Arg Ser Ser Ser Thr Ser Lys Ile Ala Ala
1 5 10 15

Leu Lys Glu Lys Ile Ala Ala Leu Lys Glu Lys Ile Ala Ala Leu Lys
20 25 30

Glu Ala Ser Glu Phe Glu Leu
35

<210> 56
<211> 1128
<212> DNA
<213> Artificial

<220>
<223> Flag-VH-linker- VSAL E3 ox coiled coil- HA-Flag-LPETG-linker 20-SrtA-His9

<400> 56
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agtttacgtc tgtcatgcgc ggcatctgga ttcaatataa aagatacata tattcactgg 120
gtccgccagg caccggggaa aggattagaa tgggtagctc gtatttaccc aacgaatggt 180
tatactcgct atgccgactc cgtgaagggc agatttacca tctcggcgga tacgtcaaaa 240
aacaccgcct atttgcagat gaacagcctg cgggctgaag atactgcagt ttattactgt 300
tctcgttggg gtggtgacgg gttttacgcc atggattatt ggggtcaagg taccttagtt 360
acagtgtcta gtagcctcga gggtaccggc ggtaccagcg gttctaccag cggcaccggc 420
ggcagctctc gtagcagctc taccacctgc ggcggtgaag tgagcgcgct ggaaaaagaa 480
gttagcgcgc tggaaaagga agtgagcgcc ctggaaaaag cgagcgaatt cgagctctac 540
ccatacgatg ttccagatta cgctaaggat tataaagatg aacttcccga gaccggctca 600
tccggtggcg gtggctcggg cggtggtggg ggtggttcct ctagatcttc cggaagccag 660
gctaagccgc agattccgaa ggataagtcg aaggtcgcgg gatatattga aattcccgac 720
gccgatataa aggaaccagt gtatcctggg ccagccactc ctgaacagtt aaatcggggg 780
gtgagctttg cagaagaaaa tgaaagcctg gacgaccaga acatttcaat tgcgggccat 840
acgttcatcg accgtccgaa ctaccagttc accaatctga aggcggccaa gaagggttcc 900
atggtttatt ttaaagtggg caacgaaaca cgcaagtata aaatgacatc tatcagagat 960
gttaaaccga cagatgtagg agttttagat gaacaaaagg gtaaggataa gcaactcacg 1020
ctgataactt gcgatgacta caatgagaag acgggcgttt gggaaaagcg taagatcttt 1080
gtcgctaccg aggtcaagca ccatcatcat catcaccatc accatcat 1128

<210> 57
<211> 1086
<212> DNA
<213> Artificial

<220>
<223> VL-linker-VelB1 coiled coil- myc-LPETG-linker 20- SrtA-His9

<400> 57
gatatccaga tgactcagag cccgagtagc ttgtccgcat cggtgggtga ccgtgttaca 60
atcacgtgtc gtgcgtctca ggatgttaac actgccgtgg cttggtatca gcaaaaaccg 120
ggcaaagctc ccaaactgct gatttactcg gcgtcattct tatattctgg cgtcccatct 180
cgttttagcg gaagtcgctc cgggaccgat tttacactca ccattagctc actgcaacct 240
gaagactttg caacctatta ttgccagcaa cactatacga ccccgccaac ctttggtcag 300
ggtacaaagg tagagataaa agcgctcgag ggtaccggca gcagcaccgg ttctagcacc 360
ggcccgggcg gcagctctcg tagcagctct accagcgtgg atgaactgca ggcggaagtg 420
gatcagctgg aagatgaaaa ctacgcgctg aaaaccaaag tggcccagct gcgtaaaaag 480

gtggaaaaac tgagcgaaga attcgagctc gagcaaaagt taattagtga agaagatctt 540

aagcttcccg agaccggctc atccggtggc ggtggctcgg gcggtggtgg gggtggttcc 600

tctagatctt ccggaagcca ggctaagccg cagattccga aggataagtc gaaggtcgcg 660

ggatatattg aaattcccga cgccgatata aaggaaccag tgtatcctgg gccagccact 720

cctgaacagt taaatcgggg ggtgagcttt gcagaagaaa atgaaagcct ggacgaccag 780

aacatttcaa ttgcgggcca tacgttcatc gaccgtccga actaccagtt caccaatctg 840

aaggcggcca agaagggttc catggtttat tttaaagtgg gcaacgaaac acgcaagtat 900

aaaatgacat ctatcagaga tgttaaaccg acagatgtag gagttttaga tgaacaaaag 960

ggtaaggata agcaactcac gctgataact tgcgatgact acaatgagaa gacgggcgtt 1020

tgggaaaagc gtaagatctt tgtcgctacc gaggtcaagc atcatcatca tcaccatcac 1080

catcat 1086