**DRAWINGS**



FIG. 1

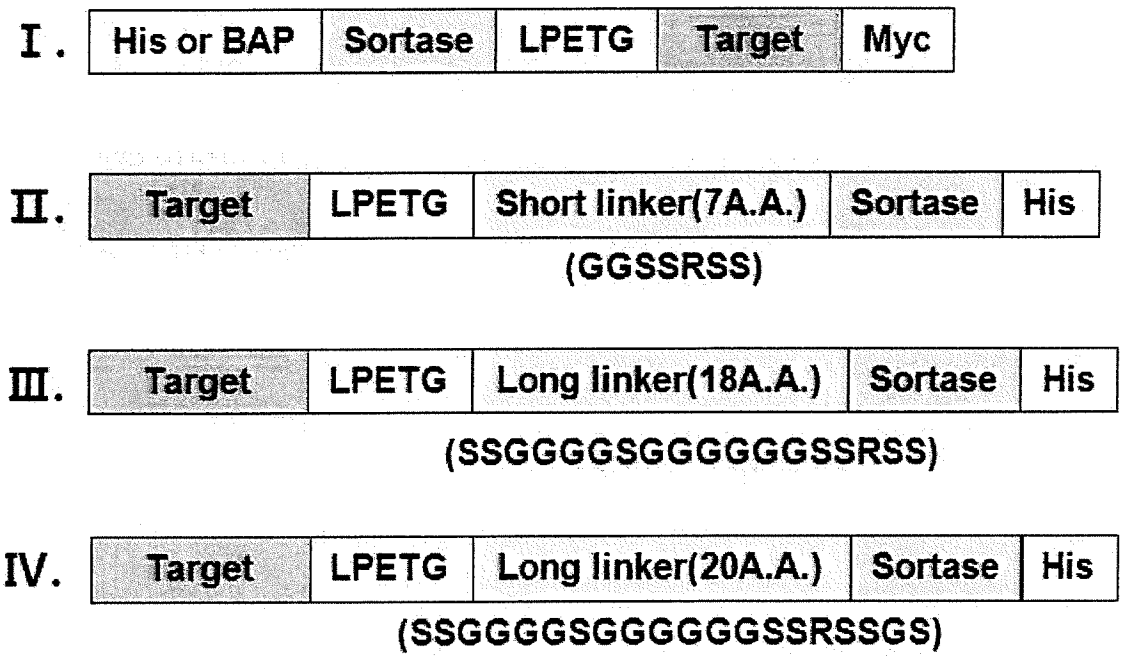



FIG. 2

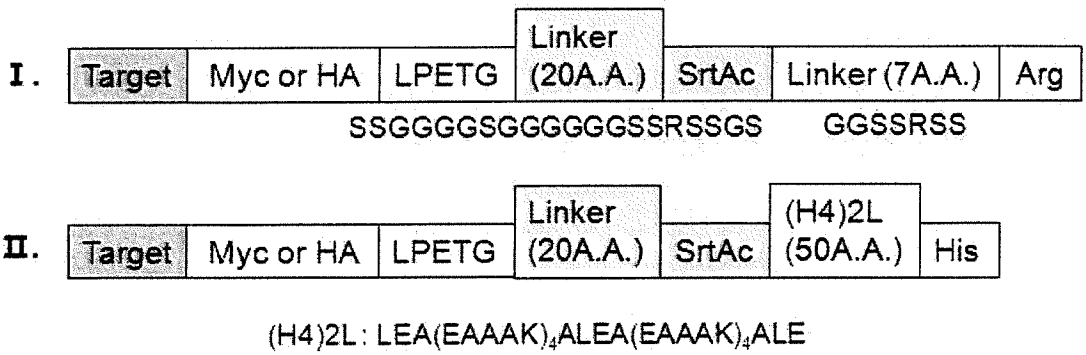


FIG. 3

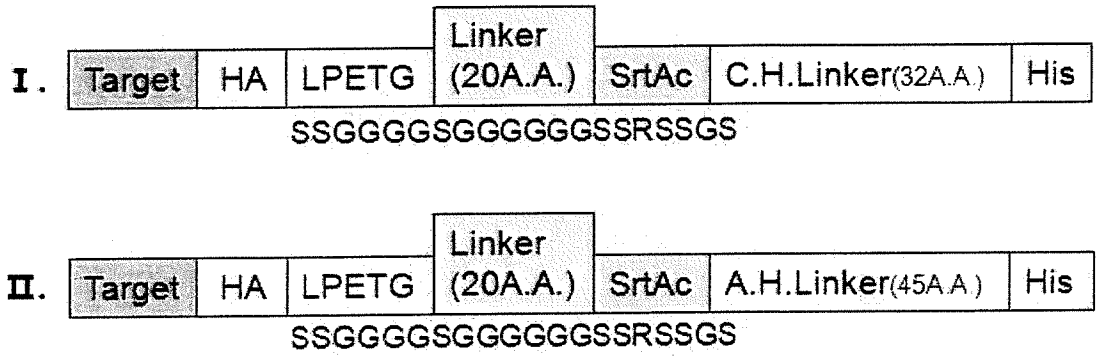

I.
Target
HA
LPETG
Linker (20A.A.)
SrtAc
C.H.Linker(32A.A.)
His
SSGGGGSGGGGGGSSRSSGS
II.
Target
HA
LPETG
Linker (20A.A.)
SrtAc
A.H.Linker(45A.A.)
His
SSGGGGSGGGGGGSSRSSGS

FIG. 4

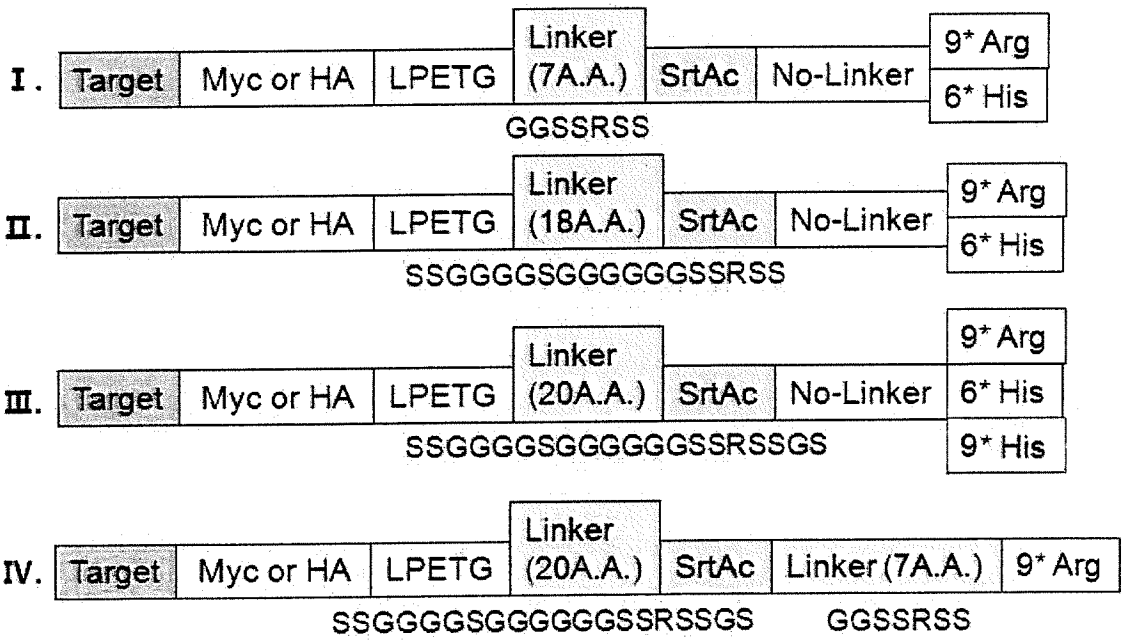

I.
Target
Myc or HA
LPETG
Linker (7A.A.)
SrtAc
No-Linker
9* Arg
6* His
GGSSRSS
II.
Target
Myc or HA
LPETG
Linker (18A.A.)
SrtAc
No-Linker
9* Arg
6* His
SSGGGGSGGGGGGSSRSS
III.
Target
Myc or HA
LPETG
Linker (20A.A.)
SrtAc
No-Linker
9* Arg
6* His
9* His
SSGGGGSGGGGGGSSRSSGS
IV.
Target
Myc or HA
LPETG
Linker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

FIG. 5

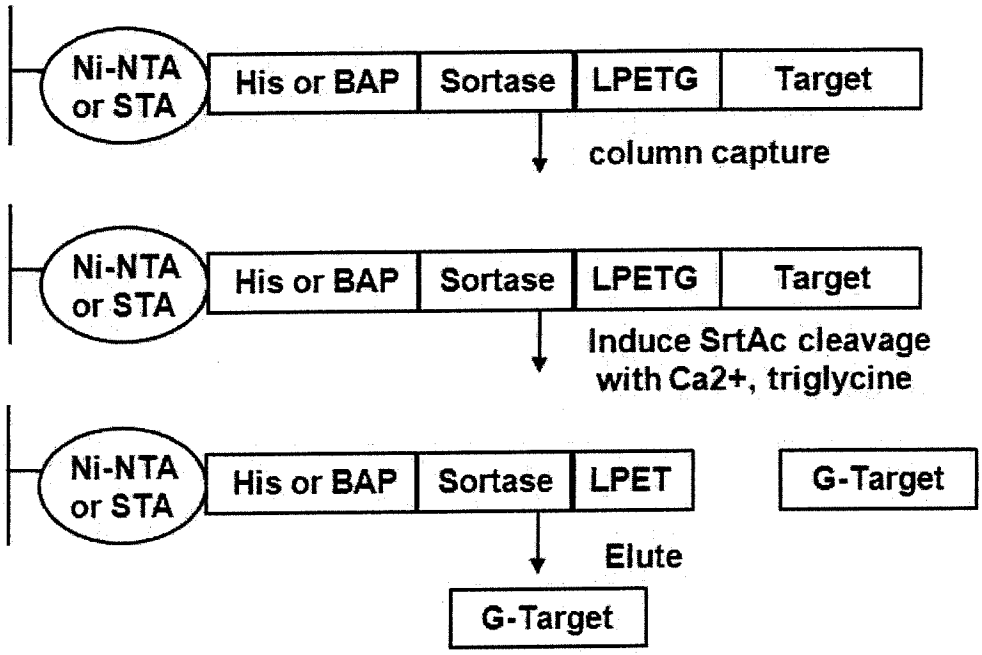

Ni-NTA or STA
His or BAP
Sortase
LPETG
Target
column capture
Ni-NTA or STA
His or BAP
Sortase
LPETG
Target
Induce SrtAc cleavage with Ca2+, triglycine
Ni-NTA or STA
His or BAP
Sortase
LPET
G-Target
Elute
G-Target

FIG. 6

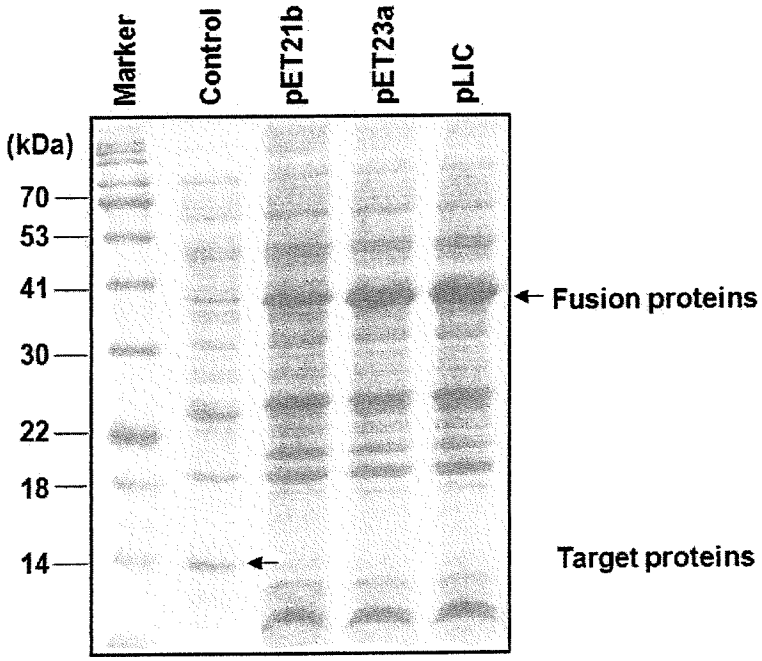

Marker
Control
pET21b
pET23a
pLIC
(kDa)
70
53
41
30
22
18
14
Fusion proteins
Target proteins

FIG. 7

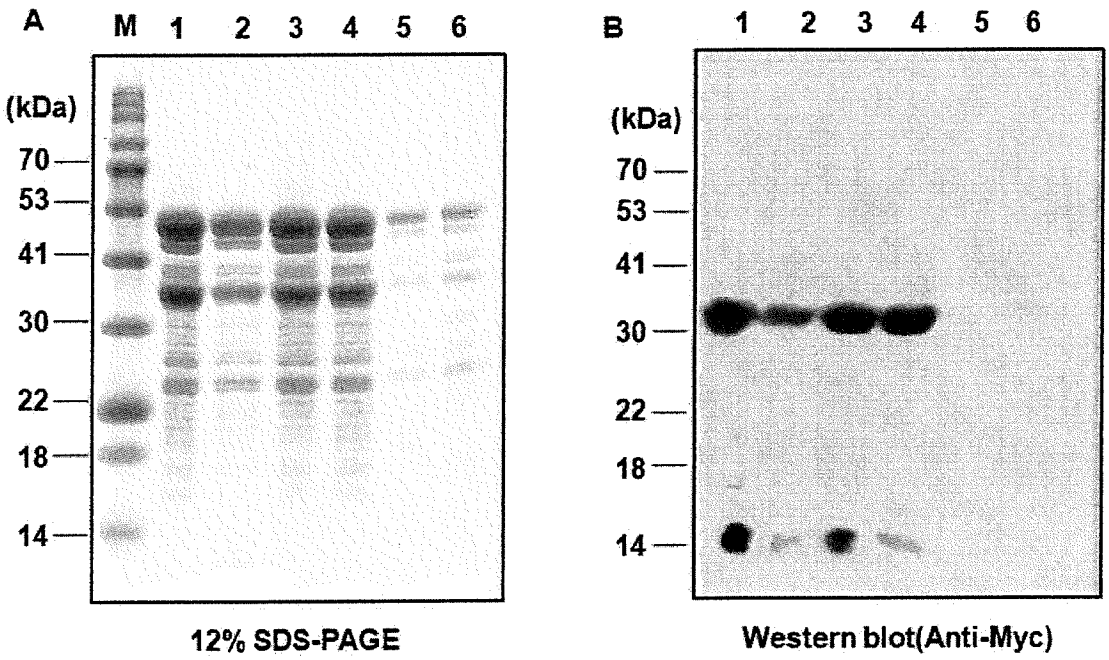

A
M 1 2 3 4 5 6
(kDa)
70
53
41
30
22
18
14
12% SDS-PAGE
B
1 2 3 4 5 6
(kDa)
70
53
41
30
22
18
14
Western blot(Anti-Myc)

FIG. 8

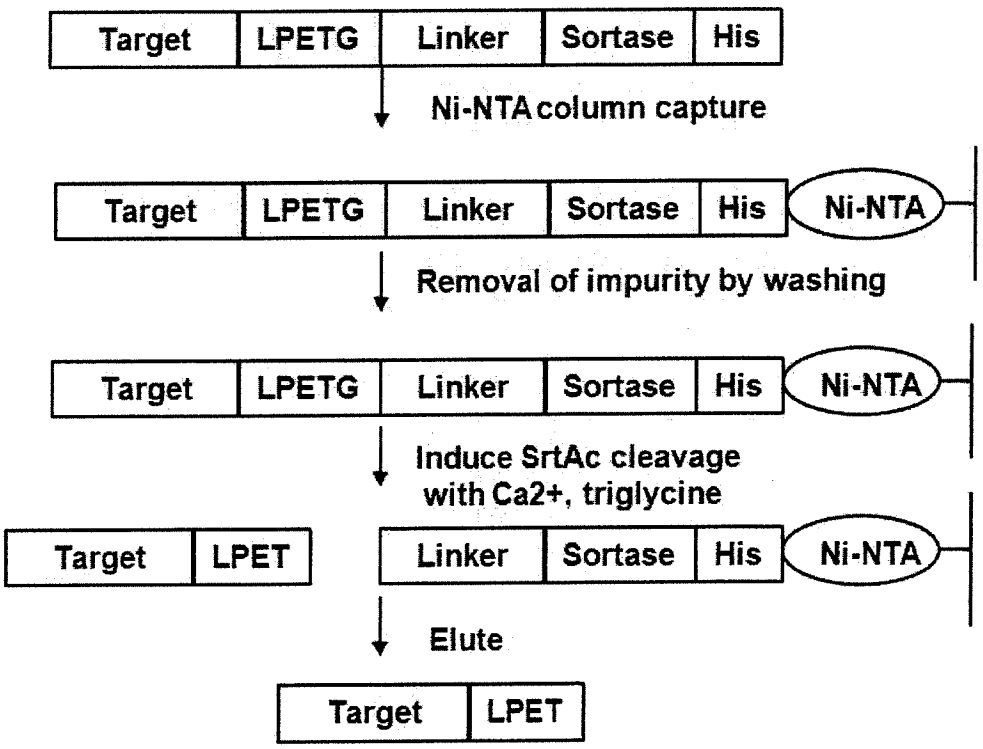

Target
LPETG
Linker
Sortase
His
Ni-NTA column capture
Ni-NTA
Removal of impurity by washing
Induce SrtAc cleavage
with Ca2+, triglycine
LPET
Elute

FIG. 9

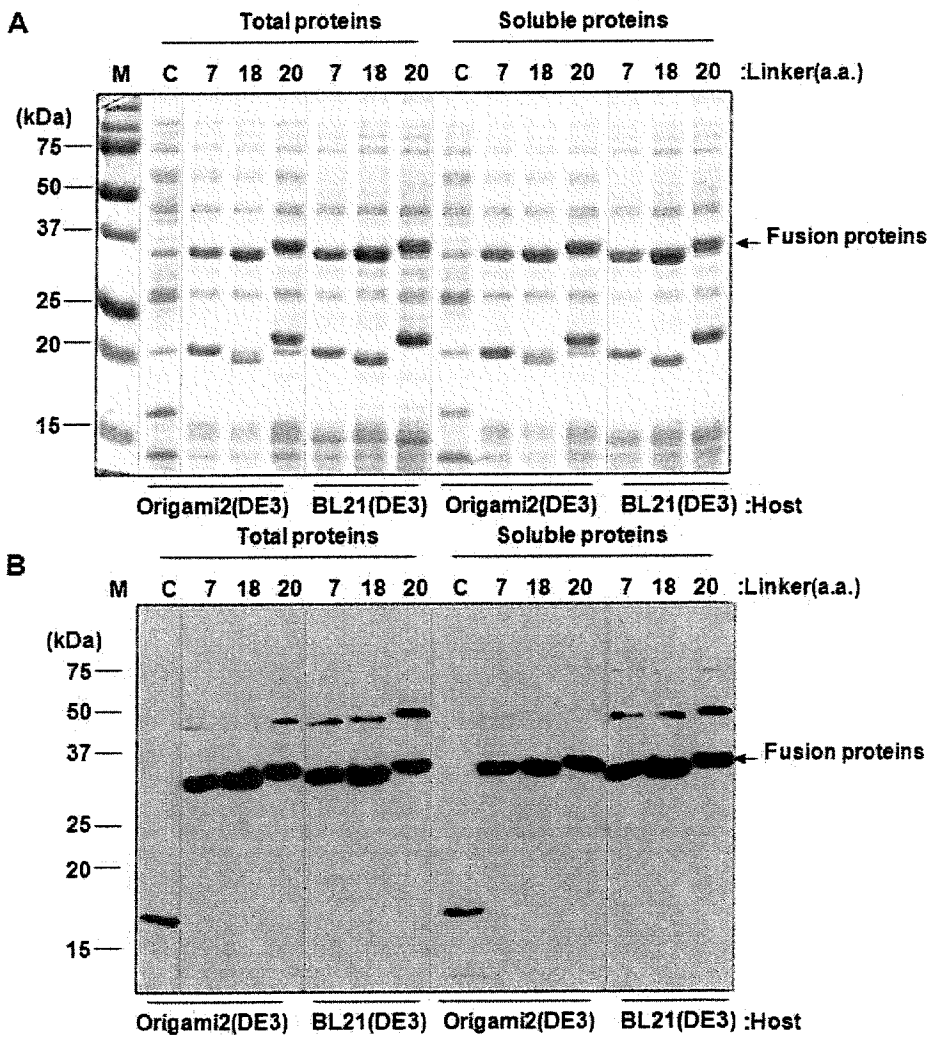

A
Total proteins
Soluble proteins
M C 7 18 20 7 18 20 C 7 18 20 7 18 20 :Linker(a.a.)
(kDa)
75
50
37
25
20
15
Fusion proteins
Origami2(DE3) BL21(DE3) Origami2(DE3) BL21(DE3) :Host
B
Total proteins
Soluble proteins
M C 7 18 20 7 18 20 C 7 18 20 7 18 20 :Linker(a.a.)
(kDa)
75
50
37
25
20
15
Fusion proteins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

FIG. 10

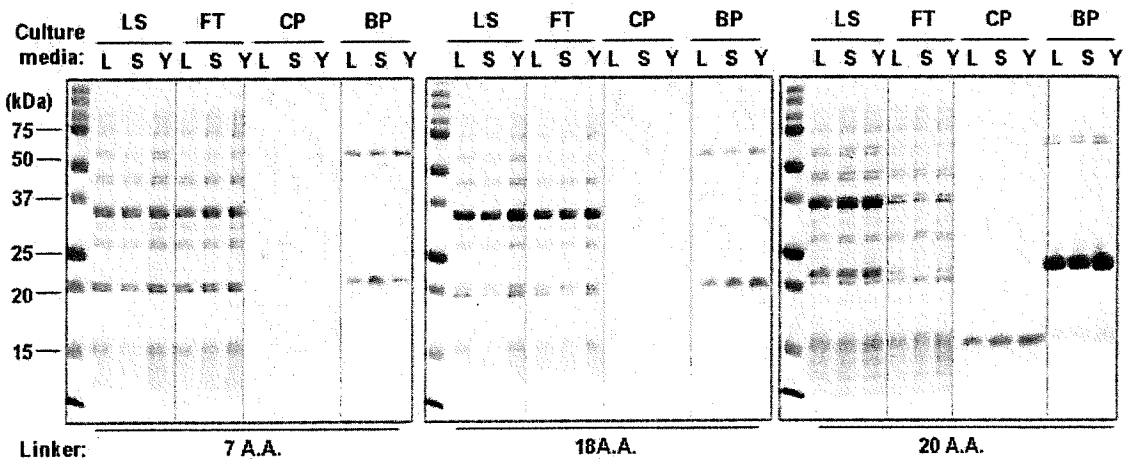

Culture media:
LS FT CP BP
L S Y L S Y L S Y L S Y
(kDa)
75
50
37
25
20
15
Linker:
7 A.A.
18A.A.
20 A.A.

FIG. 11

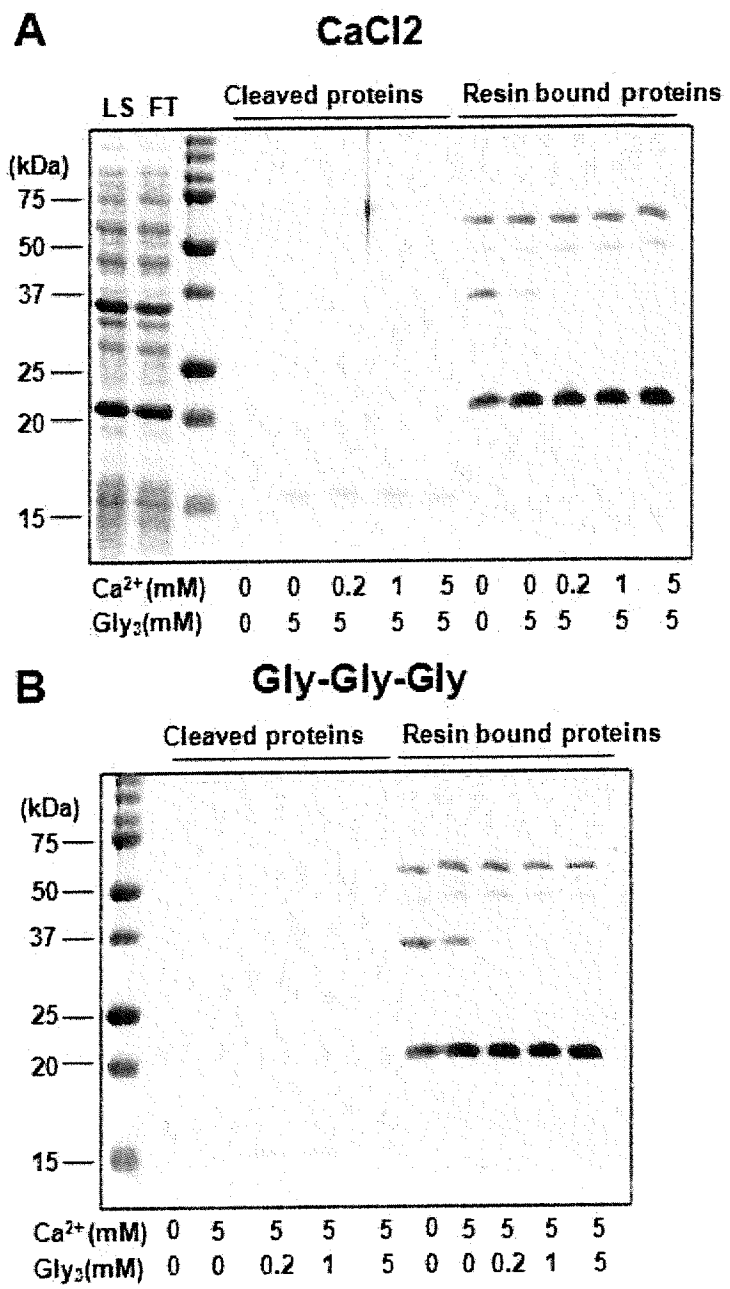

A
CaCl2
LS FT
Cleaved proteins
Resin bound proteins
(kDa)
75
50
37
25
20
15
Ca2+(mM) 0 0 0.2 1 5 0 0 0.2 1 5
Gly3(mM) 0 5 5 5 5 0 5 5 5 5
B
Gly-Gly-Gly
Cleaved proteins
Resin bound proteins
(kDa)
75
50
37
25
20
15
Ca2+(mM) 0 5 5 5 5 0 5 5 5 5
Gly3(mM) 0 0 0.2 1 5 0 0 0.2 1 5

FIG. 12

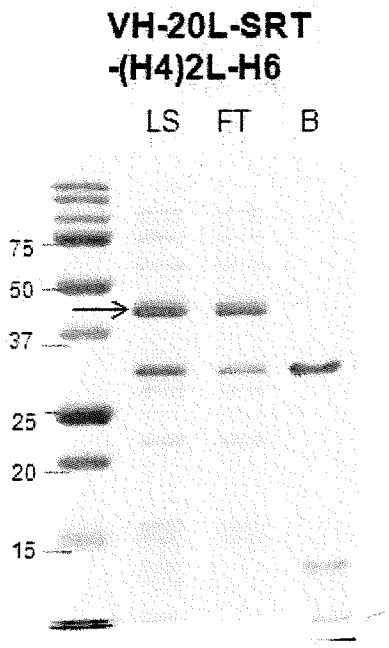

VH-20L-SRT
-(H4)2L-H6
LS
FT
B
75
50
37
25
20
15

FIG. 13

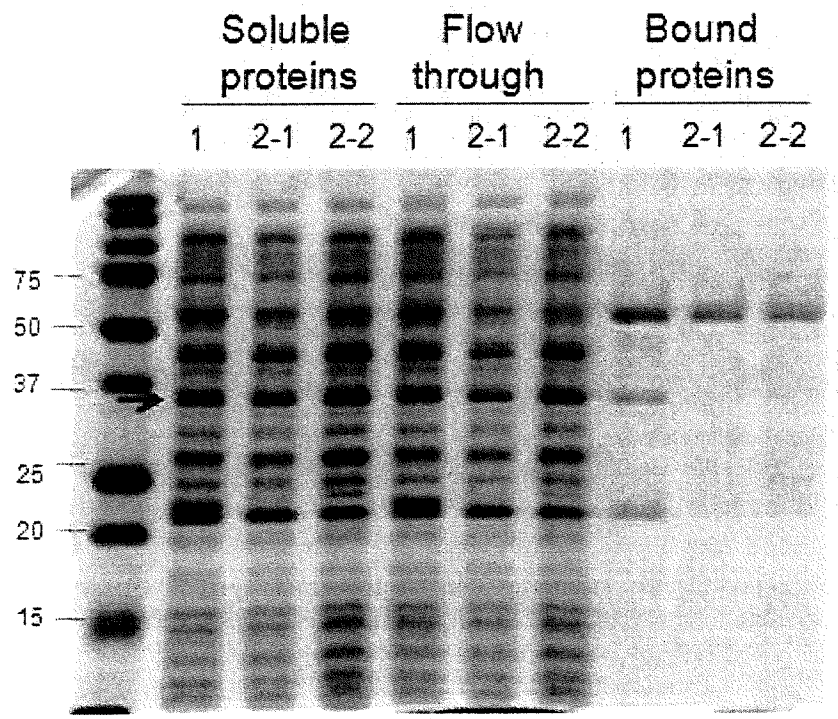

Soluble proteins
Flow through
Bound proteins
1
2-1
2-2
1
2-1
2-2
1
2-1
2-2
75
50
37
25
20
15

FIG. 14

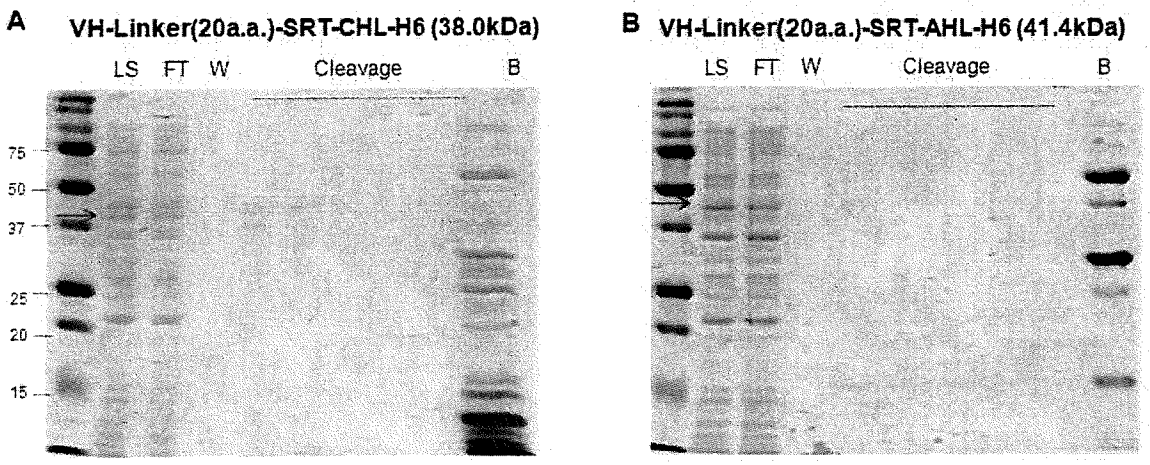

A
VH-Linker(20a.a.)-SRT-CHL-H6 (38.0kDa)
LS FT W Cleavage B
75
50
37
25
20
15
B
VH-Linker(20a.a.)-SRT-AHL-H6 (41.4kDa)
LS FT W Cleavage B

FIG. 15

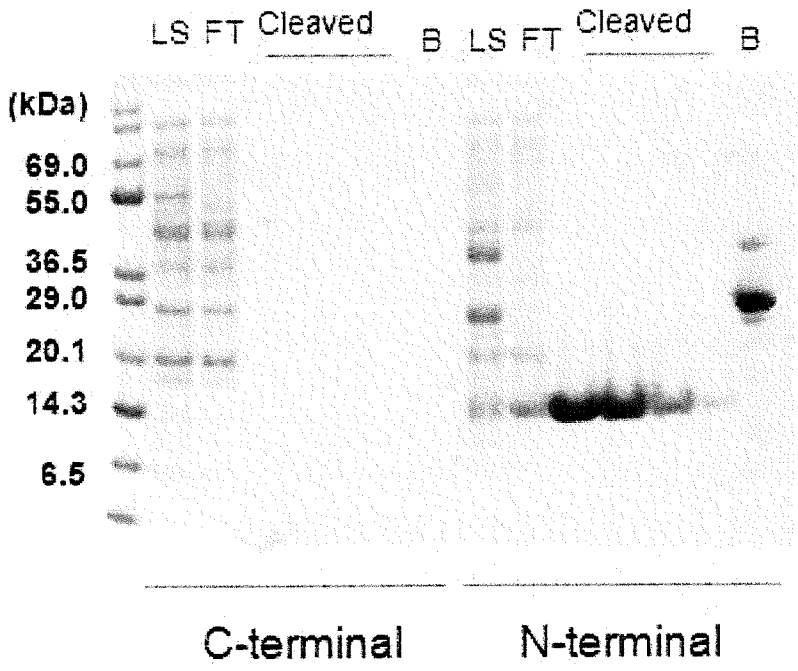
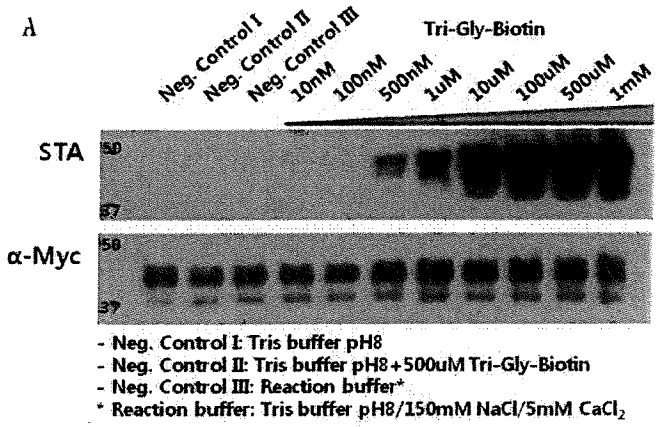

LS FT Cleaved B LS FT Cleaved B
(kDa)
69.0
55.0
36.5
29.0
20.1
14.3
6.5
C-terminal
N-terminal

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

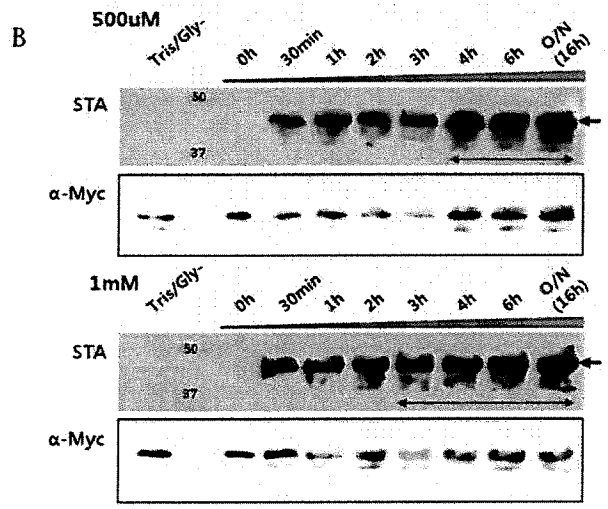
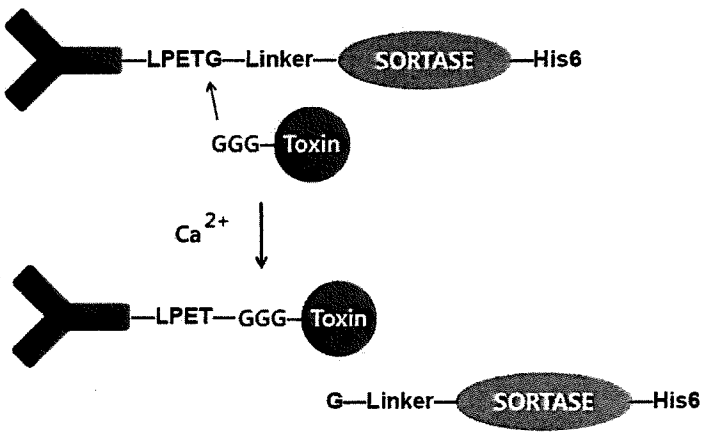

B
500uM
Tris/Gly-
0h
30min
1h
2h
3h
4h
6h
O/N (16h)
STA
α-Myc
50
37
1mM
Tris/Gly-
0h
30min
1h
2h
3h
4h
6h
O/N (16h)
STA
α-Myc
50
37

FIG. 17


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