FIGURE 1

Protein id	aa start	Sequence	aa end	SEQ ID NO:
D_AB012112	437	CLRLTKNSRDDSTC	450	23
DC_AB745660	437	CLRLTRNSRDDSTC	450	61
C1_X62389	437	CHKA**IDGR**SLYNKTLDC	453	2
CD_AB200360	437	CHKA**IDGR**SLYNKTLDC	453	
A4_EU341307	430	CVRGIITSKTKSLDEGYNKALNELC	454	62
A7_JQ954969	430	CVRGIITSKTKSLDEGYNKALNDLC	454	63
A6_FJ981696	430	CVRGIITSKTKSLDKGYNKALNDLC	454	21
A1_AF488749	430	CVRGIITSKTKSLDKGYNKALNDLC	454	
A5_EU679004	430	CVRGIITSKTKSLDEGYNKALNDLC	454	63
A3_DQ185900	426	CVRGIIPFKTKSLDEGYNKALNYLC	450	64
A2_X73423	430	CVRGIIPFKTKSLDEGYNKALNDLC	454	65
A8_KM233166	430	CVRGIIPFKTKSLDEGYNKALNDLC	454	
H_KGO15617	428	CSNSNTKNSLC	438	66
E9_JX424534	414	CKNIVSVKGIRKSIC	429	24
E12_KM370319	414	CKNIVSVKGIRKSIC	429	
E11_KF861875	411	CTNIFSPKGIRKSIC	426	67
E10_KF861917	411	CKNIVFSKGITKSIC	426	68
E7_JN695729	411	CKNIVFSKGITKSIC	426	
E8_JN695730	411	CKNIVFSKGITKSIC	426	
E5_AB037711	411	CKNIVSVKGIRKSIC	426	24
E6_AM695759	411	CKNIVFSKGIRKSIC	426	69
E4_AB088207	411	CKNIVSVKGIRKSIC	426	24
E3_EF028403	411	CKNIVSVKGIRKSIC	426	
E1_GQ244314	411	CKNIVSVKGIRKSIC	426	
E2_EF028404	411	CKNIVSVKGIRKSIC	426	
F7_GU213233	420	CKSIVSKKGTKNSLC	434	29
F5_GU213211	428	CLNSSFKKNTKKPLC	442	28
F1_GU213203	429	CKSVIPRKGTKAPPRLC	445	25
F4_GU213214	429	CKSIIPRKGTKAPPRLC	445	27
F2_GU213209	429	CKSIIPRKGTKQSPSLC	445	26
F3_GU213227	429	CKSIIPRKGTKQSPSLC	445	
F6_M92906	429	CKSVIPRKGTKAPPRLC	445	25
T_P04958	439	CKKIIPPTNIRENLYNRTASLTDLGGELC	467	30
G_X74162	436	CKPVMYKNTGKSEQC	450	31
B4_EF051570	437	CKSVKVPGIC	446	70
B8_JQ964806	437	CKSVRAPGIC	446	22
B7_JQ354985	437	CKSVKAPGIC	446	71
B6_AB302852	437	CKSVRAPGIC	446	22
B2_AB084152	437	CKSVRAPGIC	446	
B3_EF028400	437	CKSVRAPGIC	446	

FIGURE 1 CONTINUED

Protein id	aa start	Sequence	aa end	SEQ ID NO:
B1_AB232927	437	CKSVKAPGIC	446	71
B5_EF033130	437	CKSVKAPGIC	446	
X_BAQ12790	423	CPRNGLLYNAIYRNSKNYLNNIDLEDKK TTSKTNVSYPCSLLNGC	467	20

FIGURE 2

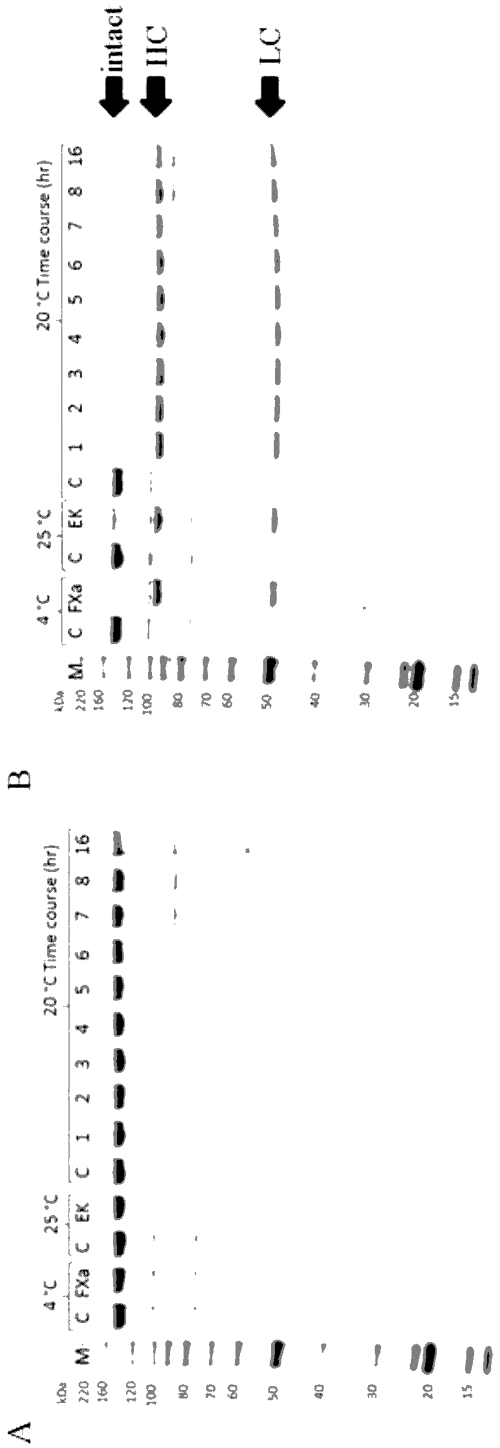

A
B
intact
HC
LC
4 °C
25 °C
20 °C Time course (hr)
M
C
FXa
EK
1
2
3
4
5
6
7
8
16
kDa
220
160
120
100
80
70
60
50
40
30
20
15

FIGURE 2 CONTINUED

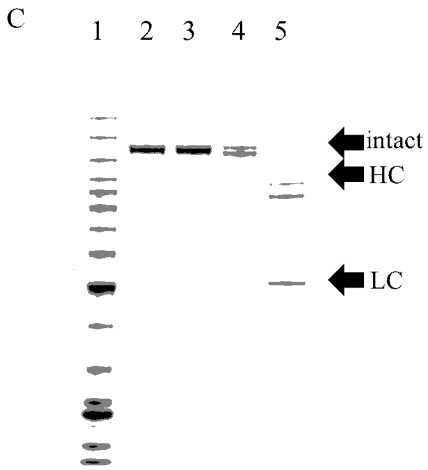


FIGURE 3

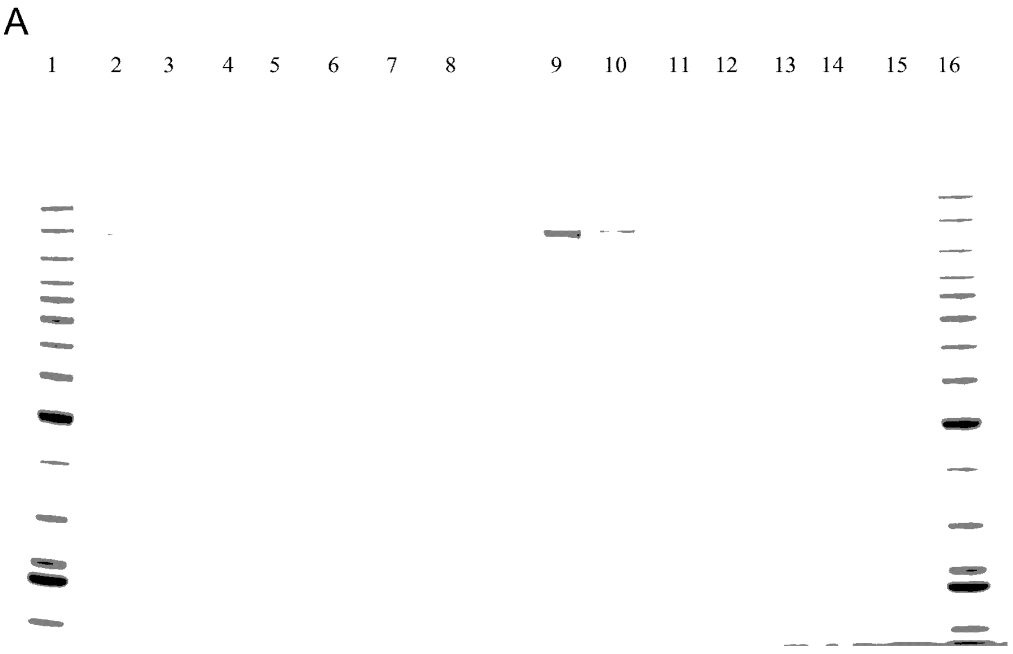


FIGURE 3 CONTINUED

B

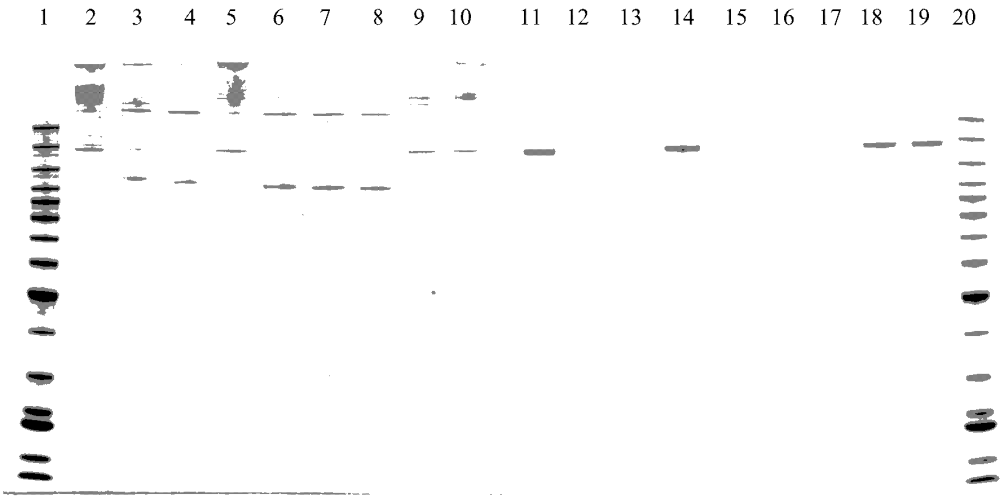


FIGURE 4

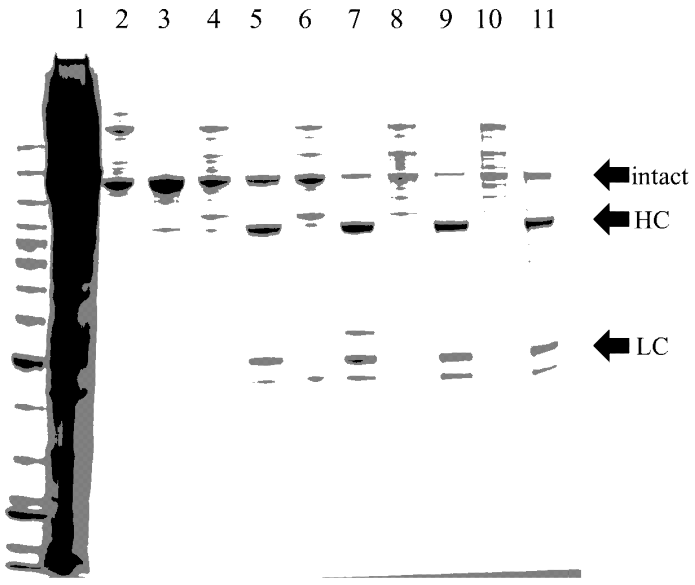
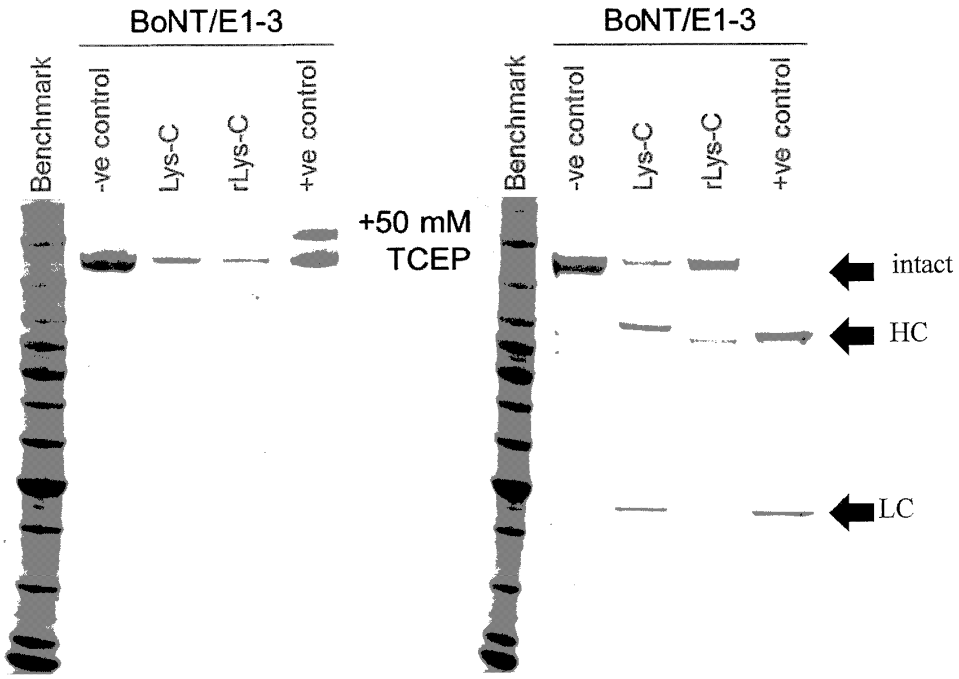


FIGURE 5

A


BoNT/E1-3
BoNT/E1-3
Benchmark
-ve control
Lys-C
rLys-C
+ve control
+50 mM TCEP
Benchmark
-ve control
Lys-C
rLys-C
+ve control
intact
HC
LC

B

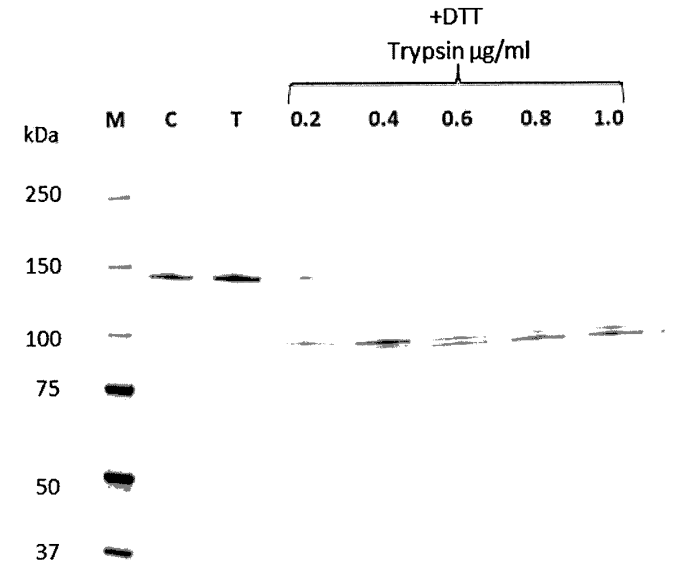

+DTT
Trypsin µg/ml
kDa
M
C
T
0.2
0.4
0.6
0.8
1.0
250
150
100
75
50
37

FIGURE 6

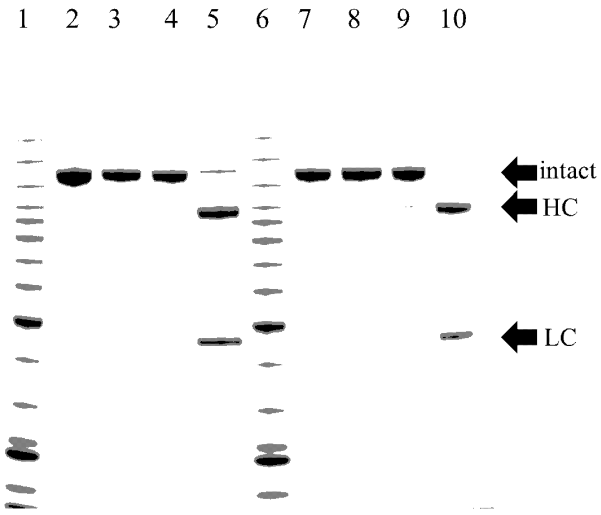


FIGURE 7

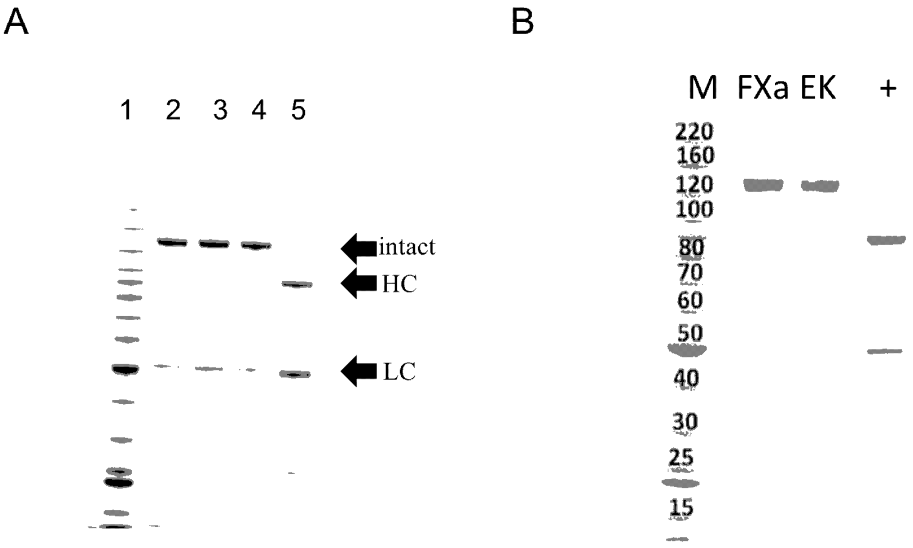
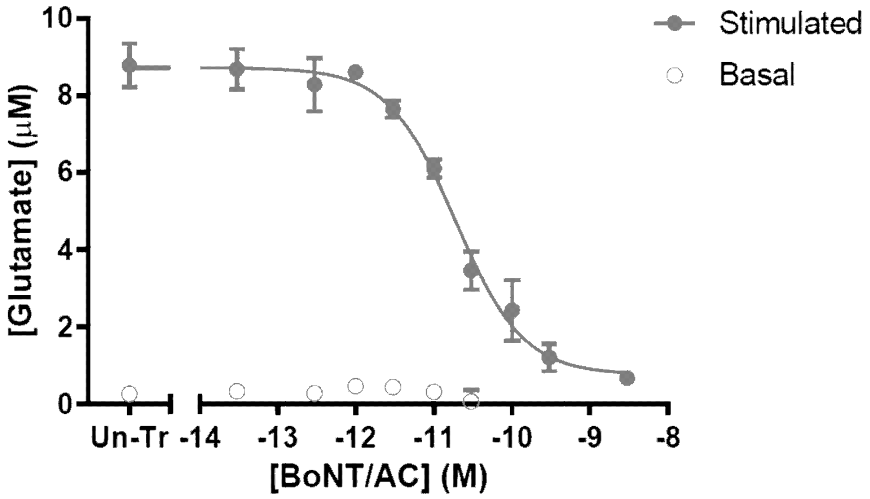


FIGURE 8

A


10
8
6
4
2
0
[Glutamate] (μM)
Un-Tr -14 -13 -12 -11 -10 -9 -8
[BoNT/AC] (M)
Stimulated
Basal

B

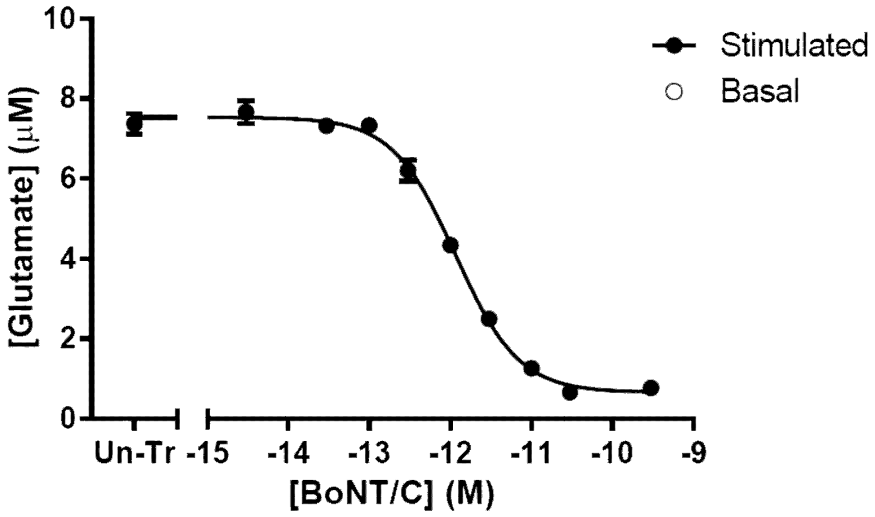

10
8
6
4
2
0
[Glutamate] (μM)
Un-Tr -15 -14 -13 -12 -11 -10 -9
[BoNT/C] (M)
Stimulated
Basal

FIGURE 9

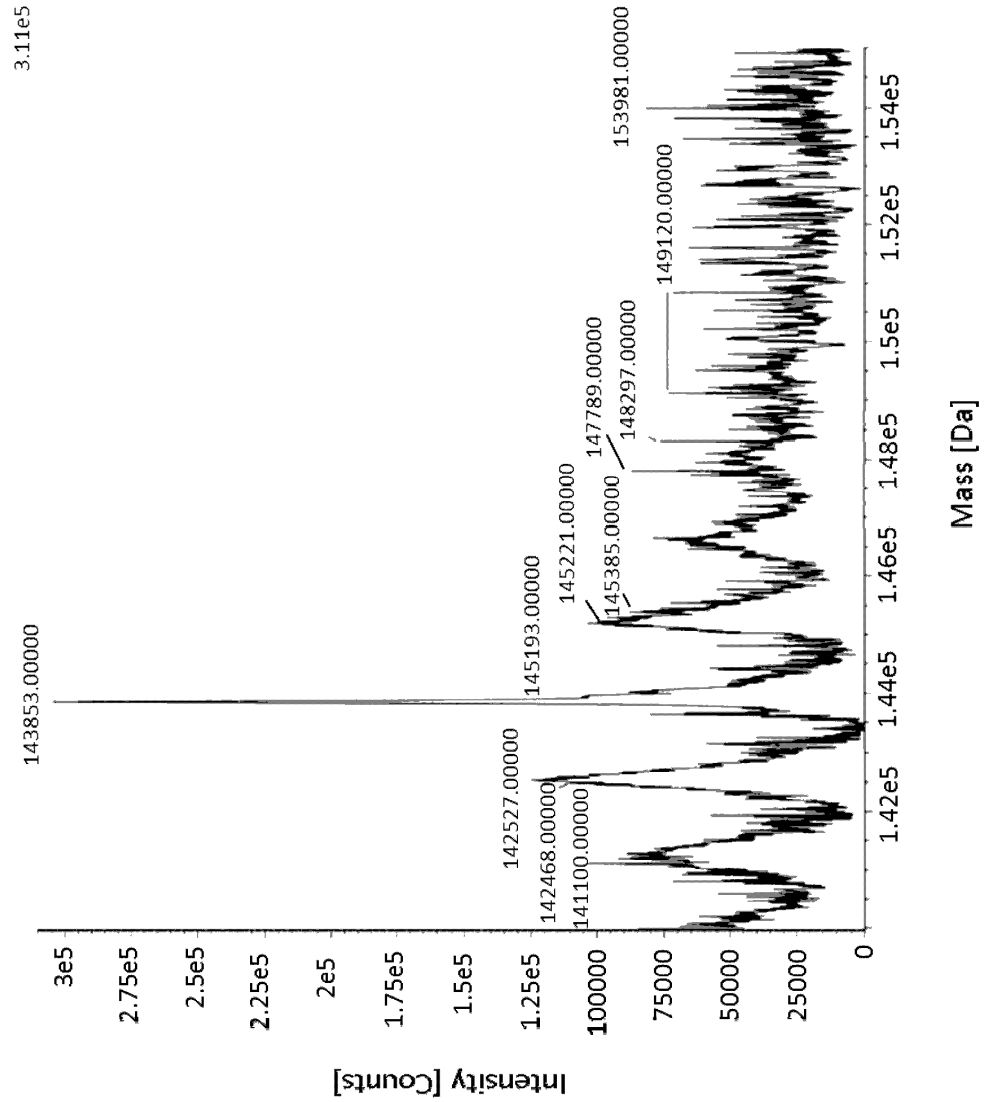

3.11e5
143853.00000
145193.00000
145221.00000
147789.00000
148297.00000
149120.00000
153981.00000
142527.00000
142468.00000
141100.00000
145385.00000
3e5
2.75e5
2.5e5
2.25e5
2e5
1.75e5
1.5e5
1.25e5
100000
75000
50000
25000
0
Intensity [Counts]
1.42e5
1.44e5
1.46e5
1.48e5
1.5e5
1.52e5
1.54e5
Mass [Da]

FIGURE 10

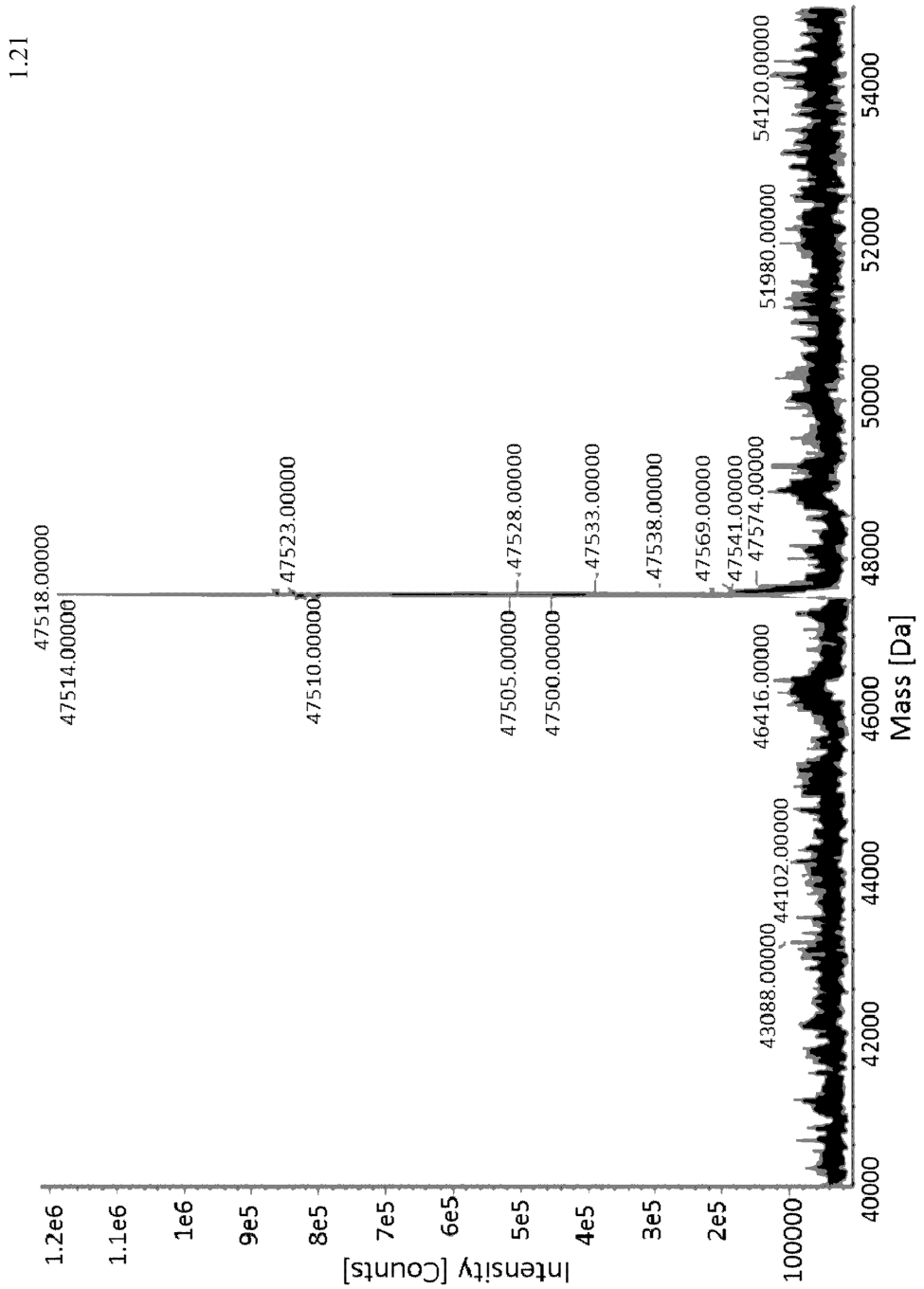

1.21
47518.00000
47514.00000
47523.00000
47510.00000
47528.00000
47505.00000
47533.00000
47500.00000
47538.00000
47569.00000
47541.00000
47574.00000
46416.00000
44102.00000
43088.00000
51980.00000
54120.00000
Intensity [Counts]
Mass [Da]

FIGURE 10 CONTINUED

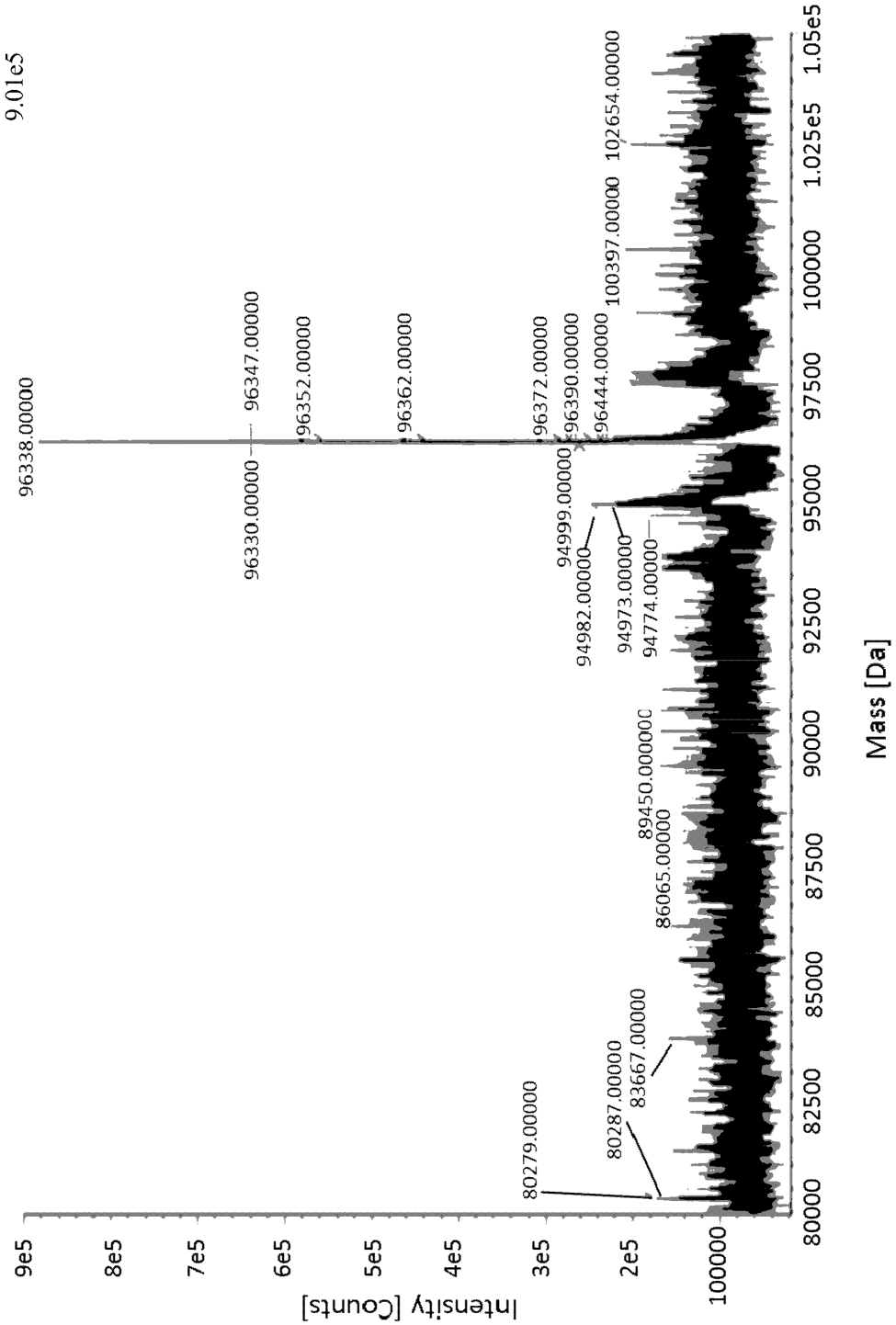


FIGURE 11

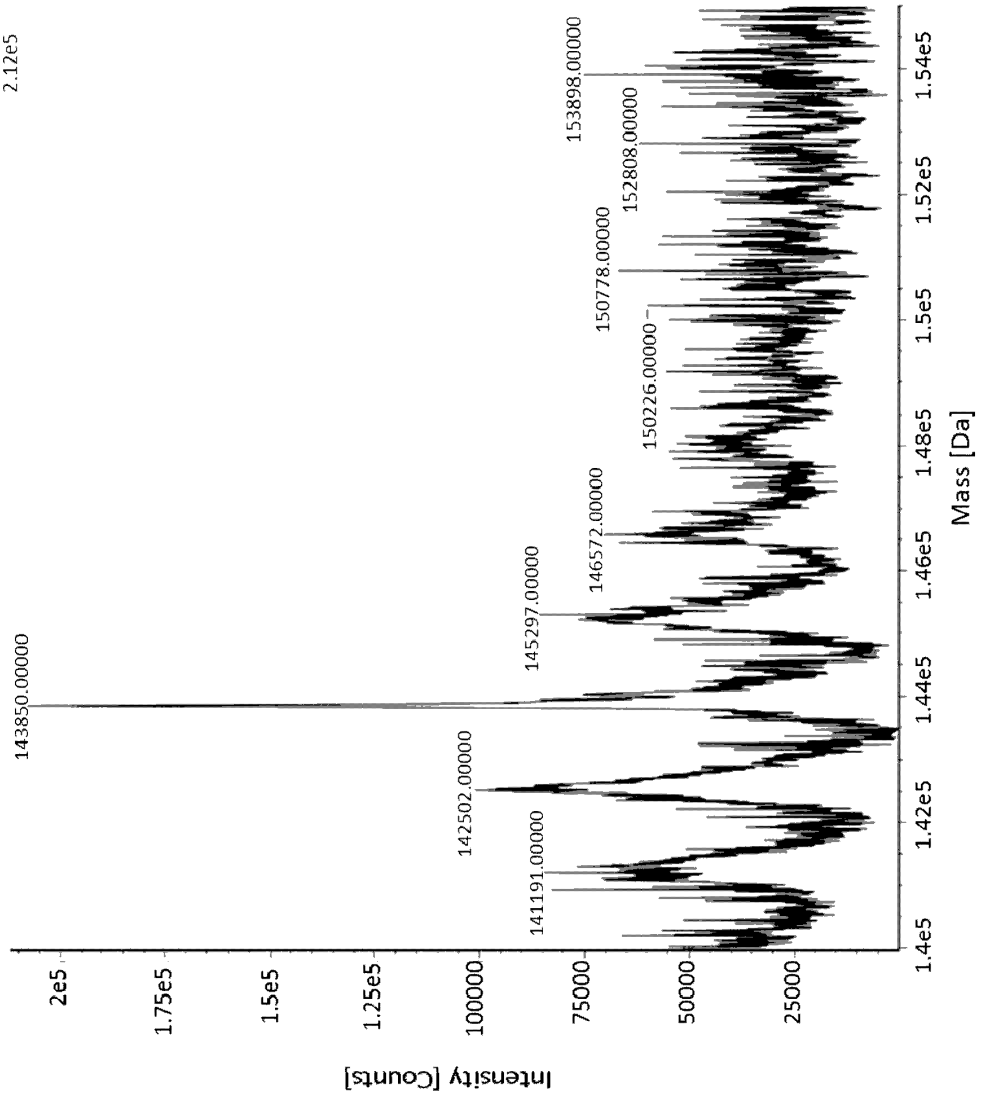

2.12e5
143850.00000
142502.00000
141191.00000
145297.00000
146572.00000
150226.00000
150778.00000
152808.00000
153898.00000
2e5
1.75e5
1.5e5
1.25e5
100000
75000
50000
25000
Intensity [Counts]
1.4e5
1.42e5
1.44e5
1.46e5
1.48e5
1.5e5
1.52e5
1.54e5
Mass [Da]

FIGURE 12

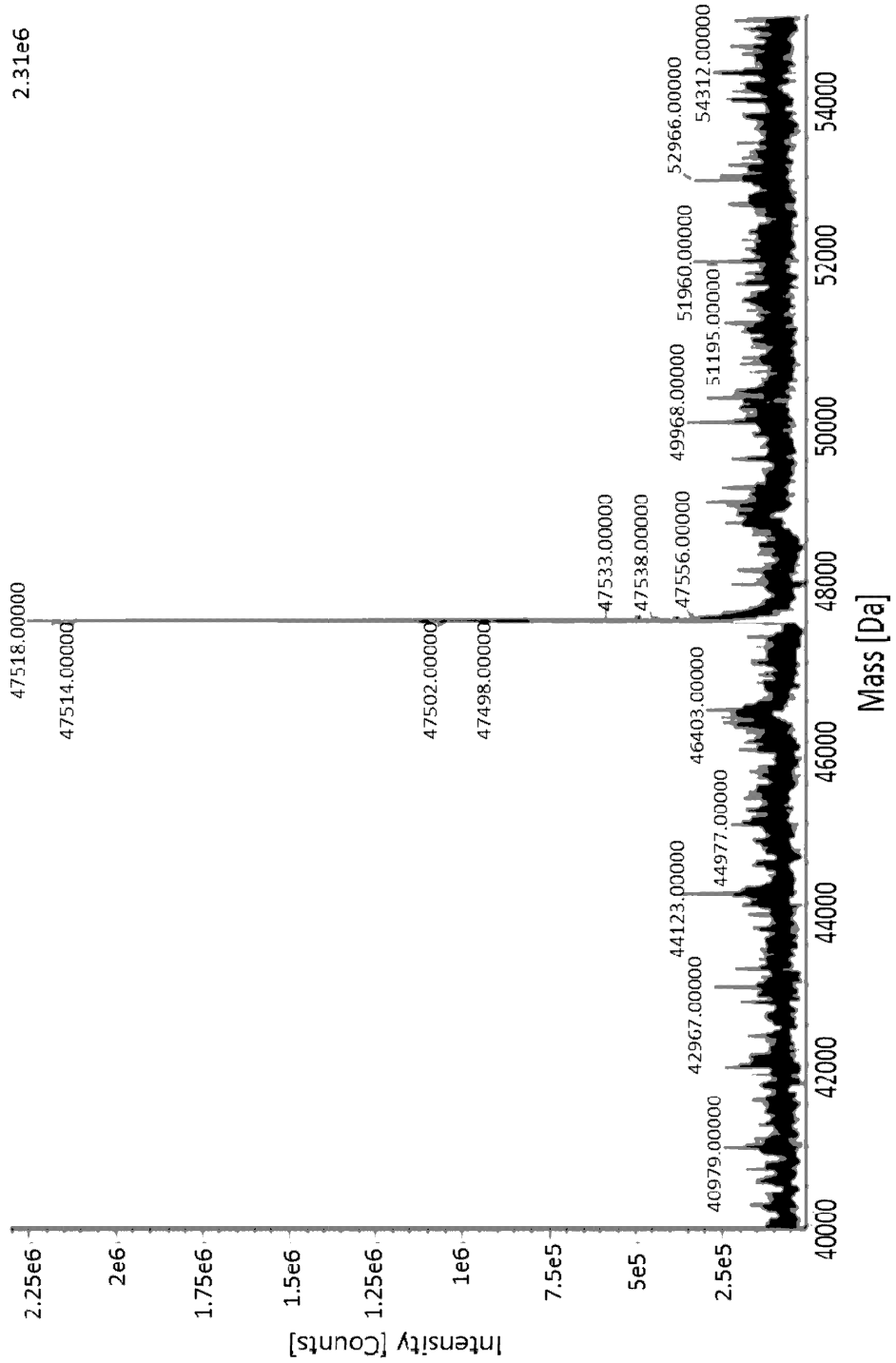

2.31e6
47518.00000
47514.00000
47502.00000
47498.00000
47533.00000
47538.00000
47556.00000
49968.00000
51960.00000
51195.00000
52966.00000
54312.00000
46403.00000
44977.00000
44123.00000
42967.00000
40979.00000
40000
42000
44000
46000
48000
50000
52000
54000
Mass [Da]
2.25e6
2e6
1.75e6
1.5e6
1.25e6
1e6
7.5e5
5e5
2.5e5
Intensity [Counts]

FIGURE 12 CONTINUED

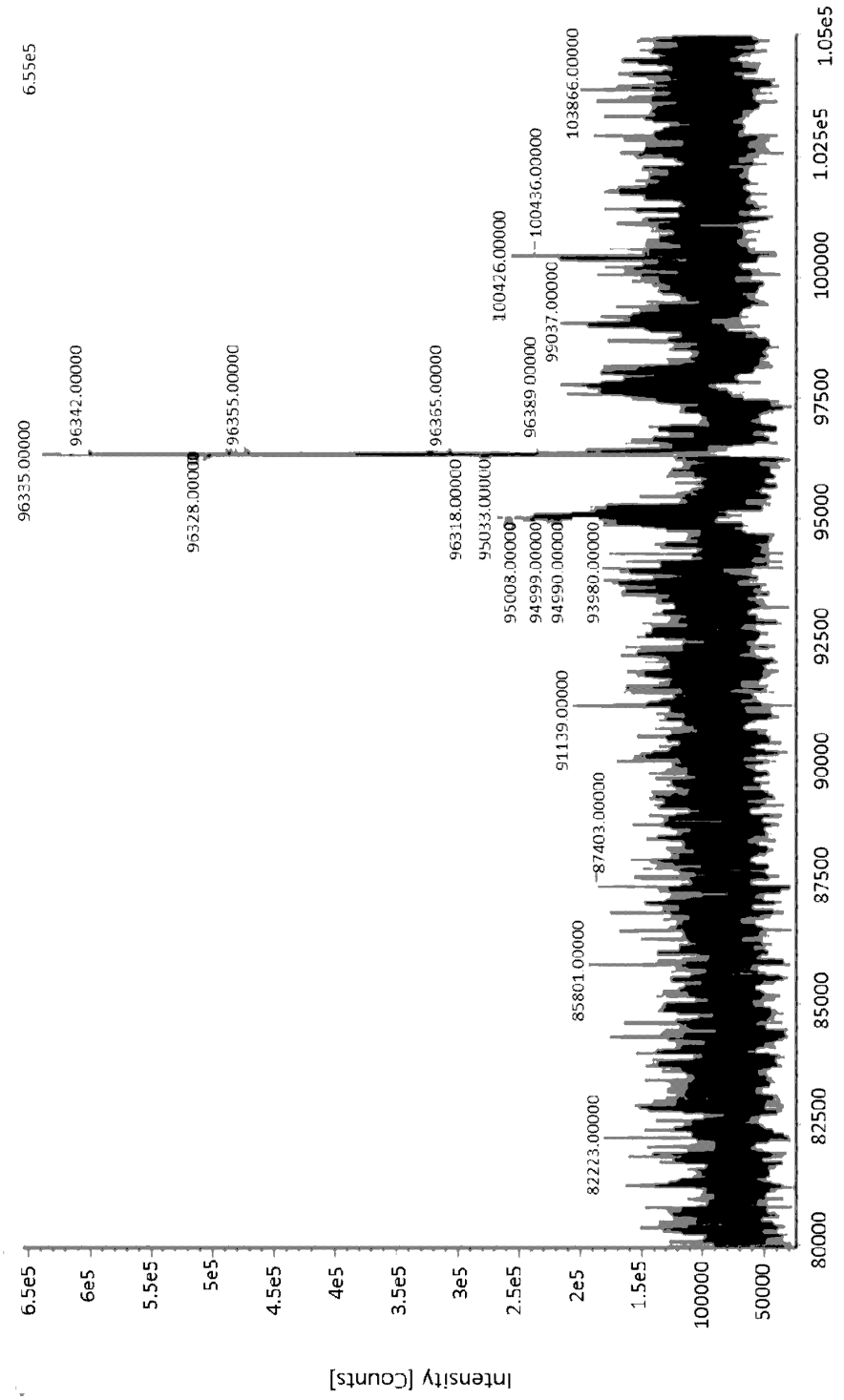


FIGURE 13

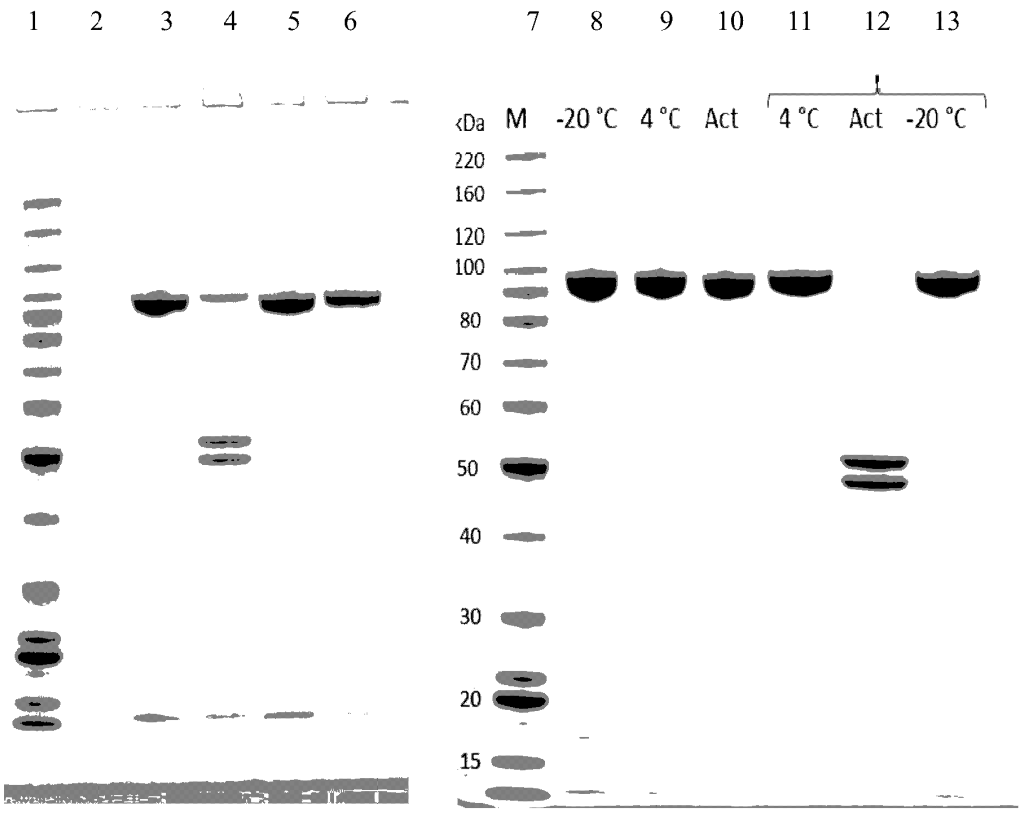

1 2 3 4 5 6
7 8 9 10 11 12 13
kDa
M
-20 °C
4 °C
Act
4 °C
Act
-20 °C
220
160
120
100
80
70
60
50
40
30
20
15

FIGURE 14

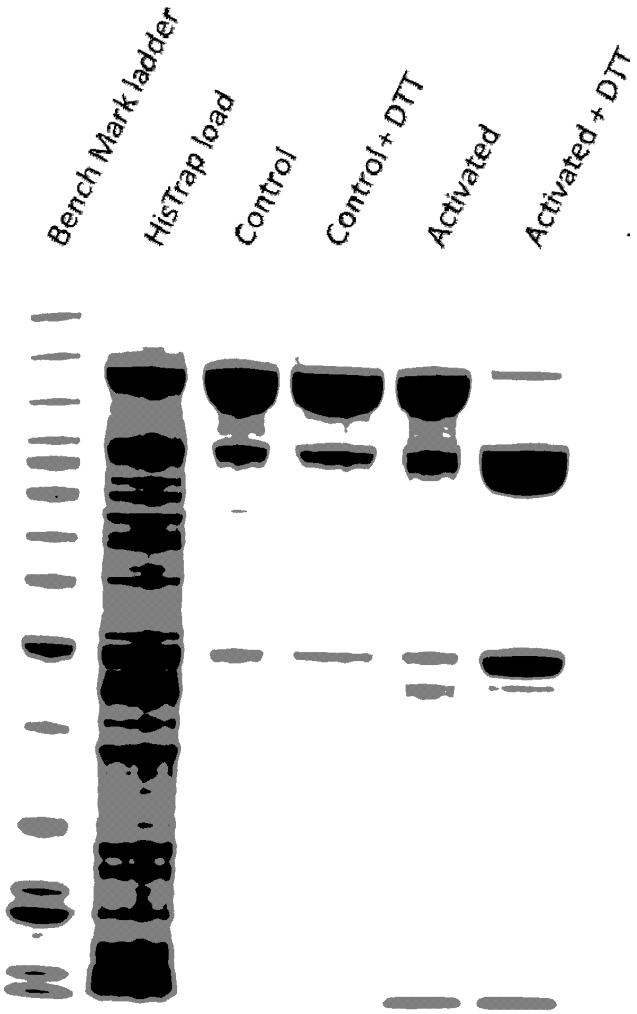
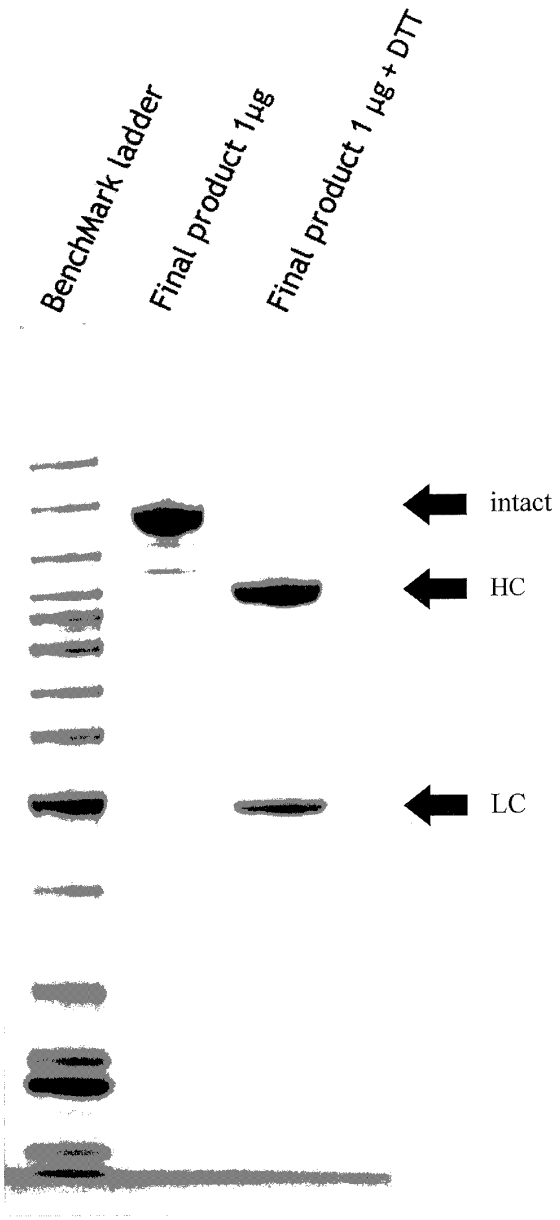

Bench Mark ladder
HisTrap load
Control
Control + DTT
Activated
Activated + DTT

FIGURE 15


BenchMark ladder
Final product 1µg
Final product 1 µg + DTT
intact
HC
LC