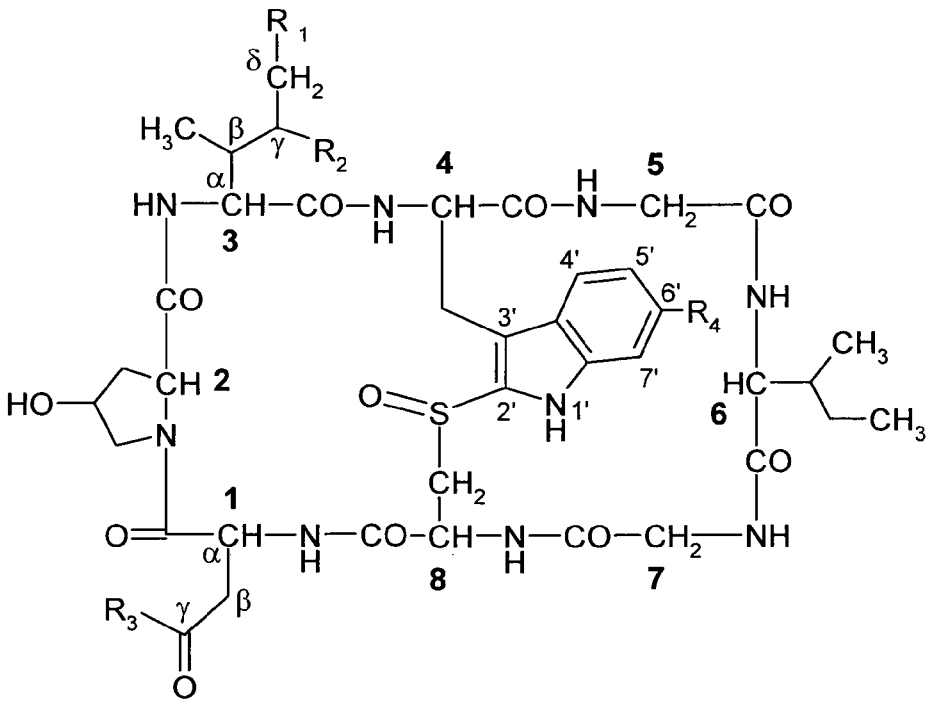
Figure 1



	R_1	R_2	R_3	R_4
α-amanitin	OH	OH	NH_2	OH
β-amanitin	OH	OH	OH	OH
γ-amanitin	H	OH	NH_2	OH
ε-amanitin	H	OH	OH	OH
amanin	OH	OH	OH	H
amaninamide	OH	OH	NH_2	H
amanullin	H	H	NH_2	OH
amanullinic acid	H	H	OH	OH

Figure 2

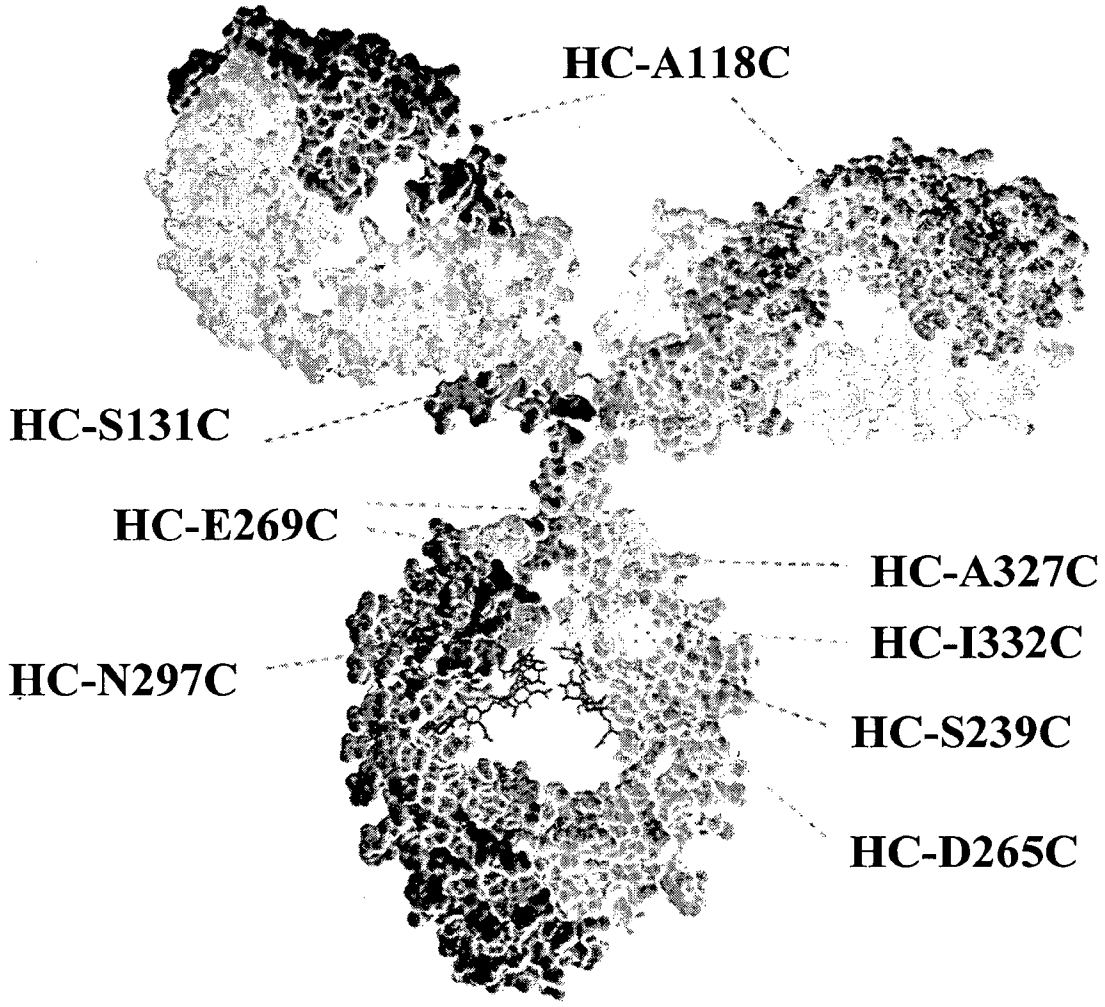
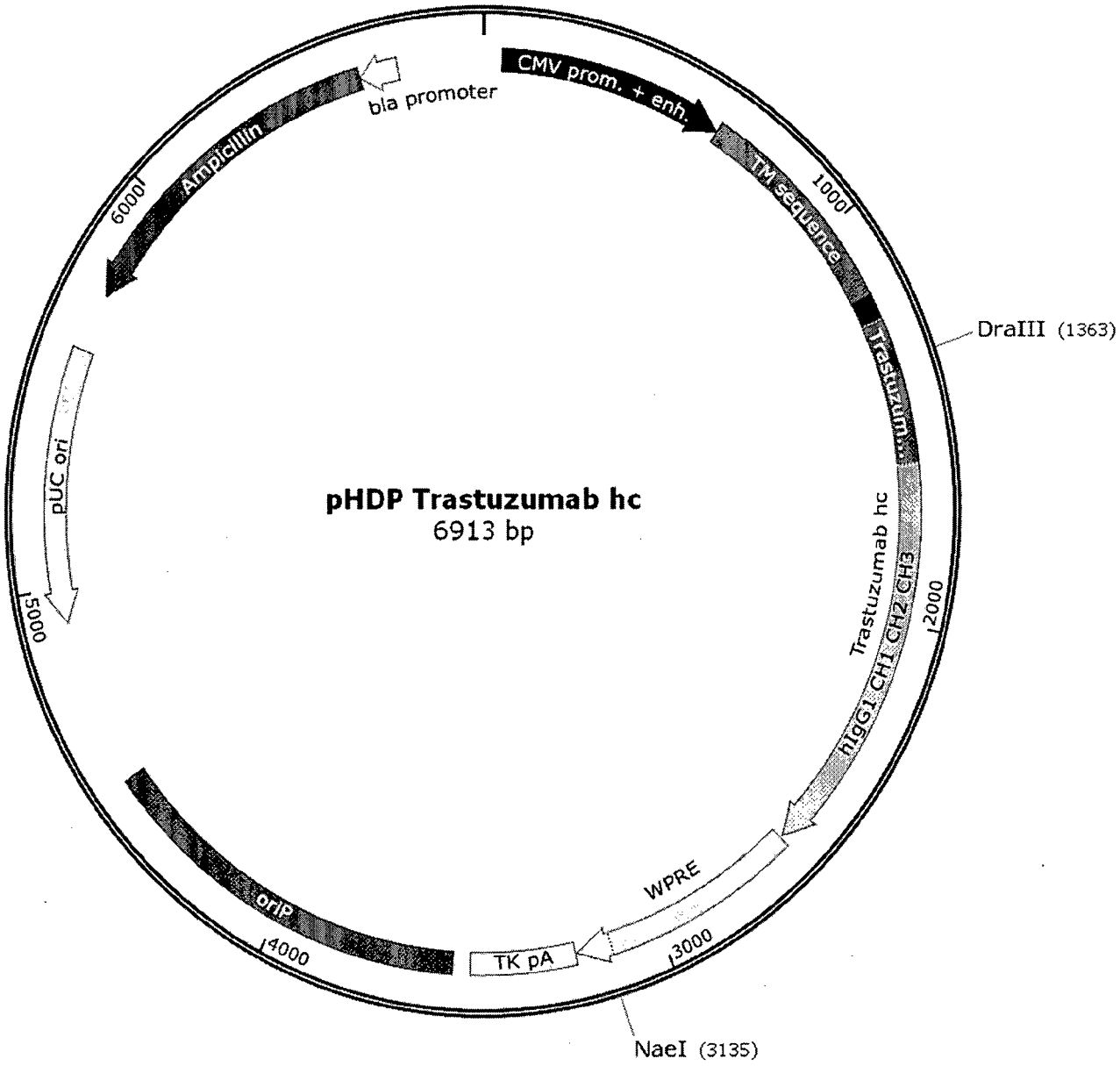

HC-A118C
HC-S131C
HC-E269C
HC-A327C
HC-I332C
HC-N297C
HC-S239C
HC-D265C

Figure 3


pHDP Trastuzumab hc
6913 bp
CMV prom. + enh.
bla promoter
Ampicillin
pUC ori
oriP
TK pA
WPRE
TM sequence
Trastuzumab hc
hIgG1 CH1 CH2 CH3
DraIII (1363)
NaeI (3135)
1000
2000
3000
4000
5000
6000

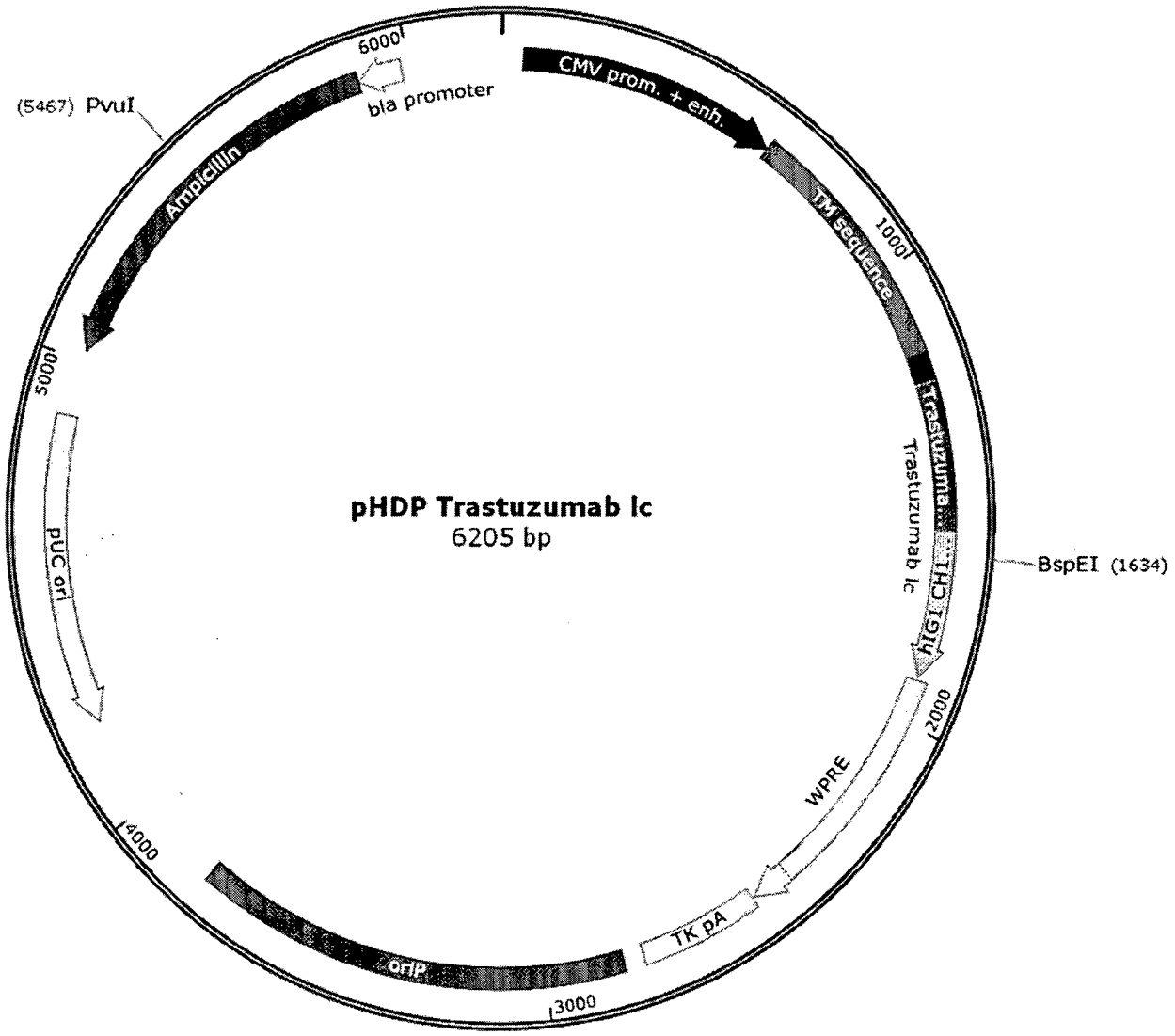
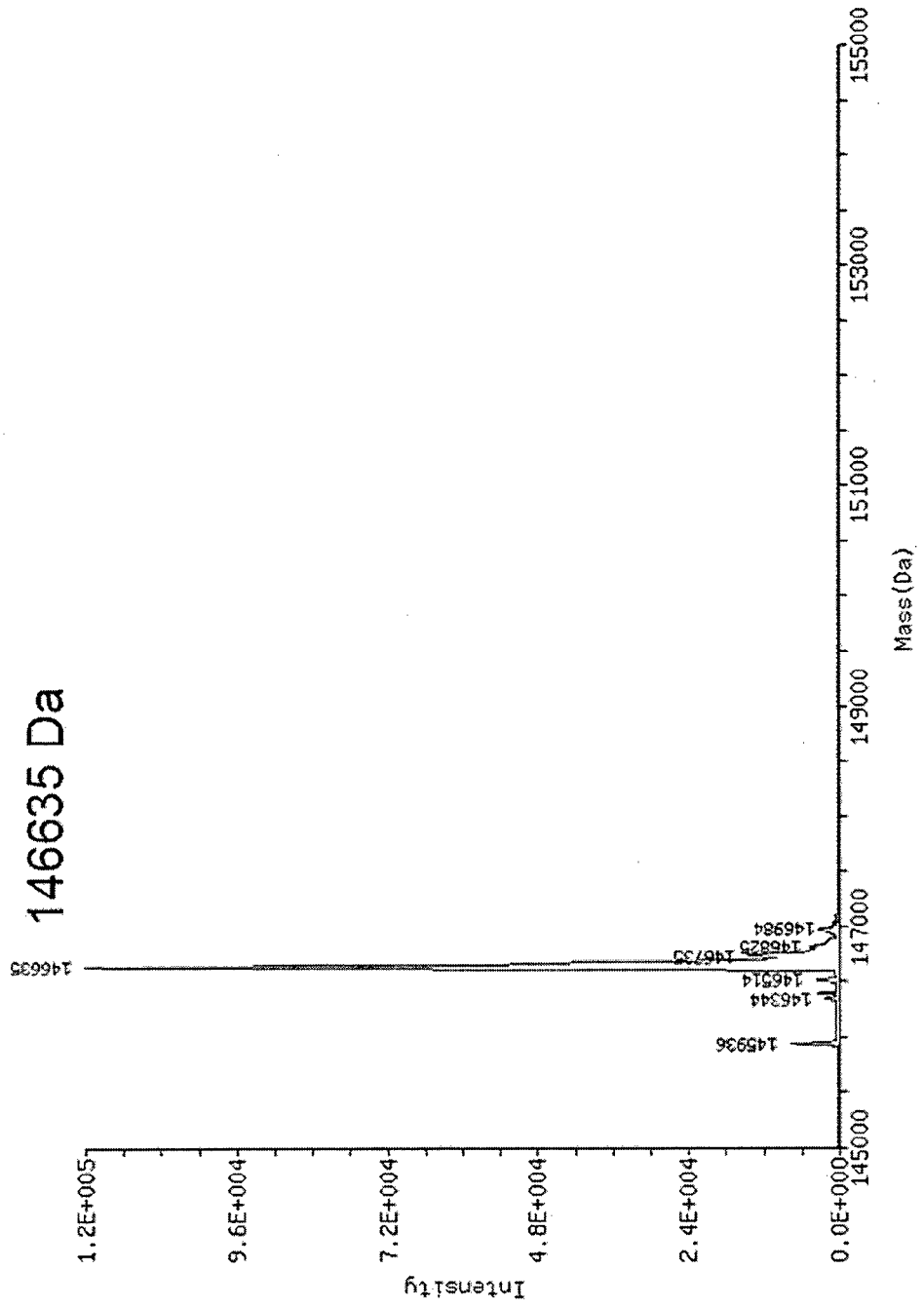

pHDP Trastuzumab lc
6205 bp
6000
bla promoter
CMV prom. + enh.
1000
TM sequence
Trastuzumab
Trastuzumab lc
hIG1 CH1
BspEI (1634)
2000
WPRE
TK pA
3000
oriP
4000
pUC ori
5000
Ampicillin
(5467) PvuI

Figure 4(A)

(a)


146635 Da
146635
145936
146344
146514
146733
146825
146984
Intensity
1.2E+005
9.6E+004
7.2E+004
4.8E+004
2.4E+004
0.0E+000
145000
147000
149000
151000
153000
155000
Mass(Da)

(b)

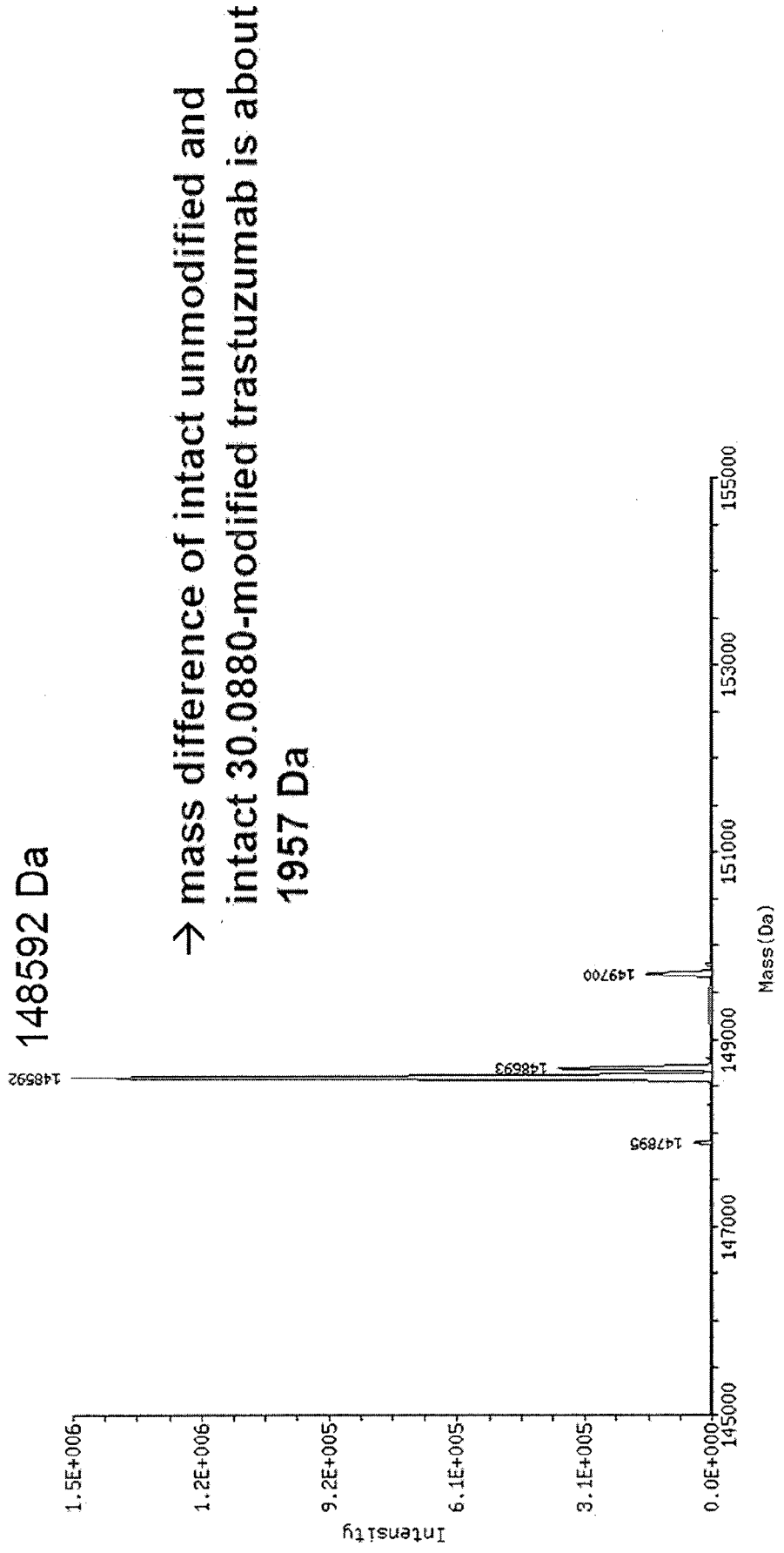
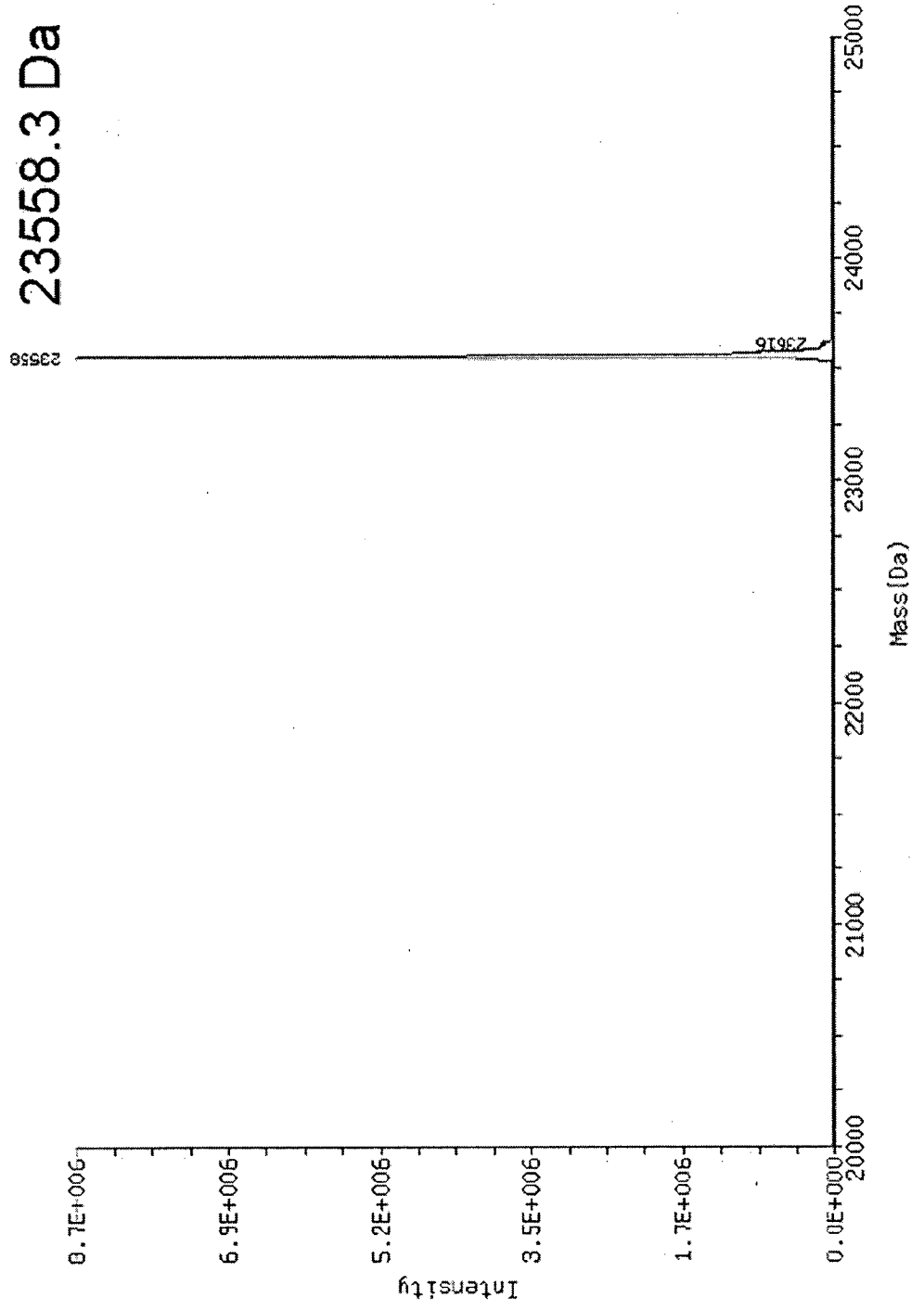

148592 Da
→ mass difference of intact unmodified and intact 30.0880-modified trastuzumab is about 1957 Da
148592
148693
149700
147895
Intensity
Mass(Da)
1.5E+006
1.2E+006
9.2E+005
6.1E+005
3.1E+005
0.0E+000
145000
147000
149000
151000
153000
155000

Figure 4(B)

(a)


23558.3 Da
23558
23616
Intensity
Mass(Da)
0.7E+006
6.9E+006
5.2E+006
3.5E+006
1.7E+006
0.0E+000
20000
21000
22000
23000
24000
25000

(b)

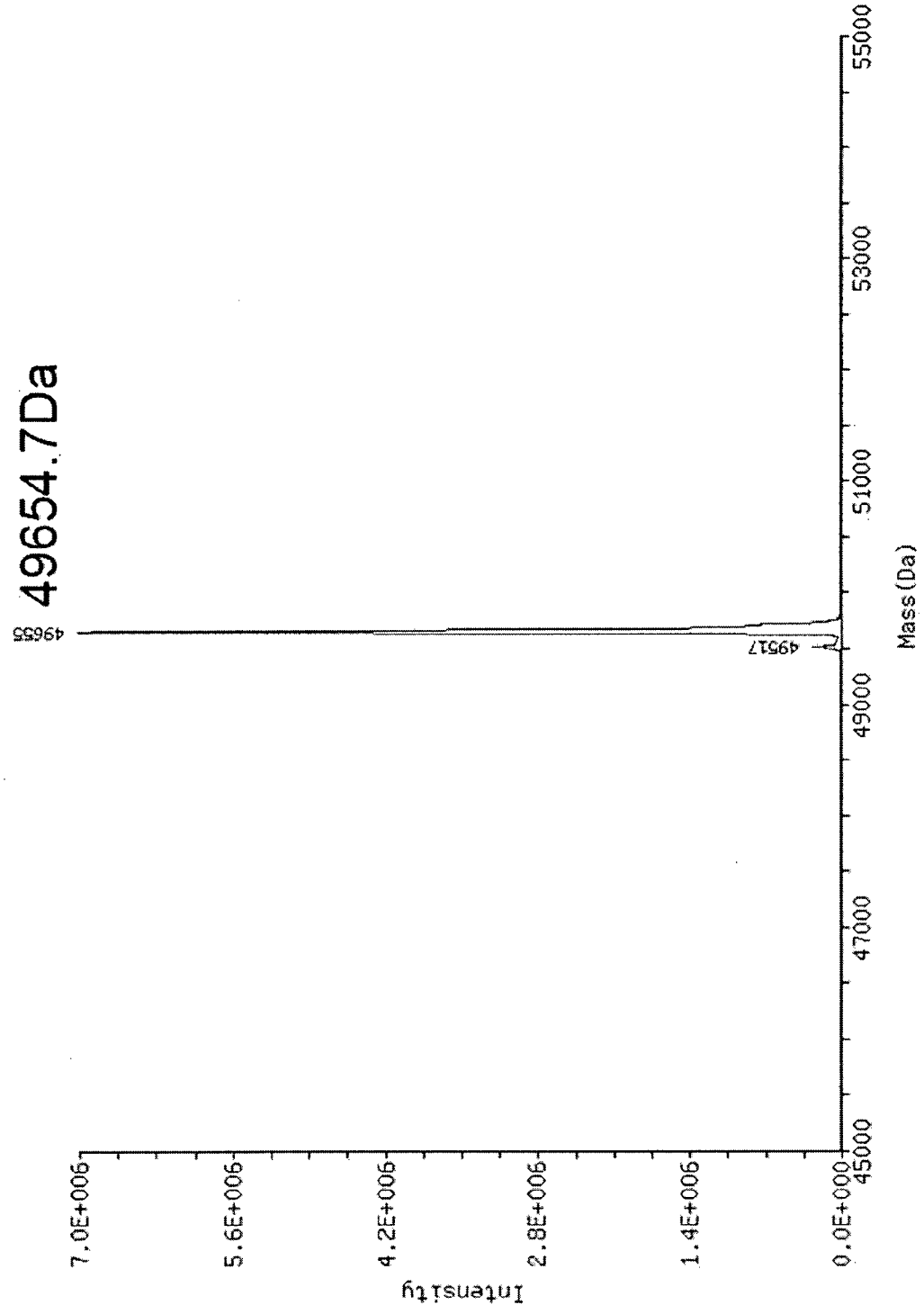
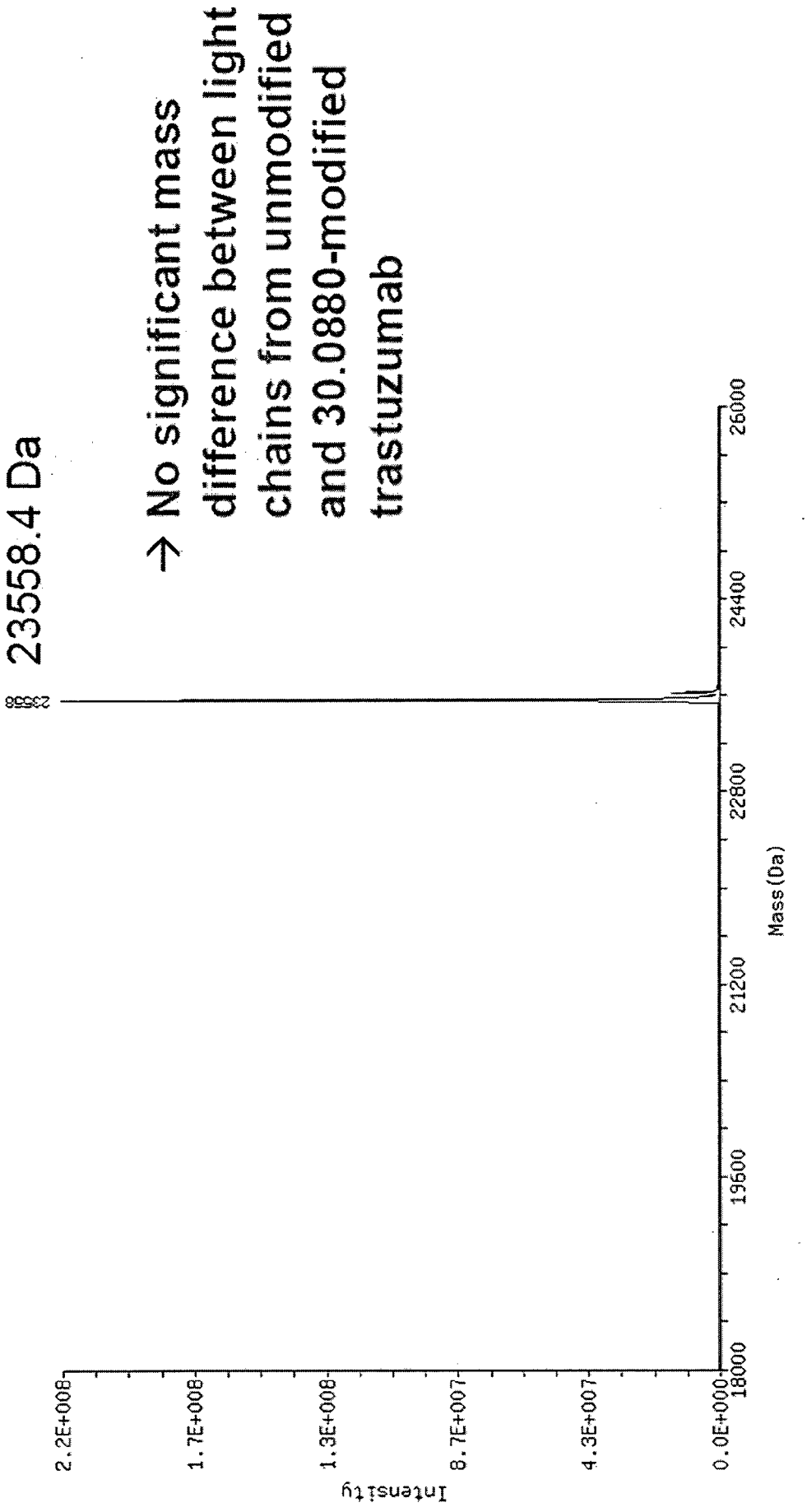

49654.7Da
49655
49517
Intensity
7.0E+006
5.6E+006
4.2E+006
2.8E+006
1.4E+006
0.0E+000
45000
47000
49000
51000
53000
55000
Mass (Da)

Figure 4(C)

(a)


23558.4 Da
→ No significant mass difference between light chains from unmodified and 30.0880-modified trastuzumab
23558
Intensity
Mass (Da)
2.2E+008
1.7E+008
1.3E+008
8.7E+007
4.3E+007
0.0E+000
18000
19600
21200
22800
24400
26000

(b)

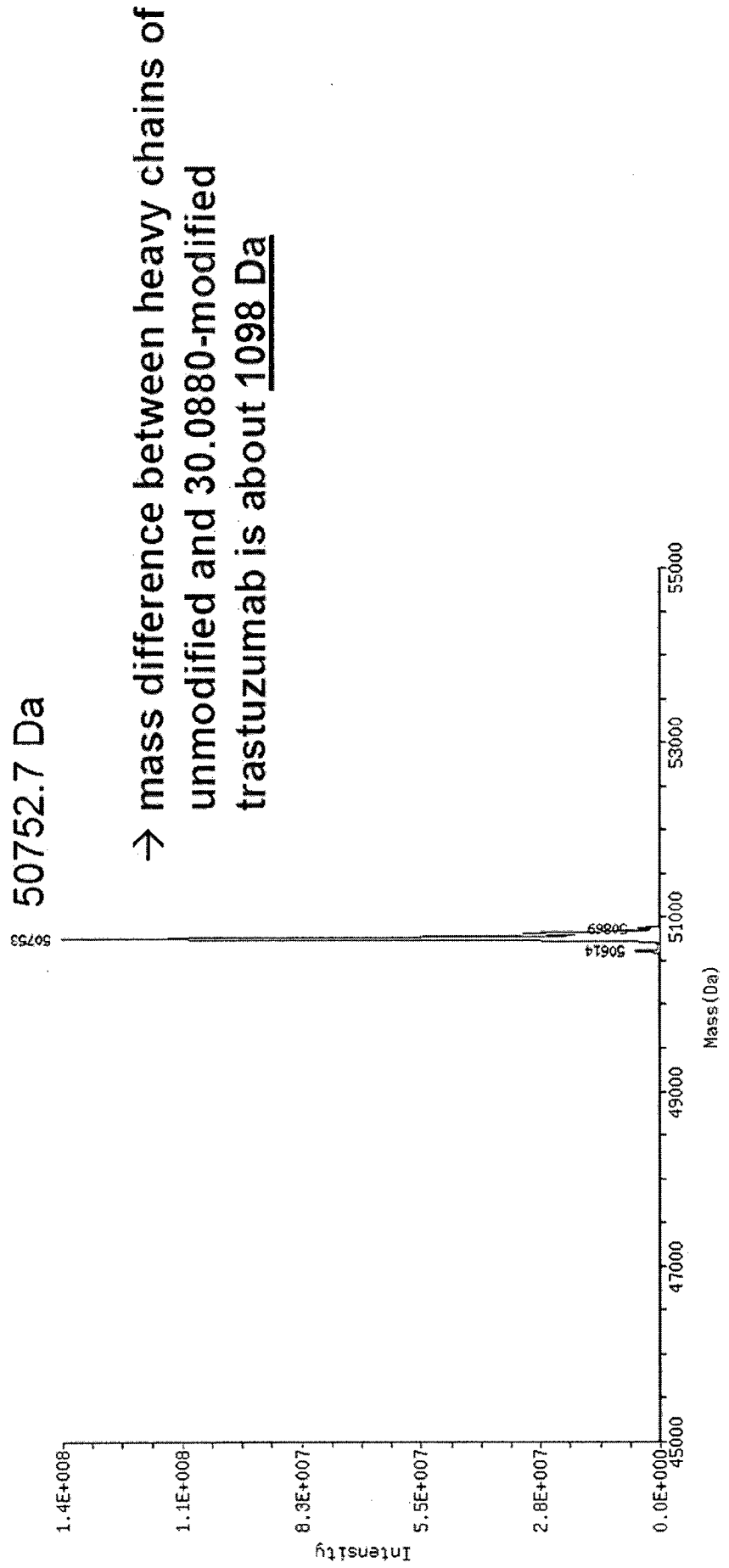
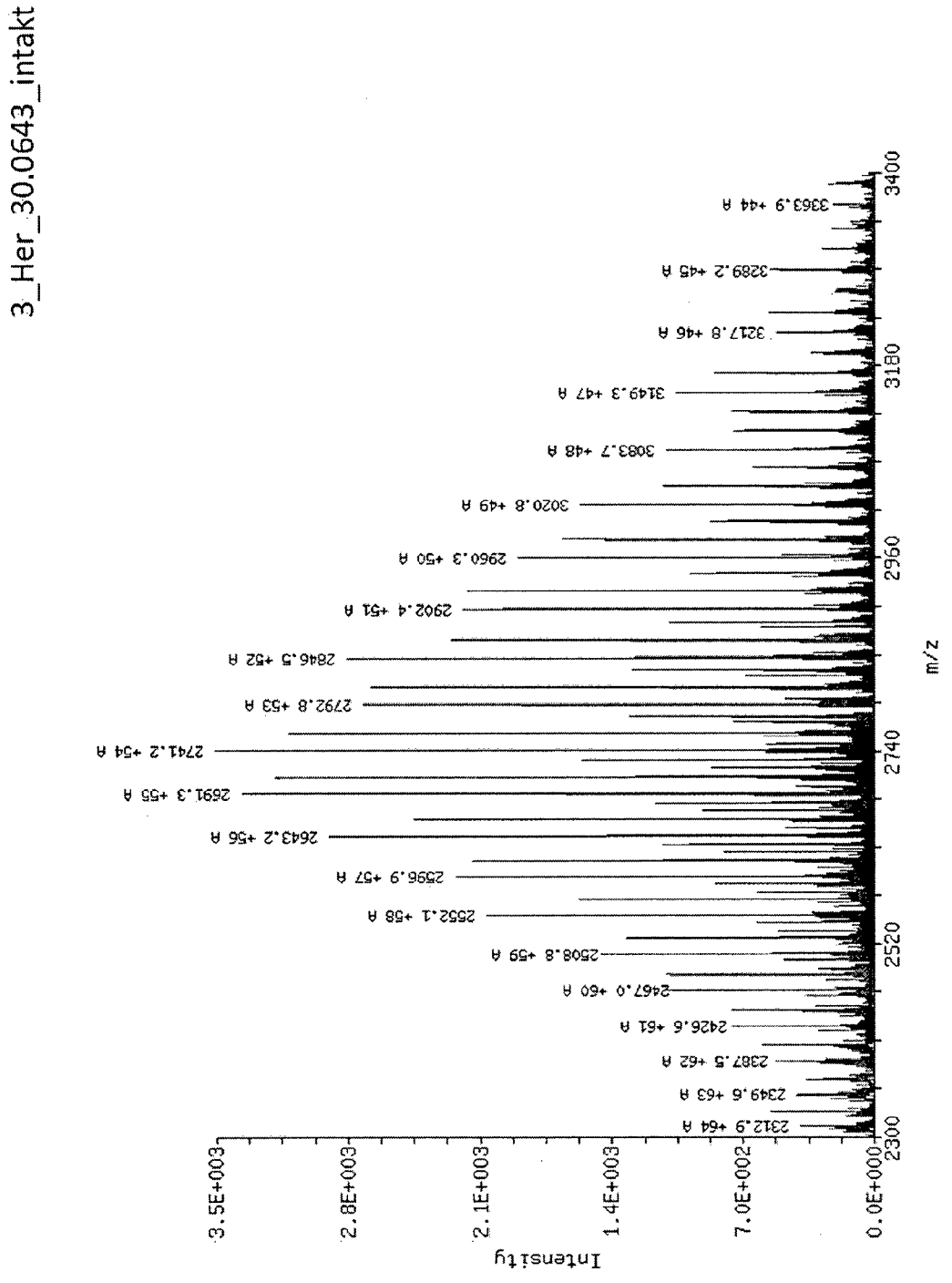
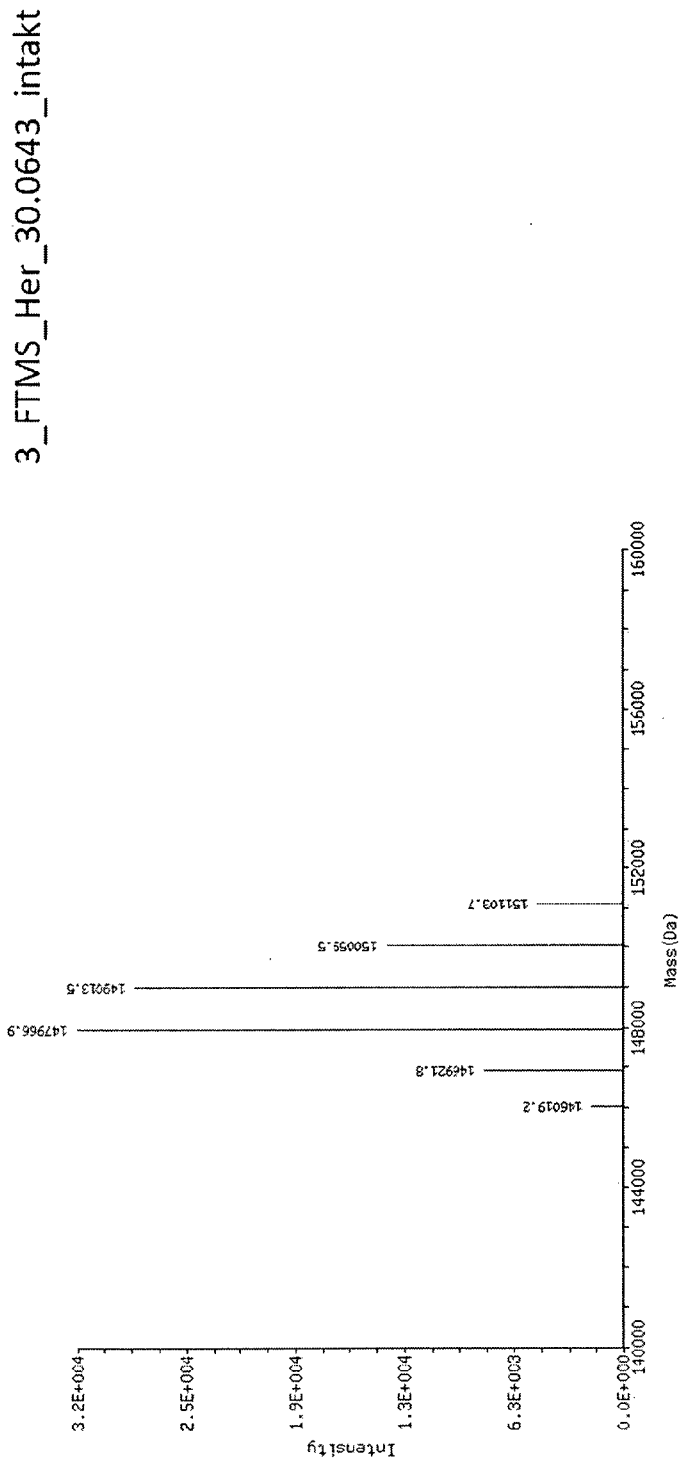

50752.7 Da
→ mass difference between heavy chains of unmodified and 30.0880-modified trastuzumab is about 1098 Da
50753
50869
50614
Intensity
1.4E+008
1.1E+008
8.3E+007
5.5E+007
2.8E+007
0.0E+000
45000
47000
49000
51000
53000
55000
Mass(Da)

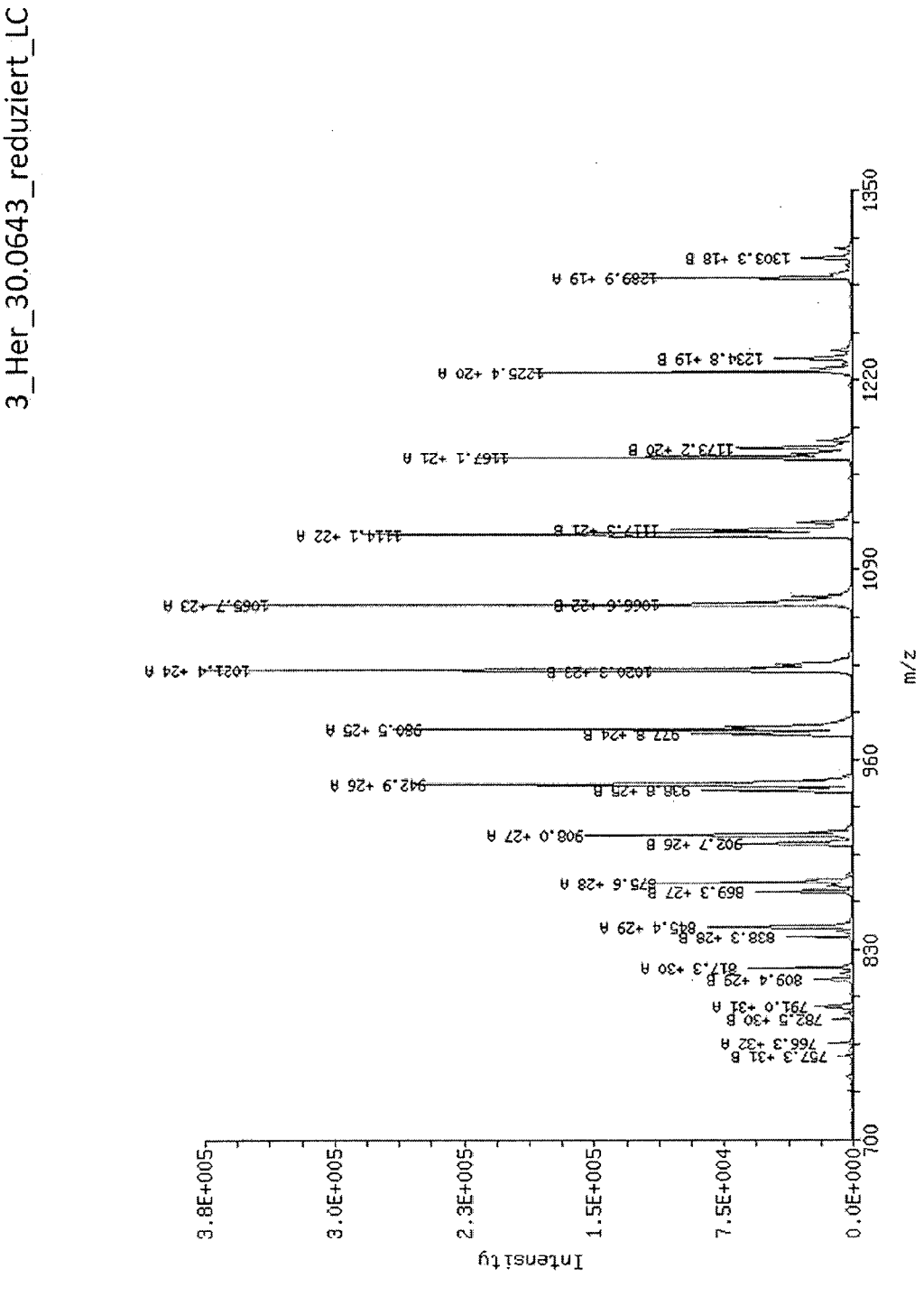
Figure 5(A)

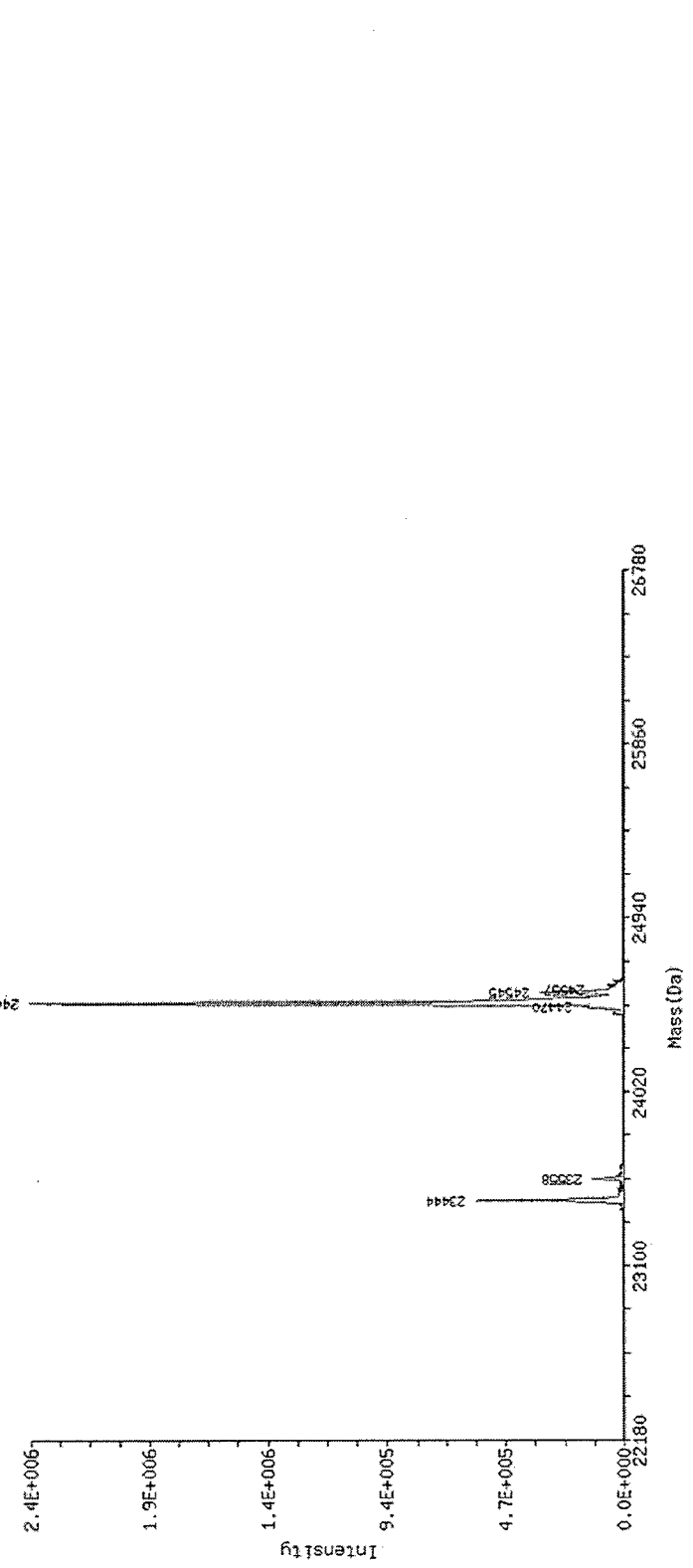

3_Her_30.0643_intakt
Intensity
3.5E+003
2.8E+003
2.1E+003
1.4E+003
7.0E+002
0.0E+000
2300
2520
2740
2960
3180
3400
m/z
2312.9 +64 A
2349.6 +63 A
2387.5 +62 A
2426.6 +61 A
2467.0 +60 A
2508.8 +59 A
2552.1 +58 A
2596.9 +57 A
2643.2 +56 A
2691.3 +55 A
2741.2 +54 A
2792.8 +53 A
2846.5 +52 A
2902.4 +51 A
2960.3 +50 A
3020.8 +49 A
3083.7 +48 A
3149.3 +47 A
3217.8 +46 A
3289.2 +45 A
3363.9 +44 A



Mass (Da)	Intensity	Score	Delta Mass	%Relative	%Total
147966.9	3.16E+004	26.33	0.0	100.00	35.89
149013.5	2.82E+004	25.40	1046.6	89.24	32.03
150059.5	1.36E+004	19.05	2092.6	43.04	15.45
146921.8	7.96E+003	14.81	-1045.1	25.19	9.04
151103.7	4.88E+003	10.38	3136.8	15.44	5.54
146019.2	1.81E+003	2.32	-1947.7	5.73	2.06

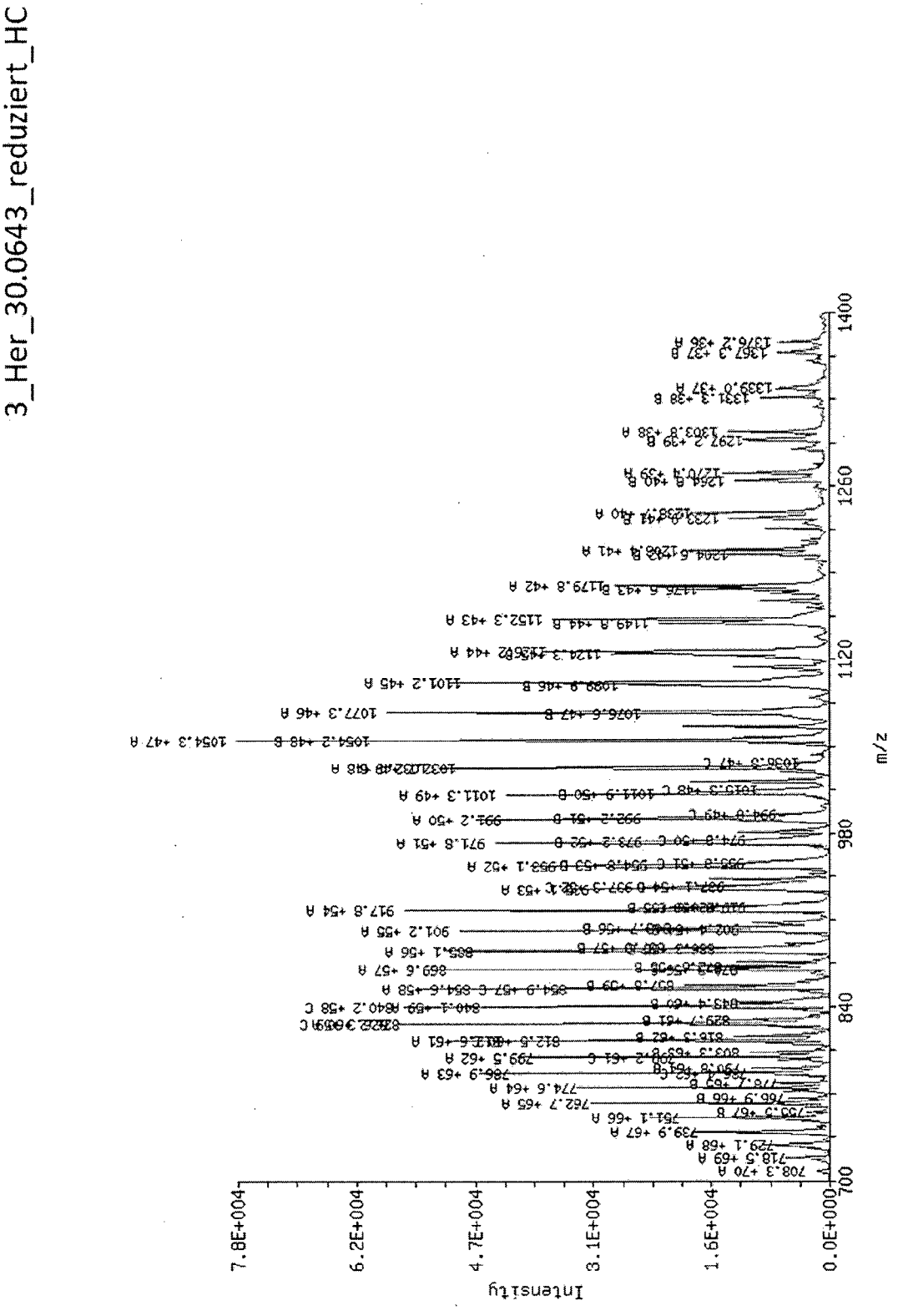
Figure 5(B)

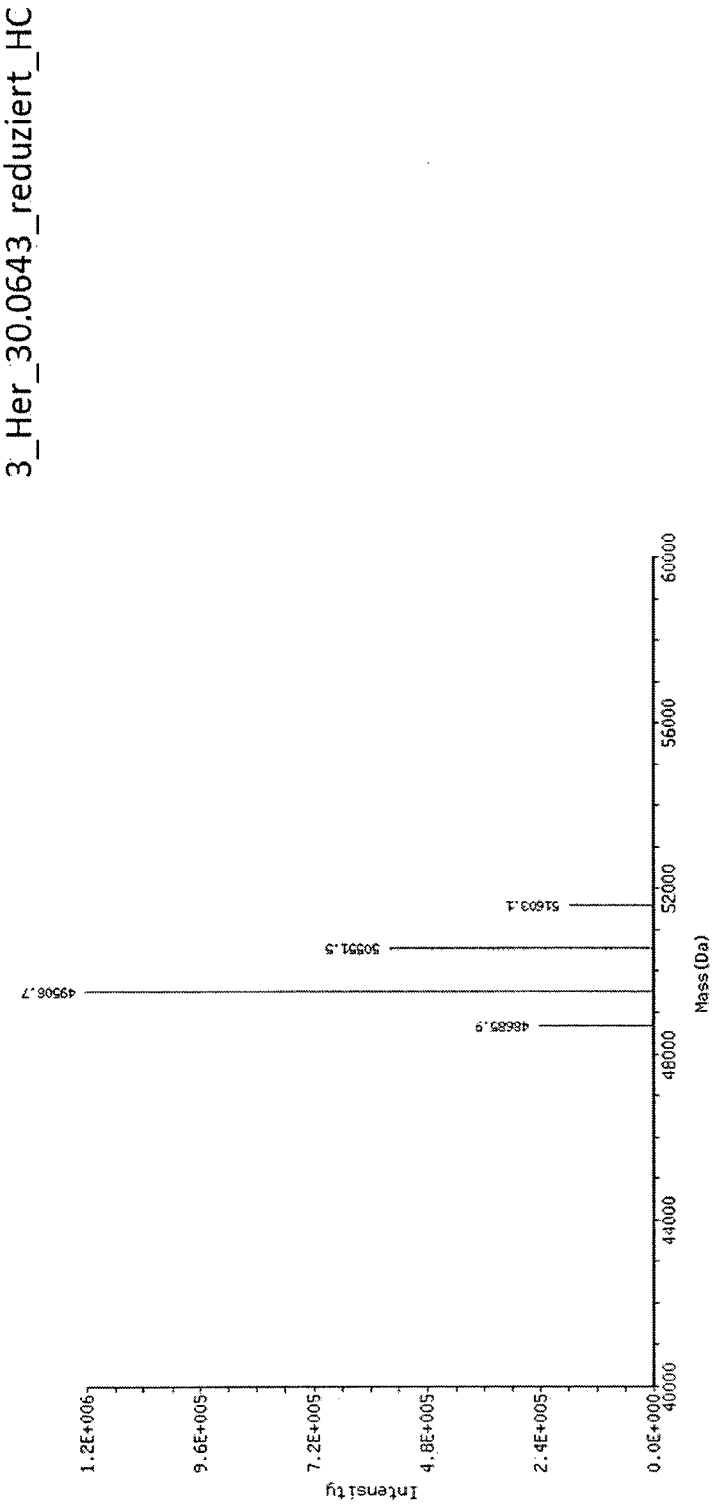

3_Her_30.0643_reduziert_LC
Intensity
3.8E+005
3.0E+005
2.3E+005
1.5E+005
7.5E+004
0.0E+000
700
830
960
1090
1220
1350
m/z
757.3 +31 B
766.3 +32 A
782.5 +30 B
791.0 +31 A
809.4 +29 B
817.3 +30 A
838.3 +28 B
845.4 +29 A
869.3 +27 B
875.6 +28 A
902.7 +26 B
908.0 +27 A
938.8 +25 B
942.9 +26 A
972.8 +24 B
980.5 +25 A
1020.3 +23 B
1024.4 +24 A
1066.6 +22 B
1065.7 +23 A
1117.3 +21 B
1114.1 +22 A
1173.2 +20 B
1167.1 +21 A
1234.8 +19 B
1225.4 +20 A
1303.3 +18 B
1289.9 +19 A



Mass (Da)	Intensity	Score	Delta Mass	%Relative	%Total
24488.5	2.35E+006	22.39	0.0	100.00	69.92
23443.9	5.82E+005	19.21	-1044.6	24.72	17.28
24551.7	3.12E+005	9.26	63.2	13.23	9.25
23557.1	1.19E+005	10.78	-931.4	5.07	3.54

Figure 5(C)


3_Her_30.0643_reduziert_HC
Intensity
m/z
7.8E+004
6.2E+004
4.7E+004
3.1E+004
1.6E+004
0.0E+000
700
840
980
1120
1260
1400



Mass (Da)	Intensity	Score	Delta Mass	%Relative	%Total
49508.7	1.20E+006	40.12	0.0	100.00	55.17
50551.5	5.57E+005	24.10	1042.8	46.42	25.61
48685.9	2.41E+005	6.35	-822.8	20.08	11.08
51603.1	1.77E+005	4.03	2094.4	14.75	8.14

Figure 6

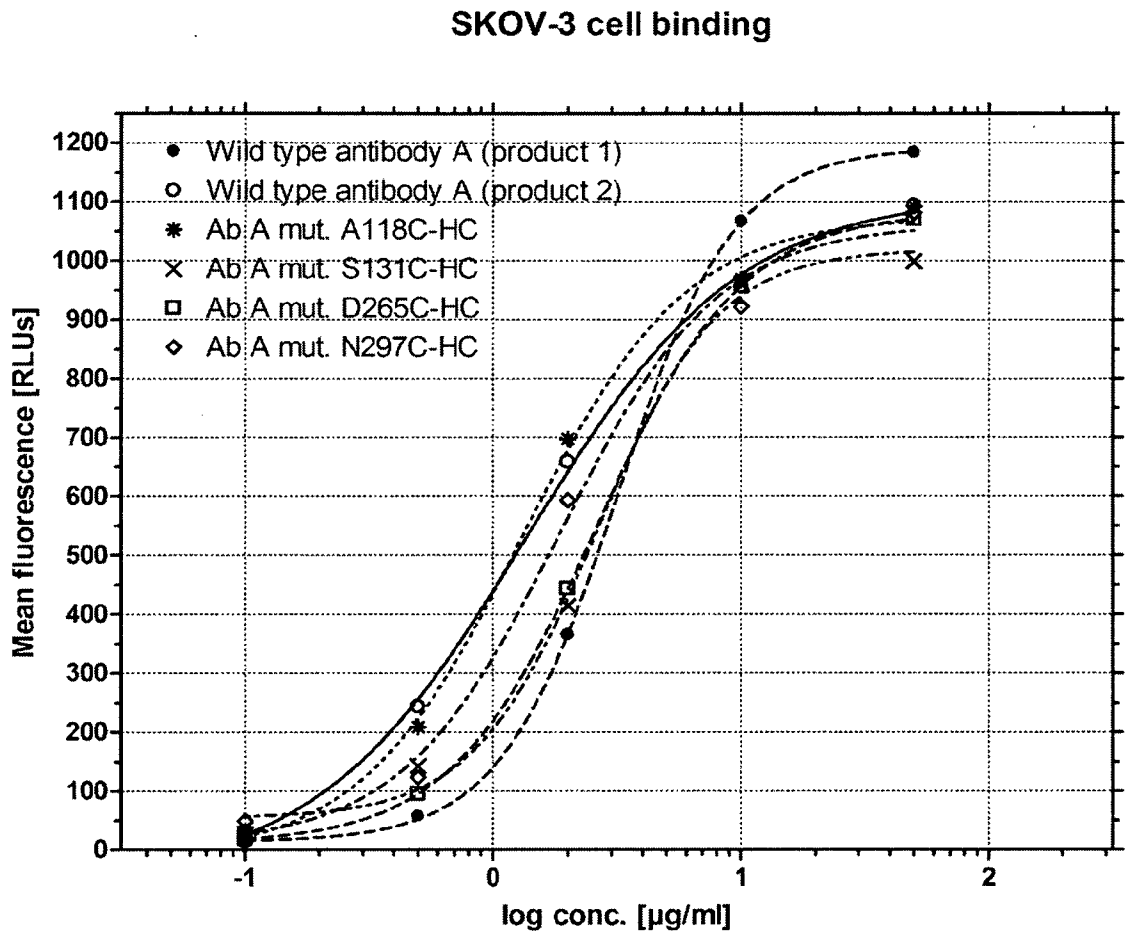
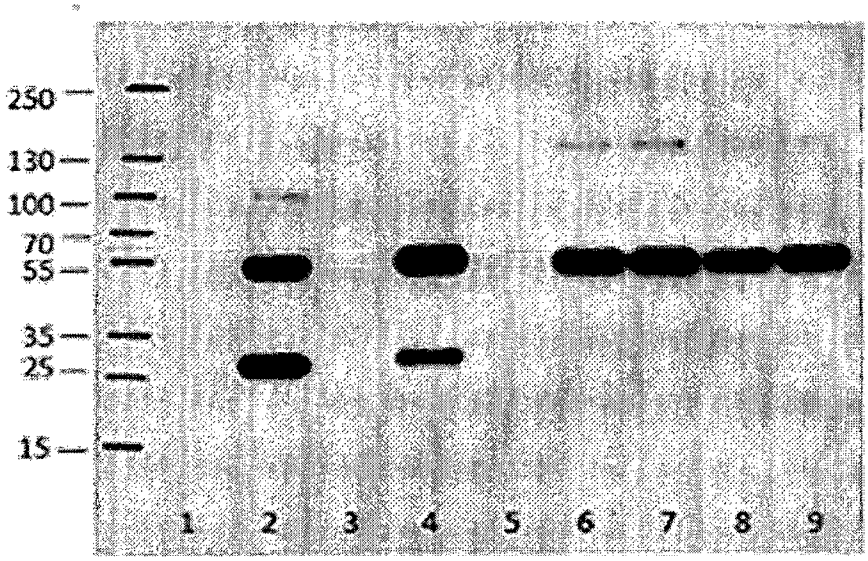

SKOV-3 cell binding
Wild type antibody A (product 1)
Wild type antibody A (product 2)
Ab A mut. A118C-HC
Ab A mut. S131C-HC
Ab A mut. D265C-HC
Ab A mut. N297C-HC
Mean fluorescence [RLUs]
log conc. [µg/ml]
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
-1
0
1
2

Figure 7(A)


250
130
100
70
55
35
25
15
1
2
3
4
5
6
7
8
9

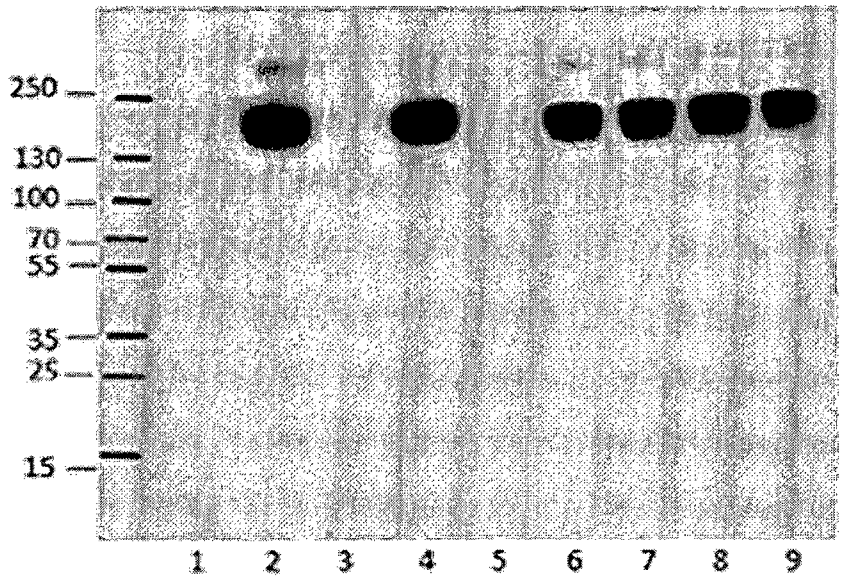
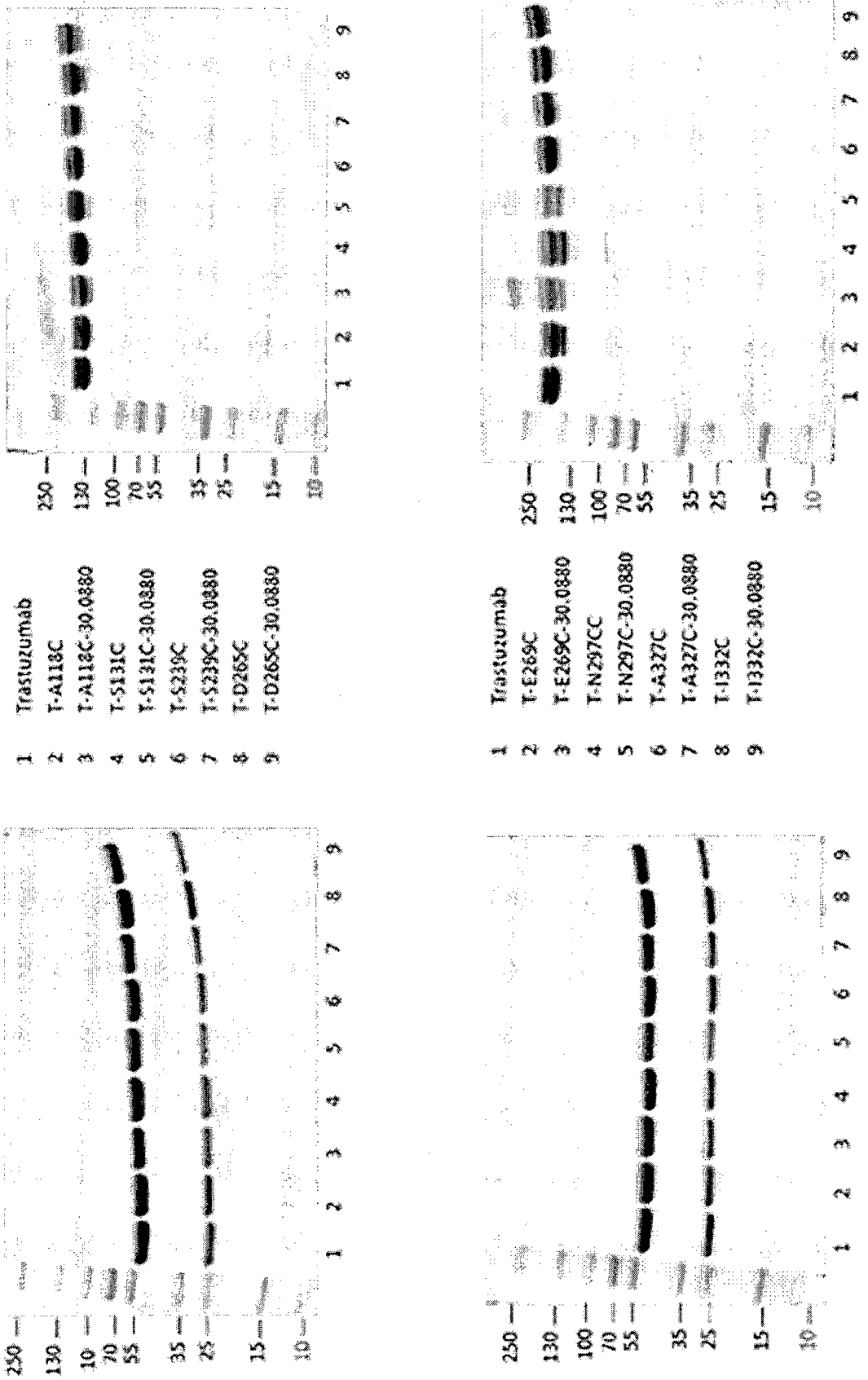

250
130
100
70
55
35
25
15
1
2
3
4
5
6
7
8
9

Figure 7(B)


1 Trastuzumab
2 T-A118C
3 T-A118C-30.0880
4 T-S131C
5 T-S131C-30.0880
6 T-S239C
7 T-S239C-30.0880
8 T-D265C
9 T-D265C-30.0880
1 Trastuzumab
2 T-E269C
3 T-E269C-30.0880
4 T-N297CC
5 T-N297C-30.0880
6 T-A327C
7 T-A327C-30.0880
8 T-I332C
9 T-I332C-30.0880
250
130
100
70
55
35
25
15
10
1 2 3 4 5 6 7 8 9

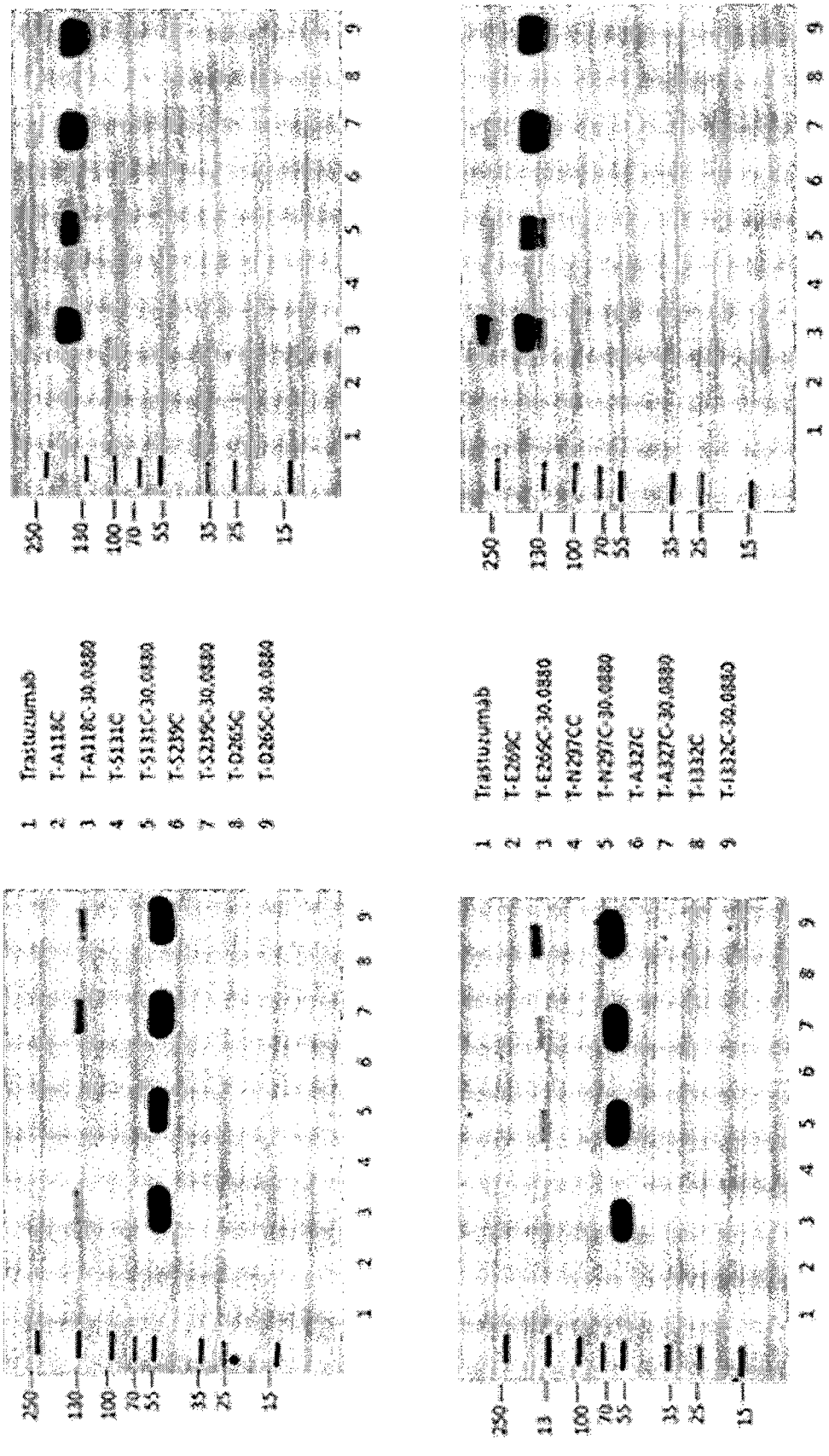
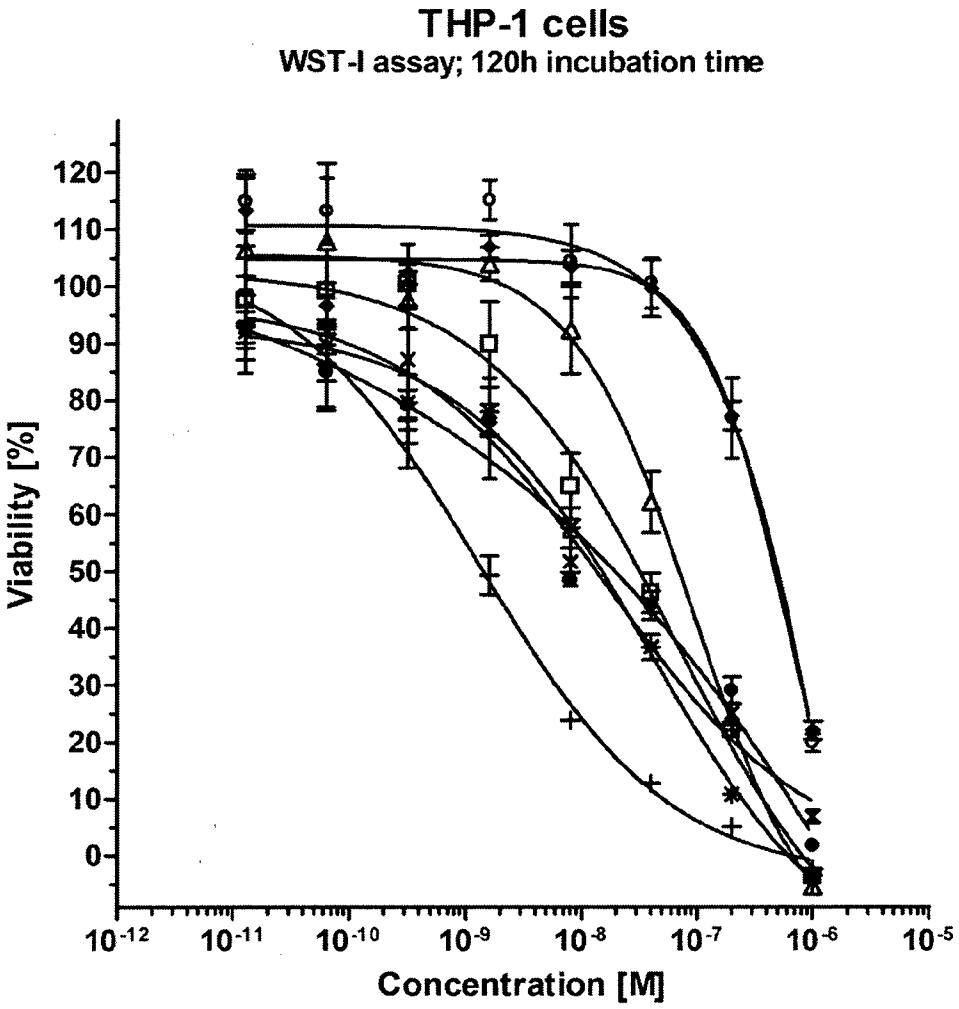

1 Trastuzumab
2 T-A118C
3 T-A118C-30.0880
4 T-S121C
5 T-S121C-30.0880
6 T-S239C
7 T-S239C-30.0880
8 T-D265C
9 T-D265C-30.0880
1 Trastuzumab
2 T-E269C
3 T-E269C-30.0880
4 T-N297CC
5 T-N297C-30.0880
6 T-A327C
7 T-A327C-30.0880
8 T-I332C
9 T-I332C-30.0880
250
130
100
70
55
35
25
15
1 2 3 4 5 6 7 8 9

Figure 8(A)



× antibody A mut. A118C-HC 30.0880
□ antibody A mut. S239C-HC 30.0880
◆ antibody A mut. D265C-HC 30.0880
△ antibody A mut. E269C-HC 30.0880
○ antibody A mut. N297C-HC 30.0880
✱ antibody A mut. A327C-HC 30.0880
● antibody A mut. I332C-HC 30.0880

\+ antibody A 30.0643

Figure 8(B)

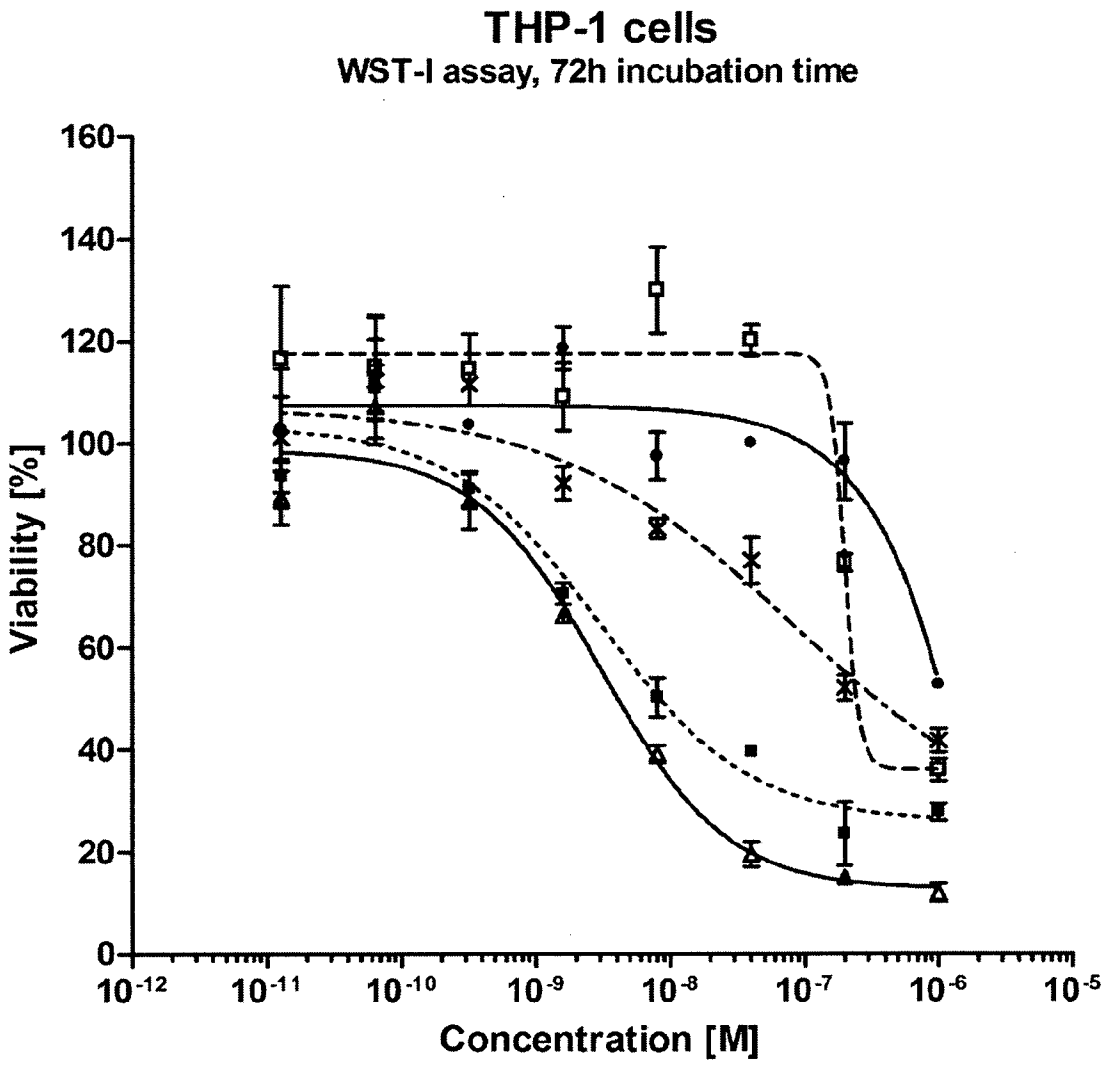
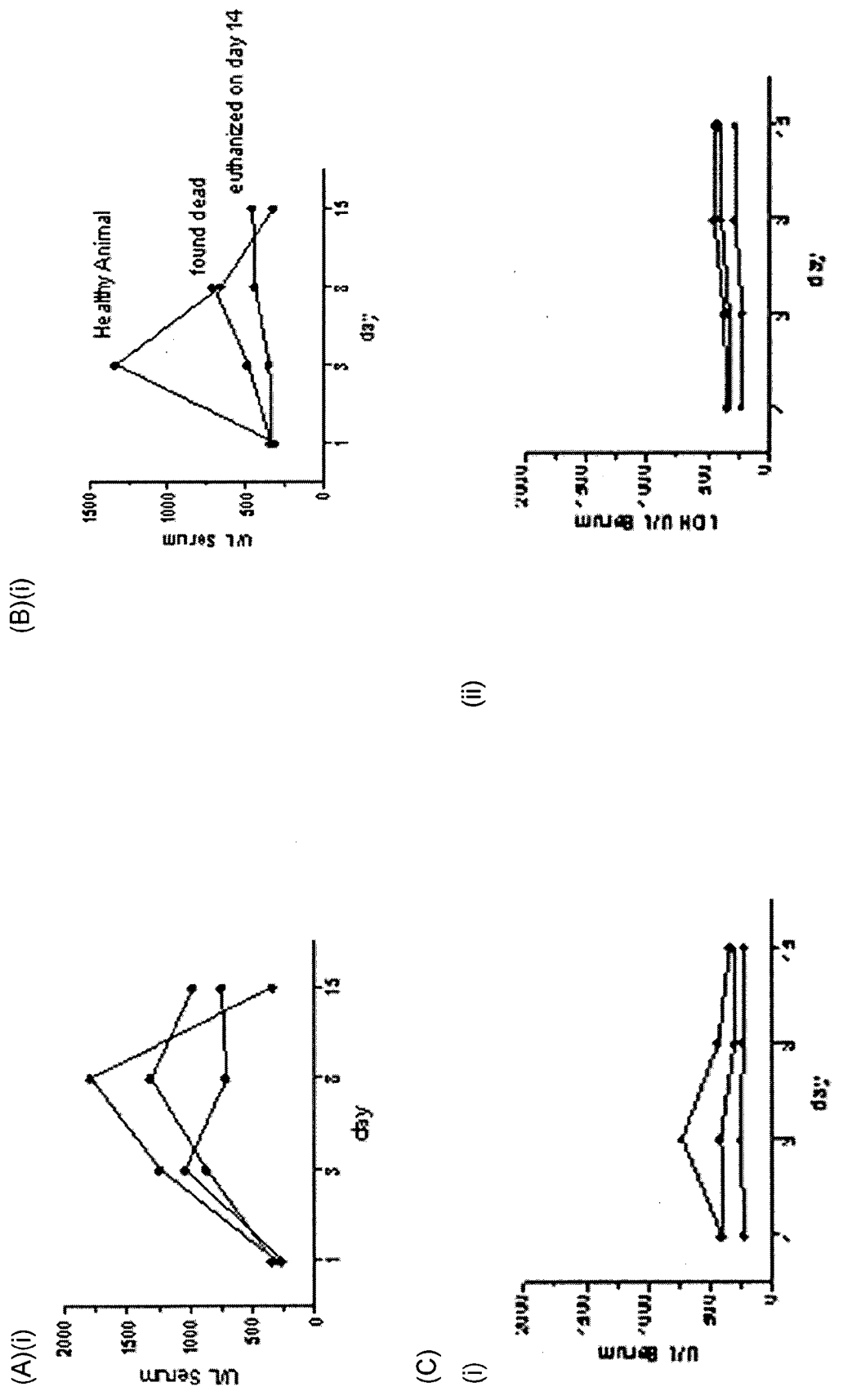
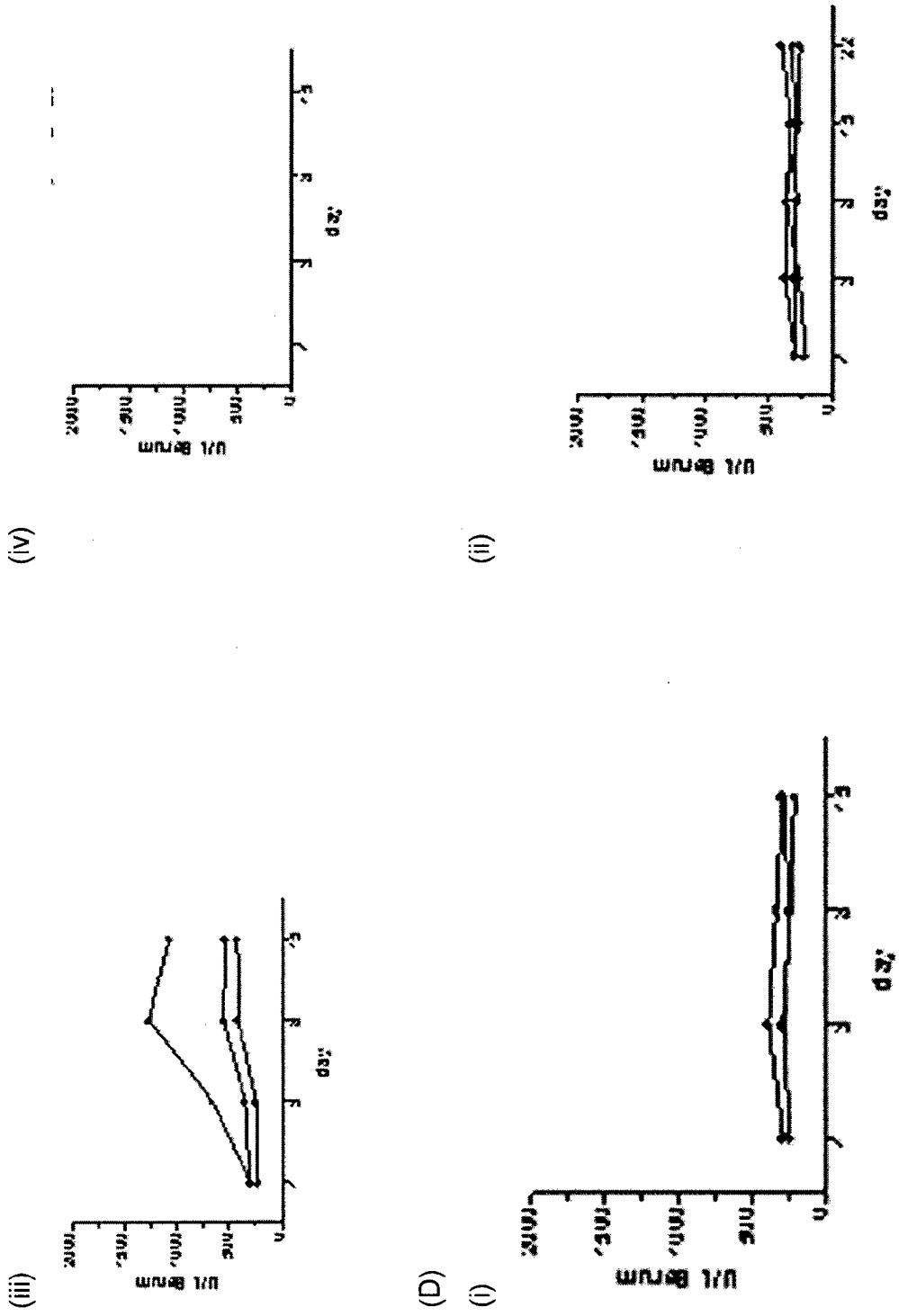

THP-1 cells
WST-I assay, 72h incubation time
Viability [%]
160
140
120
100
80
60
40
20
0
10-12
10-11
10-10
10-9
10-8
10-7
10-6
10-5
Concentration [M]
antibody B mut. A118C-HC 30.0880
antibody B mut. S131C-HC 30.0880
antibody B mut. D265C-HC 30.0880
antibody B mut. N297C-HC 30.0880
antibody B 30.0880

Figure 9

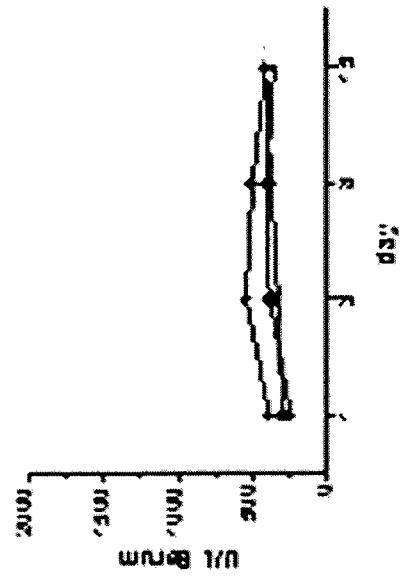
TDC	15 mg/kg	25 mg/kg	37.5 mg/kg
Ab B HC-A118C	✓	✓	✓
Ab B HC-S131C	nd*	nd*	nd*
Ab B HC-S239C	✓	✓	nd***
Ab B HC-D265C	✓	✓	nd***
Ab B HC-E269C	✓	✓	X(2/3)
Ab B HC-N297C	✓BWL	X(3/3)	nd**
Ab B HC-A327C	✓	✓	X(3/3)
Ab B HC-I332C	✓BWL	X(3/3)	nd**

Figure 10


(A)(i)
(B)(i)
(C)
(i)
(ii)
Healthy Animal
found dead
euthanized on day 14
day


(D)
(i)
(ii)
(iii)
(iv)
U/L Serum
Day

(iii)



(iv)

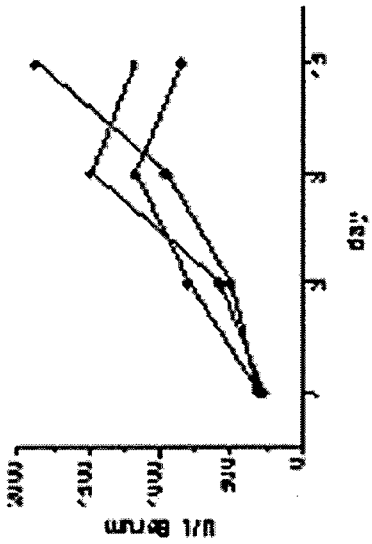
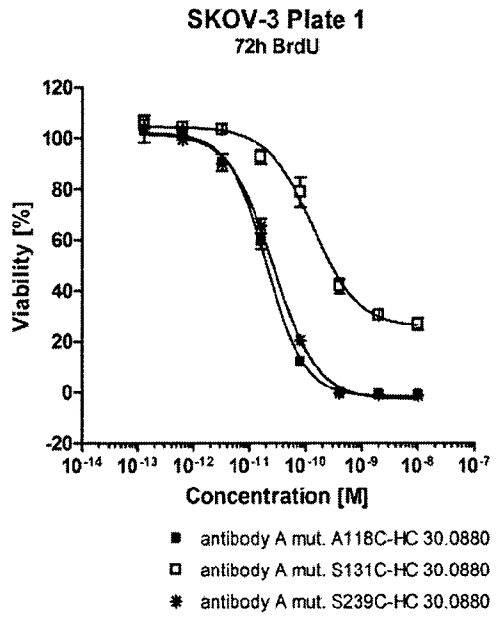
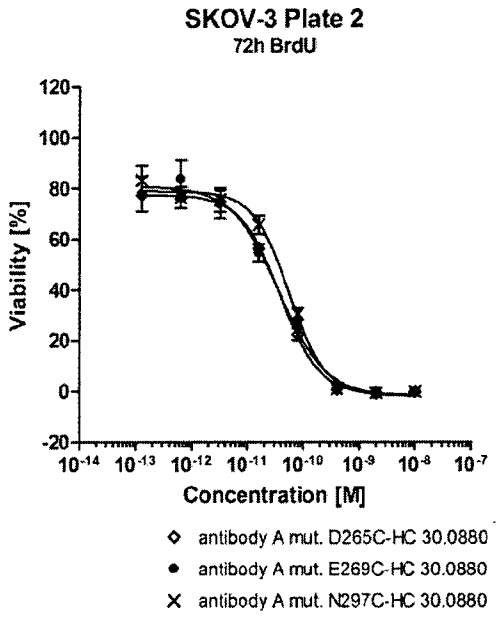


Figure 11


SKOV-3 Plate 1
72h BrdU
Viability [%]
Concentration [M]
antibody A mut. A118C-HC 30.0880
antibody A mut. S131C-HC 30.0880
antibody A mut. S239C-HC 30.0880


SKOV-3 Plate 2
72h BrdU
Viability [%]
Concentration [M]
antibody A mut. D265C-HC 30.0880
antibody A mut. E269C-HC 30.0880
antibody A mut. N297C-HC 30.0880

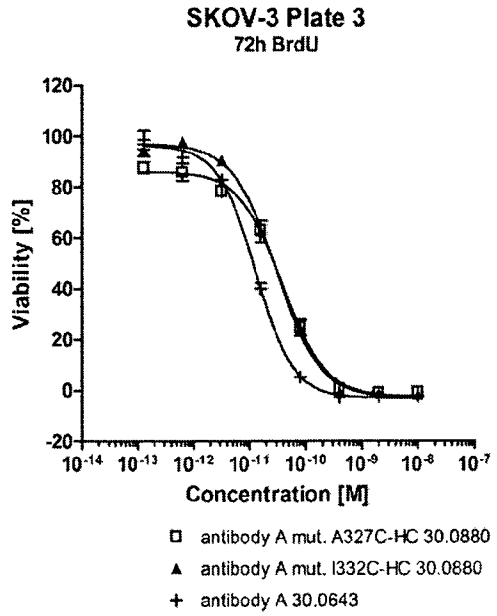

SKOV-3 Plate 3
72h BrdU
Viability [%]
Concentration [M]
antibody A mut. A327C-HC 30.0880
antibody A mut. I332C-HC 30.0880
antibody A 30.0643

Figure 12

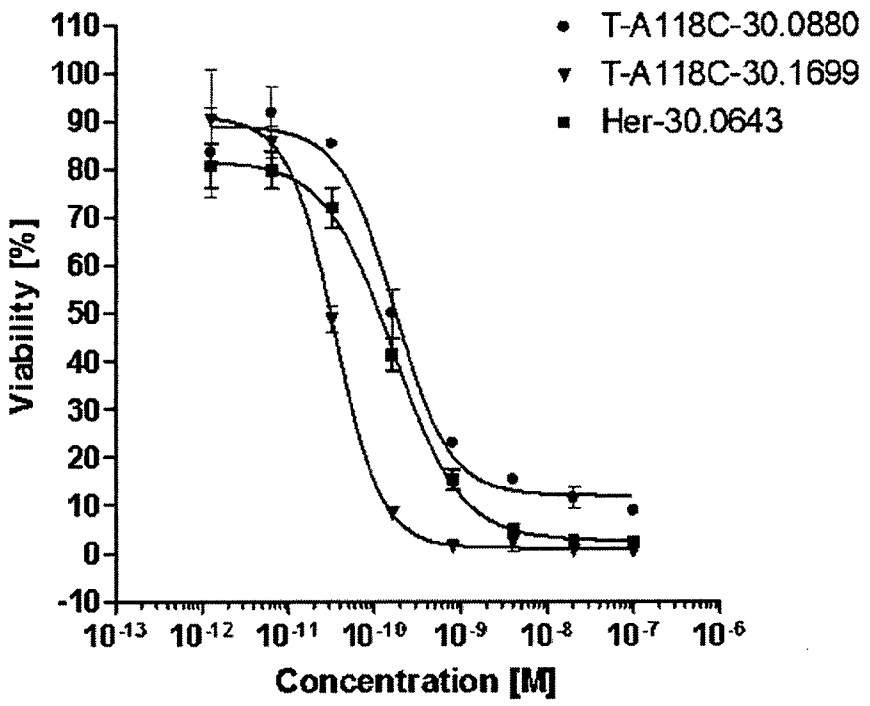
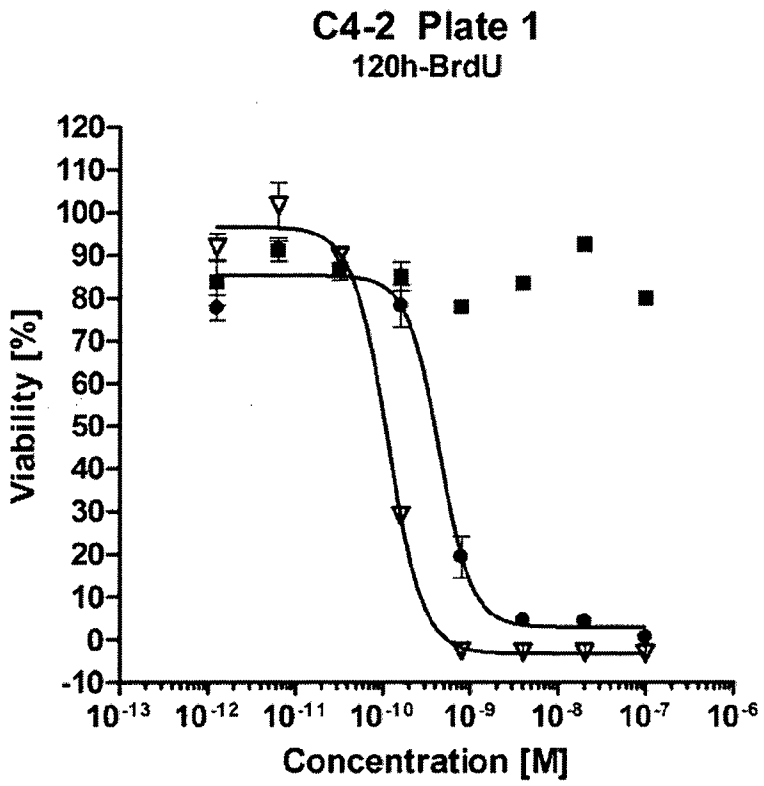
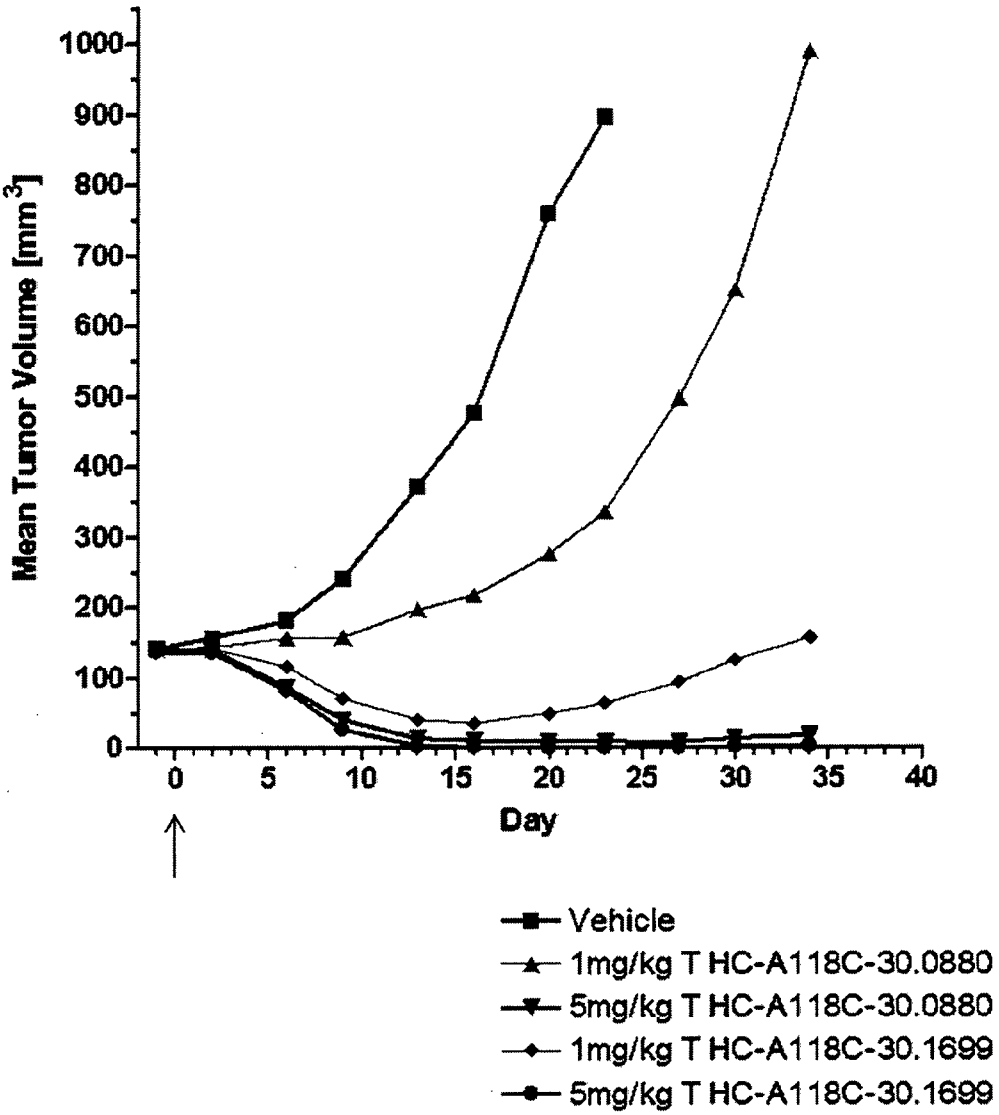

T-A118C-30.0880
T-A118C-30.1699
Her-30.0643
Viability [%]
110
100
90
80
70
60
50
40
30
20
10
0
-10
10-13
10-12
10-11
10-10
10-9
10-8
10-7
10-6
Concentration [M]

Figure 13



- antibody B mut. A118C-HC 30.0880
- antibody B mut. S131C-HC 30.0880
- antibody B mut. D265C-HC 30.0880

Figure 14


Mean Tumor Volume [mm3]
1000
900
800
700
600
500
400
300
200
100
0
0
5
10
15
20
25
30
35
40
Day
Vehicle
1mg/kg T HC-A118C-30.0880
5mg/kg T HC-A118C-30.0880
1mg/kg T HC-A118C-30.1699
5mg/kg T HC-A118C-30.1699