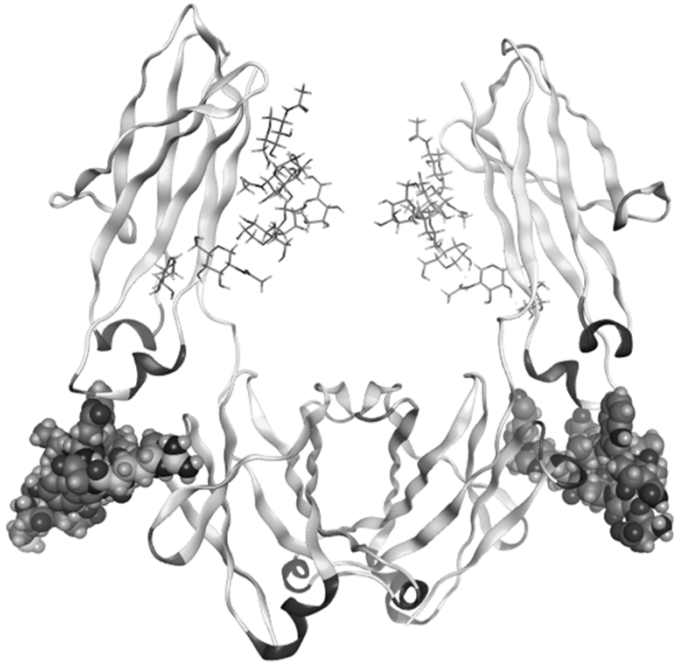
Fig. 1

(A)



(B)

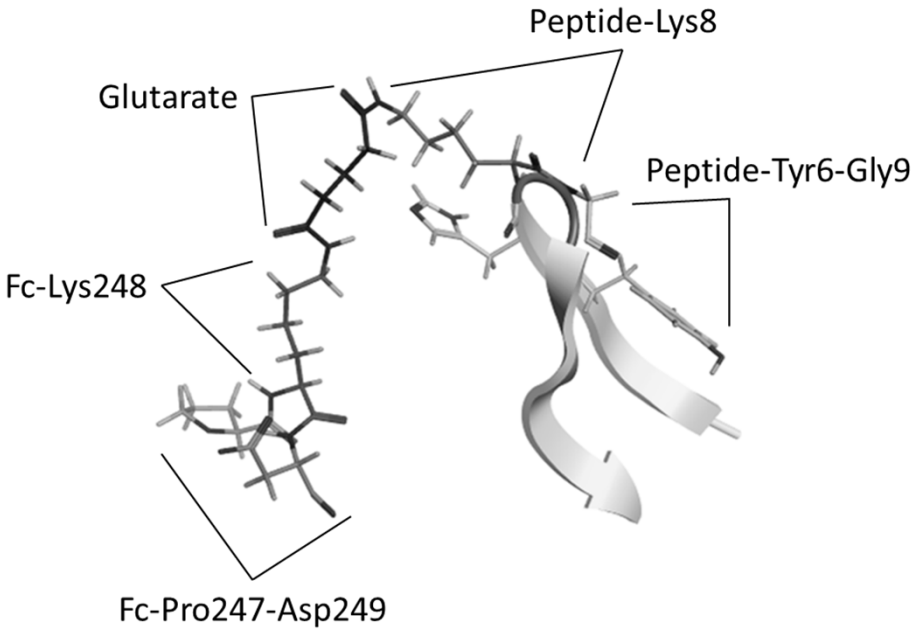

Peptide-Lys8
Glutarate
Peptide-Tyr6-Gly9
Fc-Lys248
Fc-Pro247-Asp249

Fig. 2

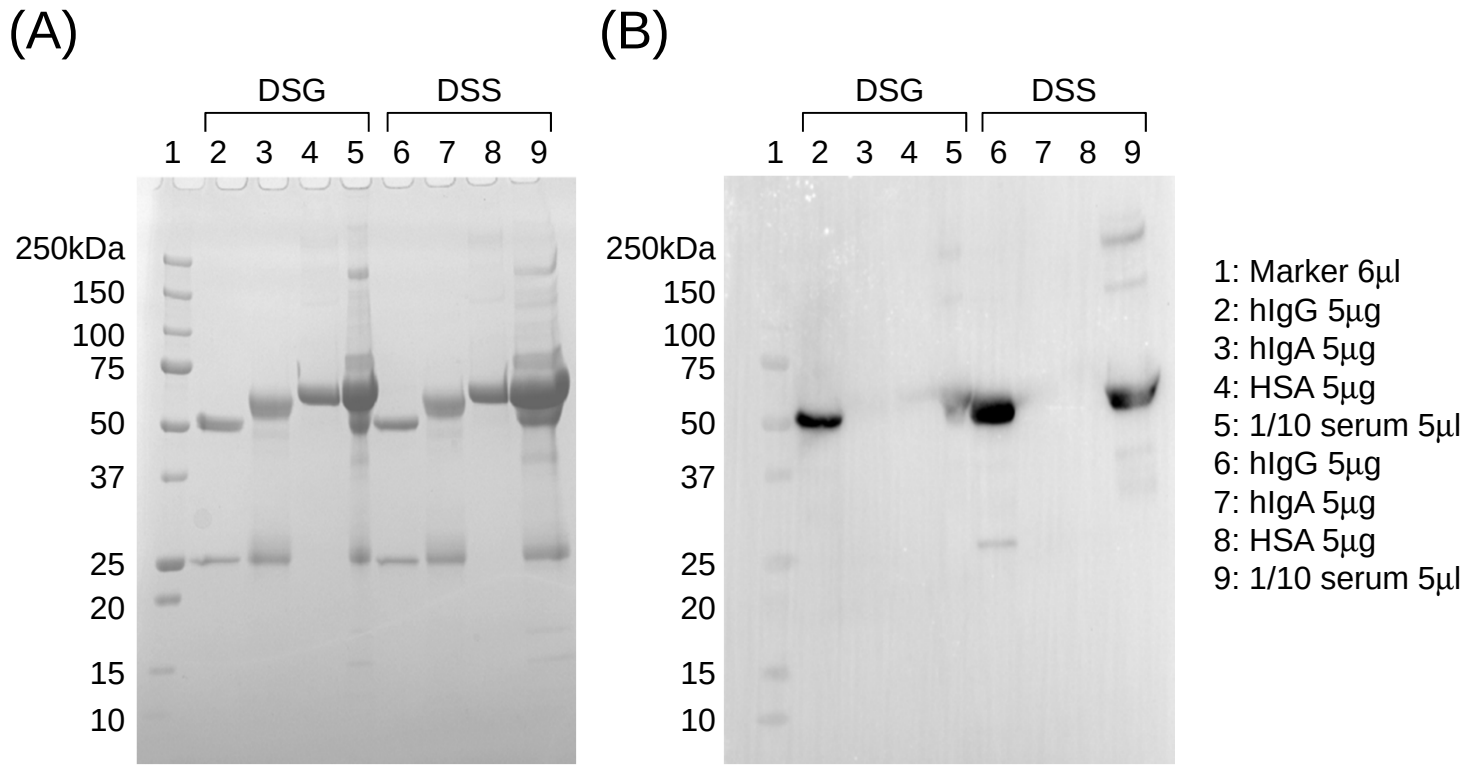

(A)
DSG
DSS
1 2 3 4 5 6 7 8 9
250kDa
150
100
75
50
37
25
20
15
10
(B)
DSG
DSS
1 2 3 4 5 6 7 8 9
250kDa
150
100
75
50
37
25
20
15
10
1: Marker 6µl
2: hIgG 5µg
3: hIgA 5µg
4: HSA 5µg
5: 1/10 serum 5µl
6: hIgG 5µg
7: hIgA 5µg
8: HSA 5µg
9: 1/10 serum 5µl

Fig. 3

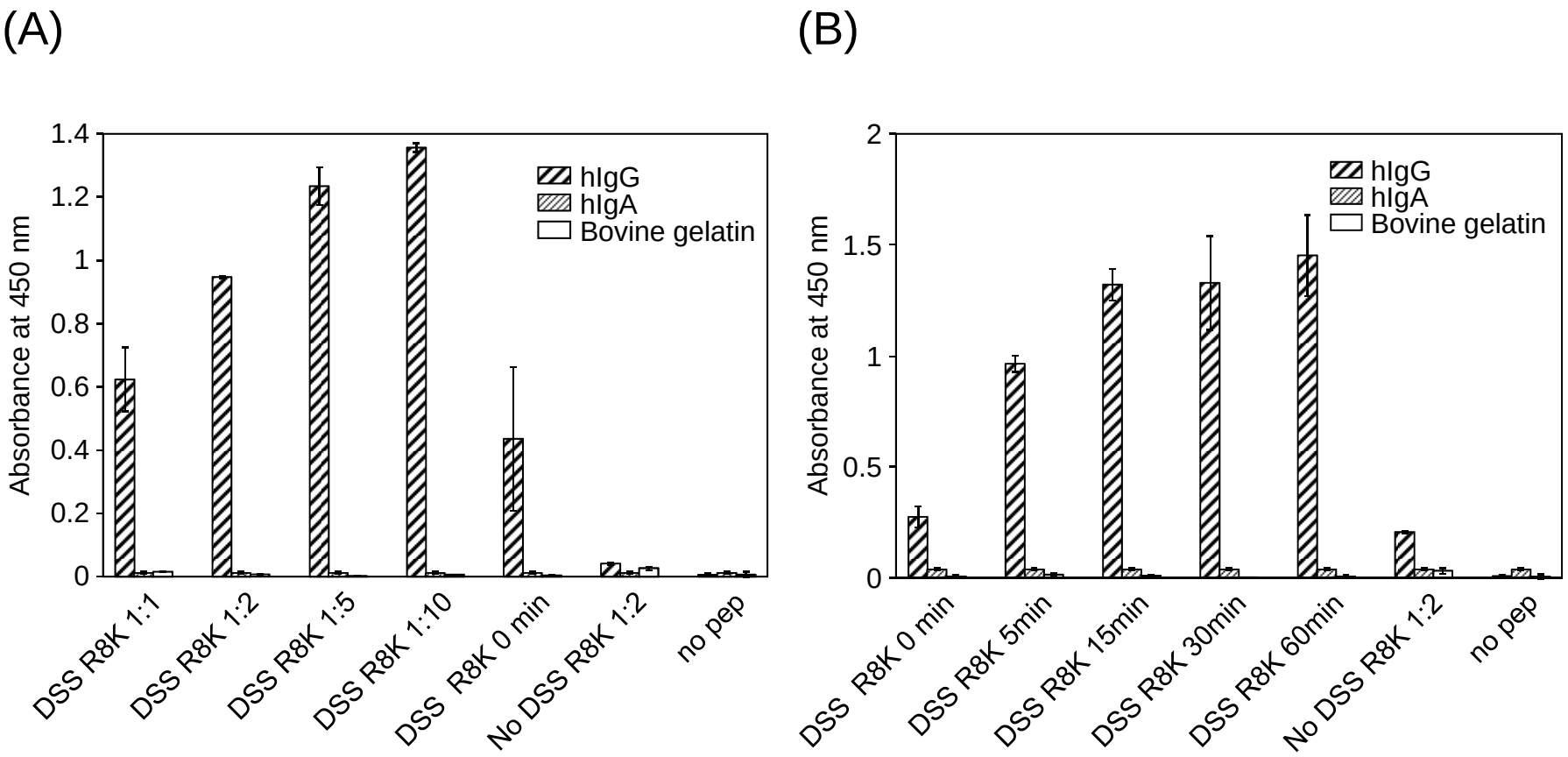

(A)
Absorbance at 450 nm
1.4
1.2
1
0.8
0.6
0.4
0.2
0
hIgG
hIgA
Bovine gelatin
DSS R8K 1:1
DSS R8K 1:2
DSS R8K 1:5
DSS R8K 1:10
DSS R8K 0 min
No DSS R8K 1:2
no pep
(B)
Absorbance at 450 nm
2
1.5
1
0.5
0
hIgG
hIgA
Bovine gelatin
DSS R8K 0 min
DSS R8K 5min
DSS R8K 15min
DSS R8K 30min
DSS R8K 60min
No DSS R8K 1:2
no pep

Fig. 4

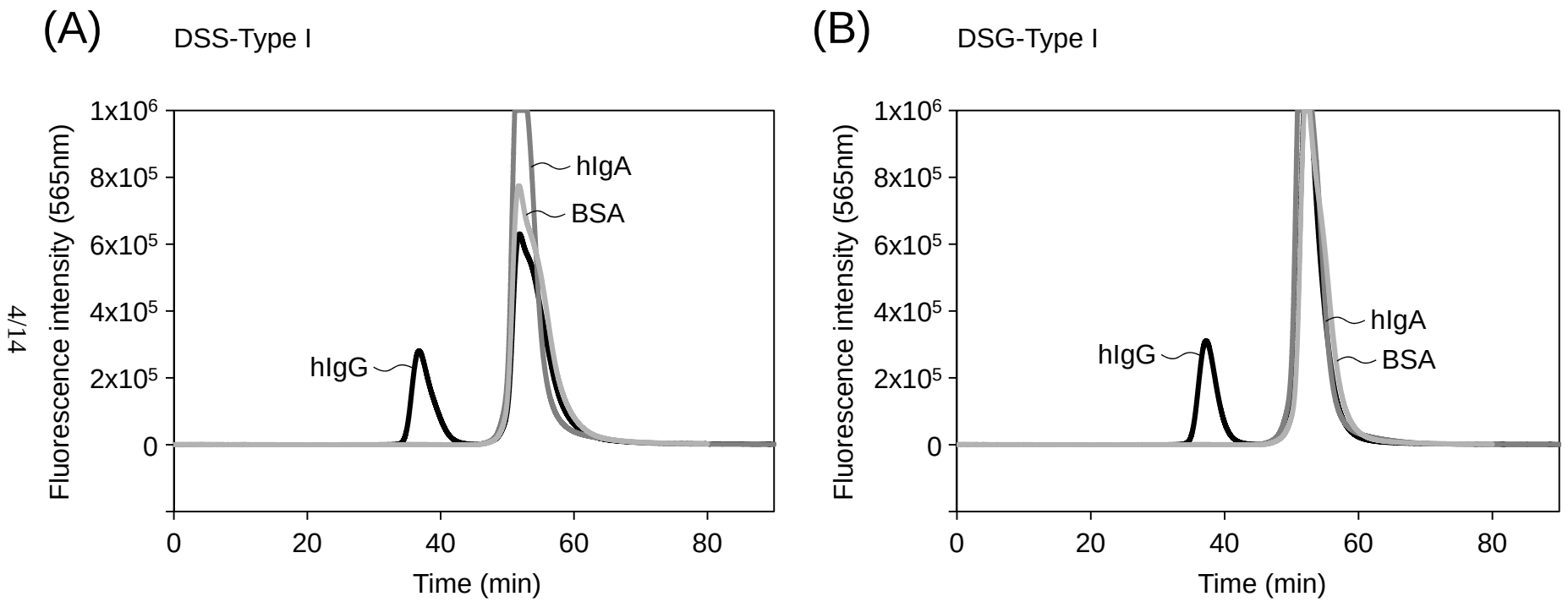
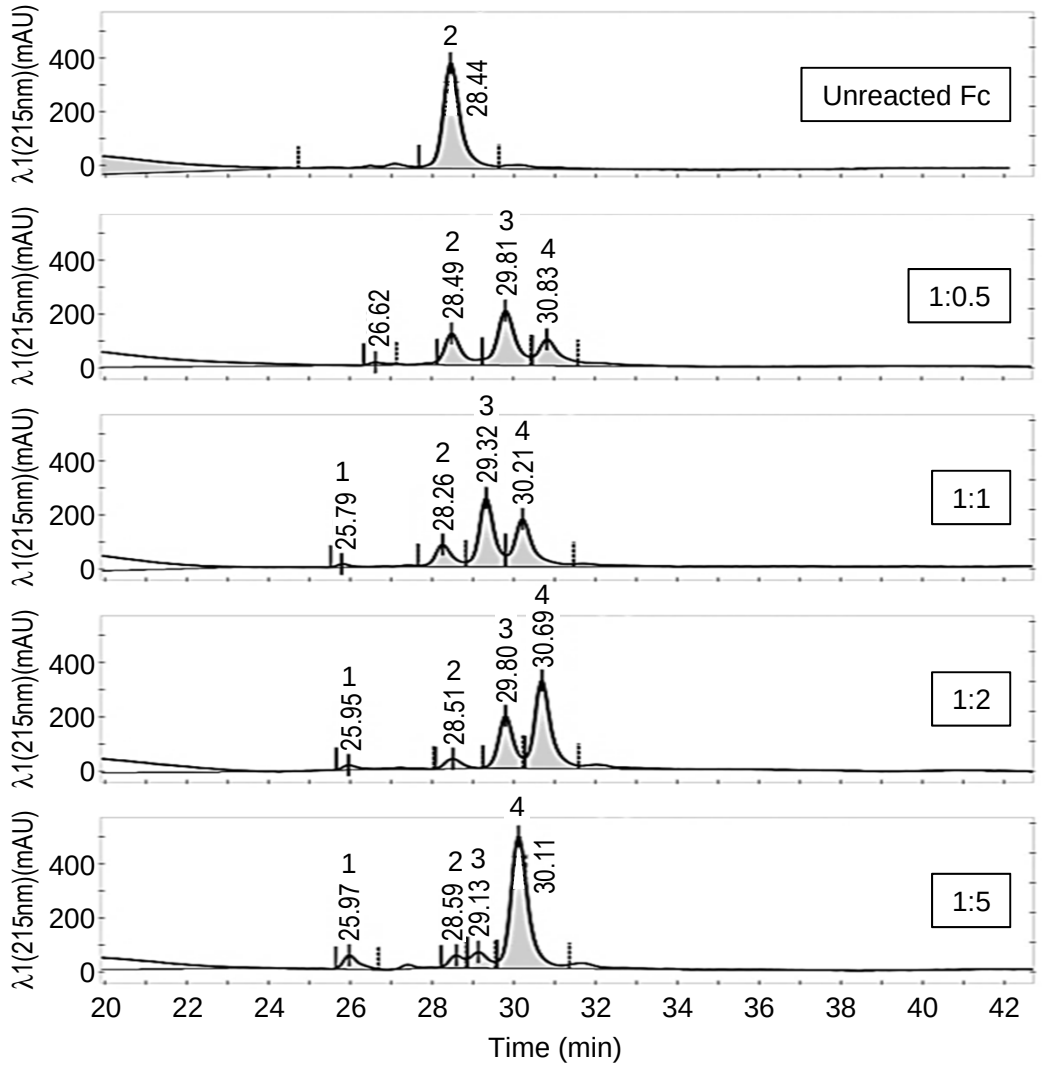

(A)
DSS-Type I
Fluorescence intensity (565nm)
1x10^6
8x10^5
6x10^5
4x10^5
2x10^5
0
hIgG
hIgA
BSA
0
20
40
60
80
Time (min)
(B)
DSG-Type I
Fluorescence intensity (565nm)
1x10^6
8x10^5
6x10^5
4x10^5
2x10^5
0
hIgG
hIgA
BSA
0
20
40
60
80
Time (min)

Fig. 5

(A)


λ1(215nm)(mAU)
400
200
0
Unreacted Fc
2
28.44
1:0.5
26.62
28.49
29.81
30.83
1:1
25.79
28.26
29.32
30.21
1:2
25.95
28.51
29.80
30.69
1:5
25.97
28.59
29.13
30.11
20
22
24
26
28
30
32
34
36
38
40
42
Time (min)

(B)

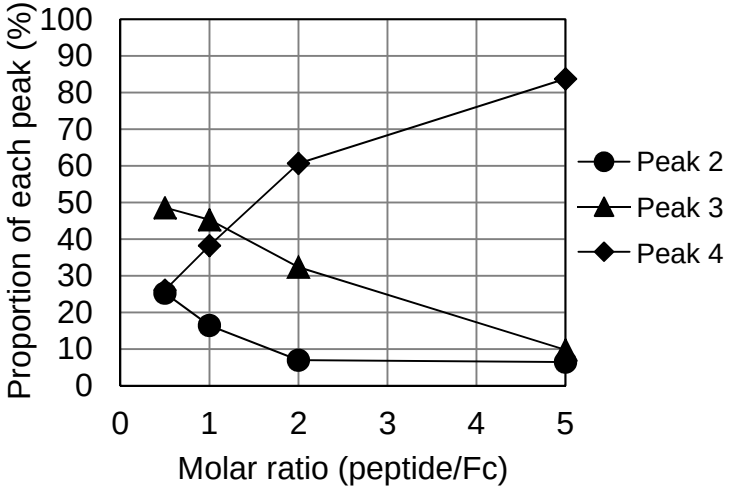

Proportion of each peak (%)
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
Molar ratio (peptide/Fc)
Peak 2
Peak 3
Peak 4

Fig. 6

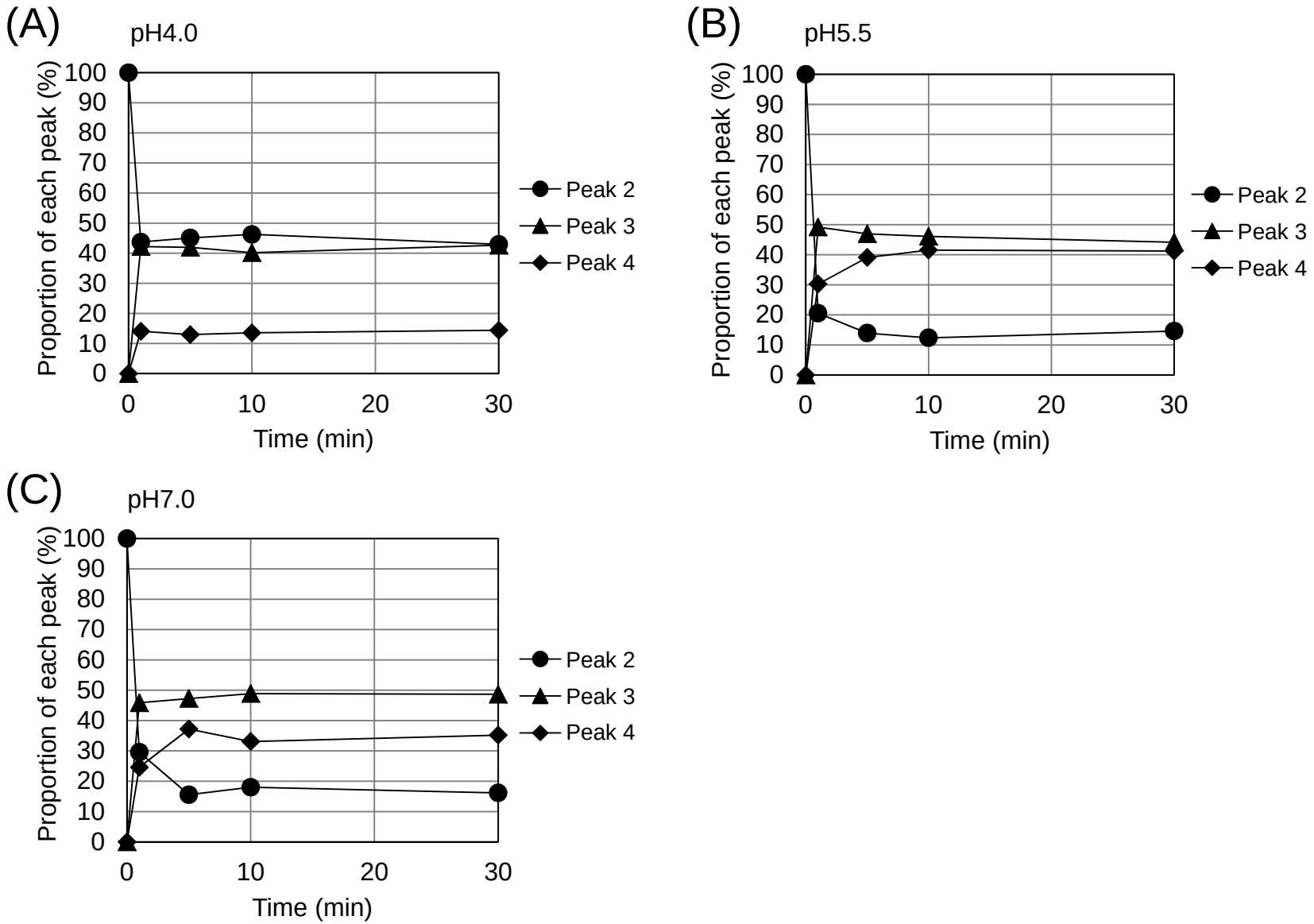
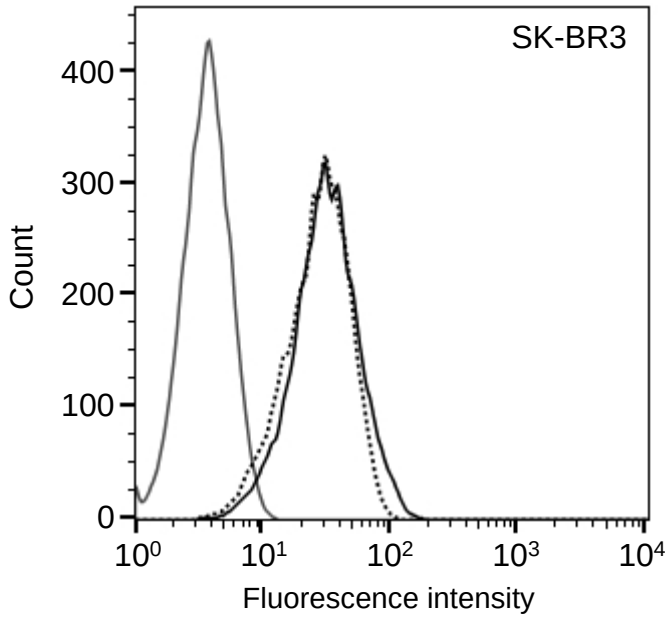

(A)
pH4.0
Proportion of each peak (%)
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
Time (min)
Peak 2
Peak 3
Peak 4
(B)
pH5.5
Proportion of each peak (%)
Time (min)
Peak 2
Peak 3
Peak 4
(C)
pH7.0
Proportion of each peak (%)
Time (min)
Peak 2
Peak 3
Peak 4

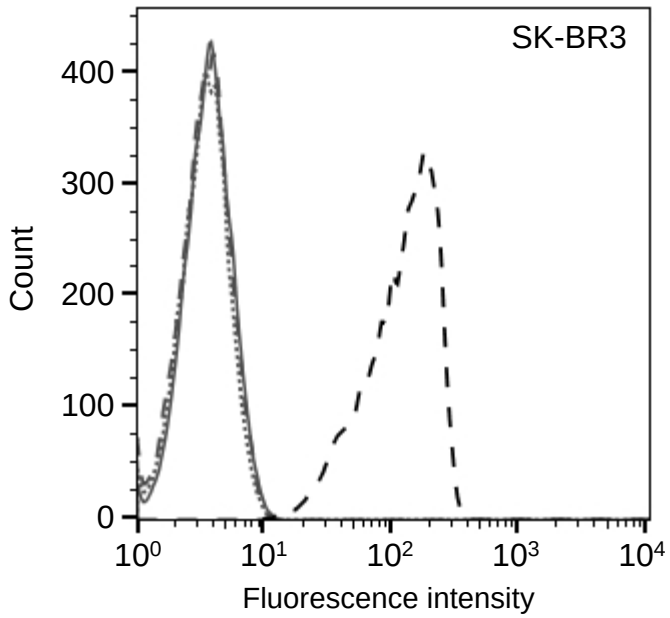
Fig. 7

(A)



	4D5-Fc	Secondary antibody
—	-	Biotinylated IgG-binding peptide + SA-PE label
—	+	Biotinylated IgG-binding peptide + SA-PE label
···	+	Anti hIgG mAb-biotin label + SA-PE label

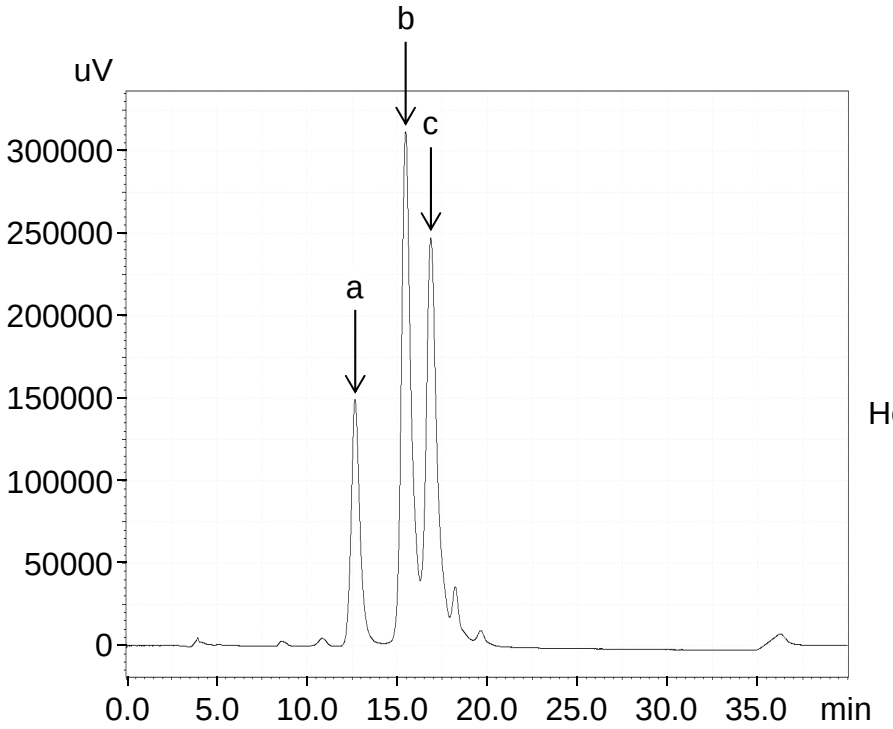
(B)



	4D5-Fc	Secondary antibody
—	-	Biotinylated IgG-binding peptide + SA-PE label
···	-	Anti hIgG mAb-biotin label + SA-PE label
---	-	Anti hIgG mAb-PE label
---	+	Anti hIgG mAb-PE label

Fig. 8

(A)


uV
300000
250000
200000
150000
100000
50000
0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
min
a
b
c

(B)

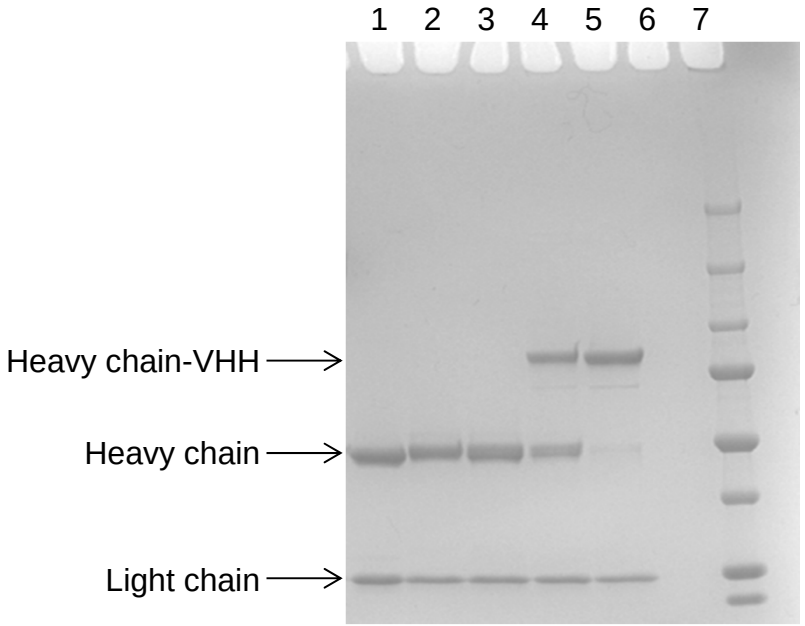

1
2
3
4
5
6
7
Heavy chain-VHH
Heavy chain
Light chain

Fig. 9

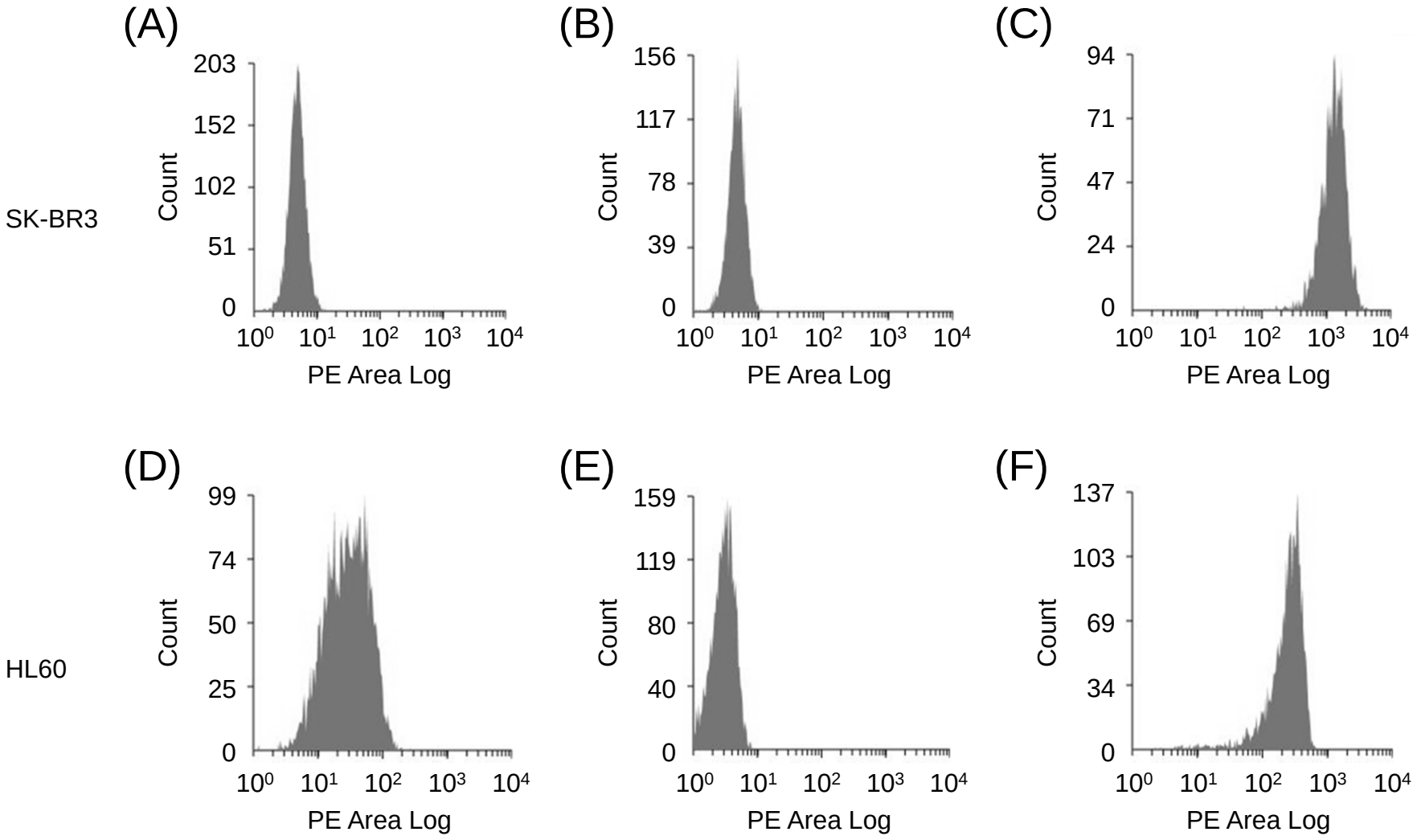

(A)
(B)
(C)
(D)
(E)
(F)
SK-BR3
HL60
Count
PE Area Log
203
152
102
51
0
156
117
78
39
94
71
47
24
99
74
50
25
159
119
80
40
137
103
69
34
10^0 10^1 10^2 10^3 10^4

Fig. 10-1

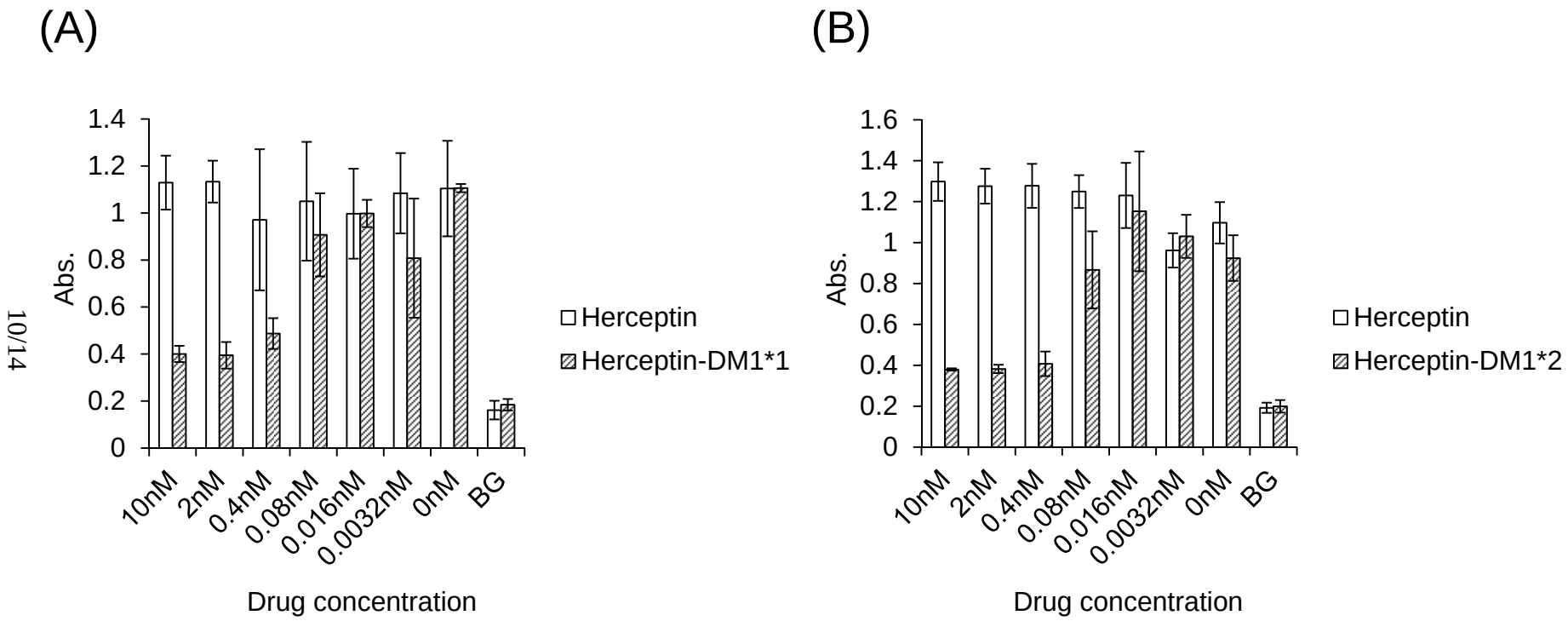
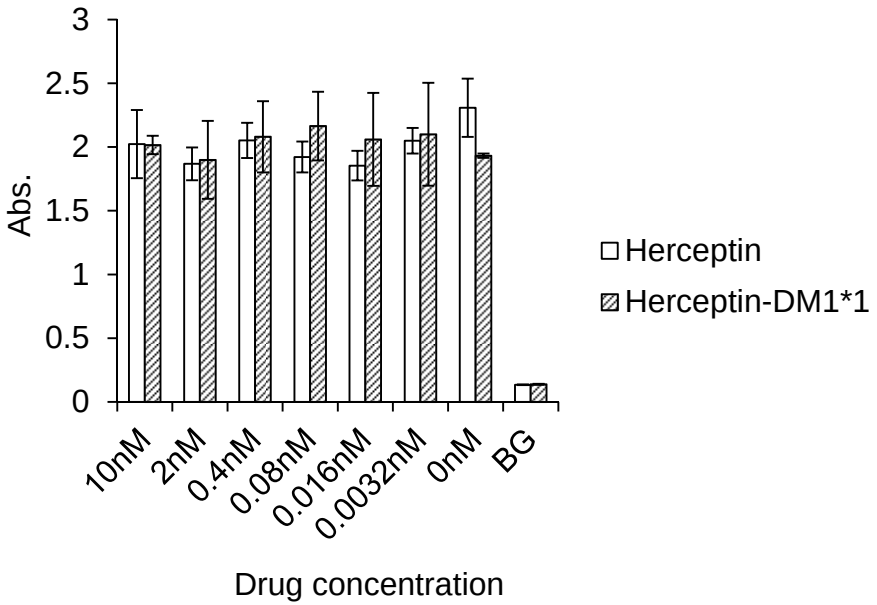

(A)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Abs.
10nM
2nM
0.4nM
0.08nM
0.016nM
0.0032nM
0nM
BG
Drug concentration
Herceptin
Herceptin-DM1*1
(B)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Abs.
10nM
2nM
0.4nM
0.08nM
0.016nM
0.0032nM
0nM
BG
Drug concentration
Herceptin
Herceptin-DM1*2

Fig. 10-2

(C)


3
2.5
2
1.5
1
0.5
0
Abs.
10nM
2nM
0.4nM
0.08nM
0.016nM
0.0032nM
0nM
BG
Drug concentration
Herceptin
Herceptin-DM1*1

(D)

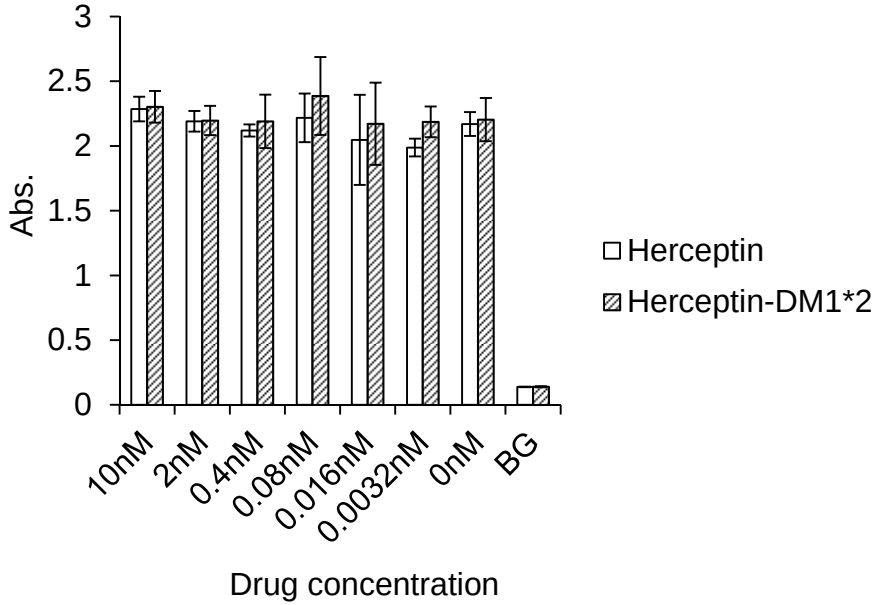

3
2.5
2
1.5
1
0.5
0
Abs.
10nM
2nM
0.4nM
0.08nM
0.016nM
0.0032nM
0nM
BG
Drug concentration
Herceptin
Herceptin-DM1*2

Fig. 11

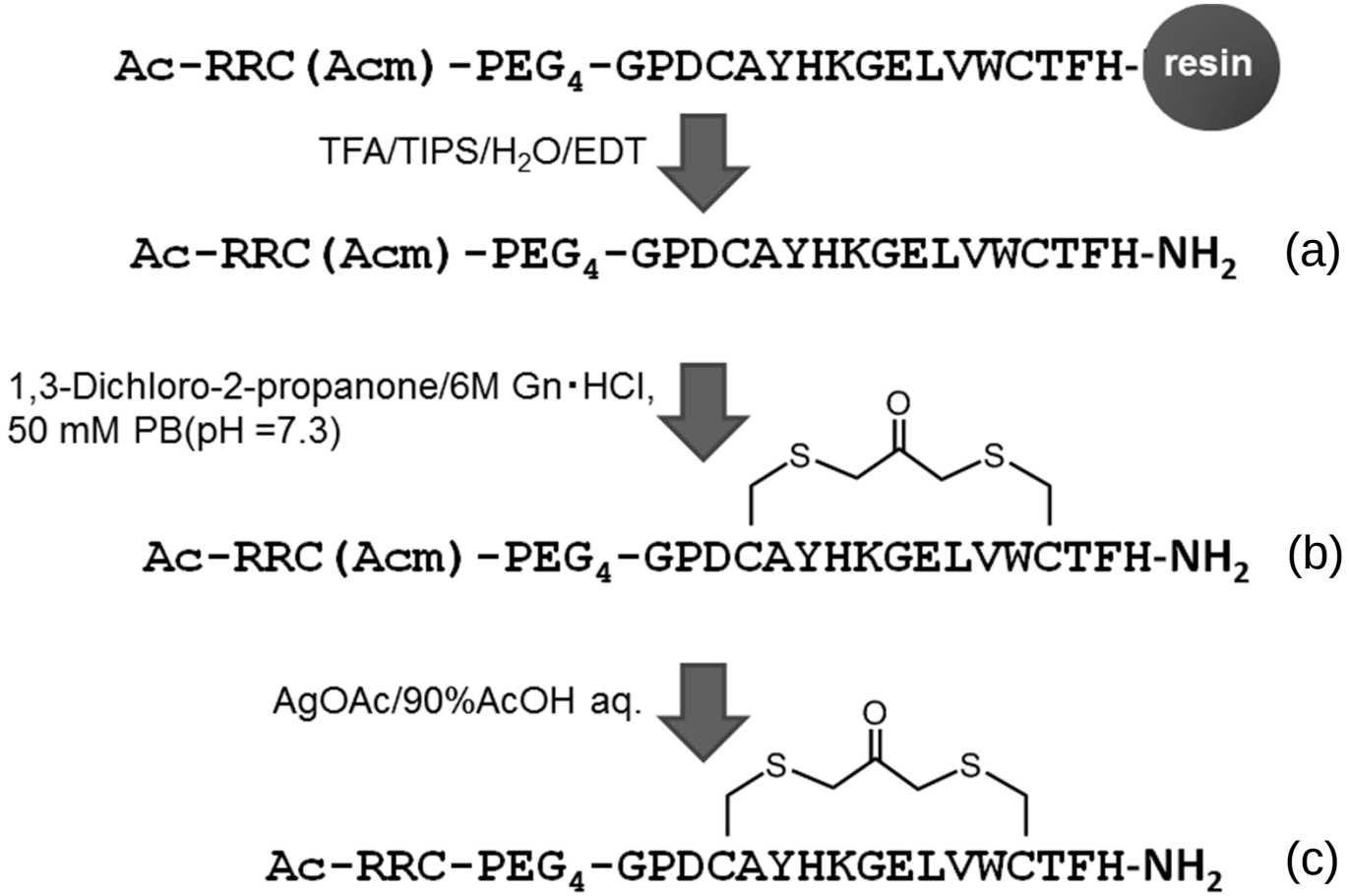

Ac-RRC(Acm)-PEG4-GPDCAYHKGELVWCTFH-
resin
TFA/TIPS/H2O/EDT
Ac-RRC(Acm)-PEG4-GPDCAYHKGELVWCTFH-NH2
(a)
1,3-Dichloro-2-propanone/6M Gn·HCl,
50 mM PB(pH =7.3)
O
S
S
Ac-RRC(Acm)-PEG4-GPDCAYHKGELVWCTFH-NH2
(b)
AgOAc/90%AcOH aq.
O
S
S
Ac-RRC-PEG4-GPDCAYHKGELVWCTFH-NH2
(c)

Fig. 12

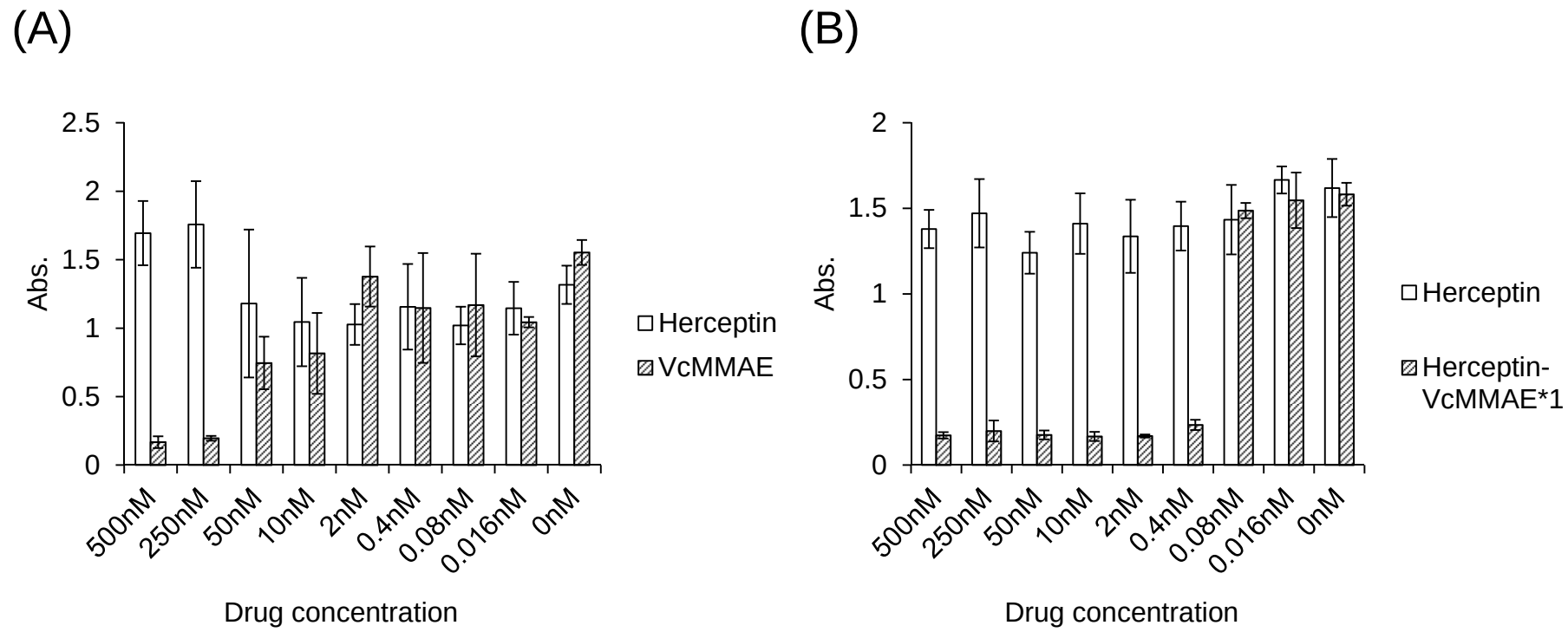
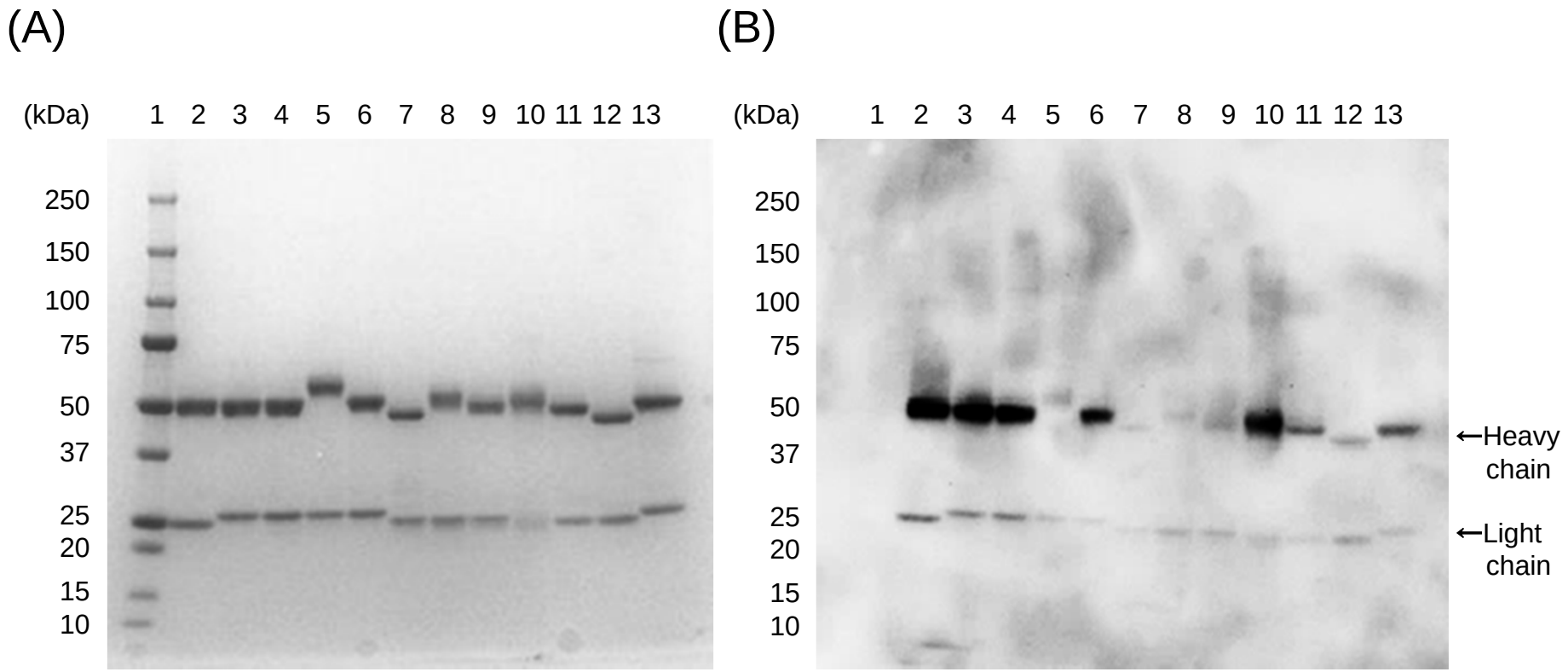

(A)
2.5
2
1.5
1
0.5
0
Abs.
500nM
250nM
50nM
10nM
2nM
0.4nM
0.08nM
0.016nM
0nM
Drug concentration
Herceptin
VcMMAE
(B)
2
1.5
1
0.5
0
Abs.
500nM
250nM
50nM
10nM
2nM
0.4nM
0.08nM
0.016nM
0nM
Drug concentration
Herceptin
Herceptin-VcMMAE*1

Fig. 13


(A)
(kDa)
1 2 3 4 5 6 7 8 9 10 11 12 13
250
150
100
75
50
37
25
20
15
10
(B)
(kDa)
1 2 3 4 5 6 7 8 9 10 11 12 13
250
150
100
75
50
37
25
20
15
10
←Heavy chain
←Light chain