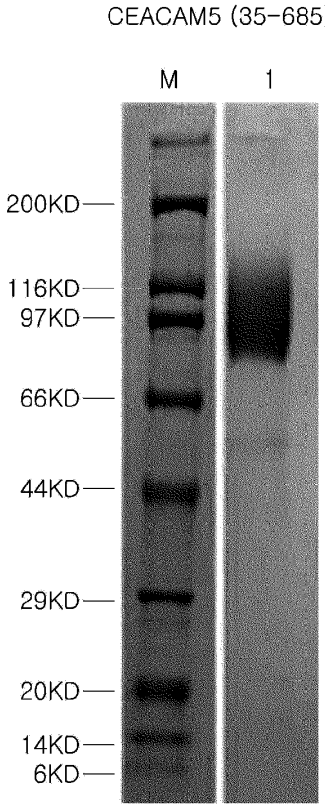
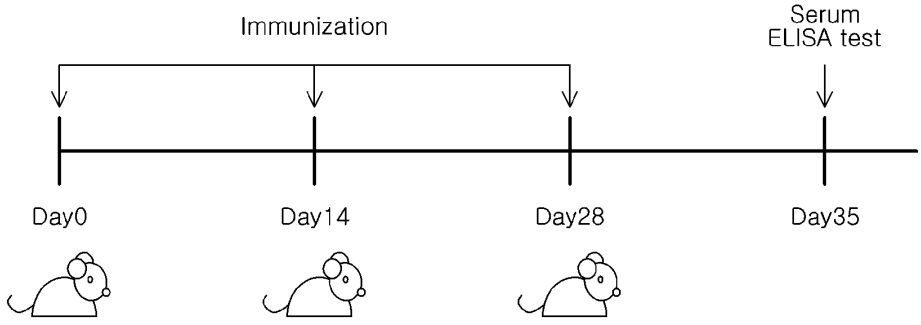
[도1]


CEACAM5 (35-685)
M
1
200KD
116KD
97KD
66KD
44KD
29KD
20KD
14KD
6KD

[도2]


Immunization
Serum
ELISA test
Day0
Day14
Day28
Day35

[도3]

Immunized mouse serum antibody titer (Cohort I)

- ELISA antigen : CEACAM5 (full)
- Coating Concentration : 0.5ug/ml, 100ul/well
- Coating buffer : Phosphate buffered saline

Sample`	1:100	1:200	1:400	1:800	1:1,600	1:3,200	1:6,400	1:12,800	1:25,600	1:51,200	Average	Titer	Coating	NC
	1	2	3	4	5	6	7	8	9	10	11			
3137	2.397	2.396	2.445	2.499	2.455	2.448	2.359	2.217	2.071	1.749	0.055	>1:51,200	A	0.066
3138	2.382	2.365	2.421	2.509	2.396	2.403	2.330	2.115	1.965	1.660	0.054	>1:51,200	A	0.073
3139	2.342	2.304	2.384	2.475	2.404	2.384	2.288	2.174	1.951	1.623	0.056	>1:51,200	A	0.061
3140	2.477	2.444	2.567	2.550	2.550	2.472	2.476	2.309	2.204	1.943	0.058	>1:51,200	A	0.082
3141	2.492	2.512	2.486	2.603	2.567	2.438	2.442	2.215	2.038	1.765	0.072	>1:51,200	A	0.055

[도4a]

Parental clones

Clone list	ELISA		
	CEACAM5 (full-length) (+)	CEACAM1 (-)	CEACAM6 (-)
3-C12	1.233	0.053	0.047
12-C12	0.806	0.061	0.051
14-B6	0.092	0.053	0.044
16-C4	0.136	0.054	0.048
22-A12	0.428	0.168	0.158
23-G6	0.245	0.047	0.048
24-H7	1.248	0.043	0.043
27-A4	1.255	0.061	0.055
27-A10	1.787	0.054	0.061
27-F9	1.975	0.049	0.113
27-H2	0.648	0.059	0.057
36-B5	2.409	0.062	0.166
36-C7	1.844	0.065	0.047
42-F2	0.98	0.053	0.043
44-G11	0.444	0.079	0.053
46-B7	1.351	0.055	0.049
46-C10	1.588	0.078	0.052
46-H1	1.157	0.055	0.056
47-A6	0.551	0.058	0.098
47-B1	0.414	0.068	0.091
47-B2	1.663	0.054	0.085
49-C4	0.405	0.056	0.085
49-F8	0.423	0.064	0.066
49-F12	1.172	0.088	0.065
nc(HT MEDIUM)	0.063	0.078	0.071
pc(ANTISERUM)	2.836	2.047	1.989

Clone list	ELISA		
	CEACAM5 (full-length) (+)	CEACAM1 (-)	CEACAM6 (-)
52-F6	0.889	0.071	0.069
60-G5	2.223	0.06	0.045
61-A1	2.051	0.064	0.043
62-G10	1.319	0.061	0.051
63-B7	2.211	0.057	0.057
64-F12	1.827	0.061	0.045
71-E11	1.618	0.083	0.051
72-F3	2.379	0.063	0.054
73-F6	2.028	0.074	0.05
77-B7	2.05	0.069	0.048
80-D12	1.745	0.07	0.054
81-E3	2.295	0.062	0.047
82-H3	2.053	0.072	0.057
83-G8	1.801	0.061	0.044
84-B11	2.025	0.061	0.046
86-E8	1.749	0.07	0.042
87-D5	1.533	0.062	0.042
88-G4	2.124	0.081	0.072
91-A10	1.658	0.098	0.045
92-A2	1.846	0.061	0.047
94-E12	1.968	0.059	0.043
96-F6	1.948	0.092	0.051
96-D8	1.883	0.061	0.043
97-C5	1.819	0.068	0.058
98-F8	2.297	0.068	0.048
99-A2	1.953	0.067	0.05

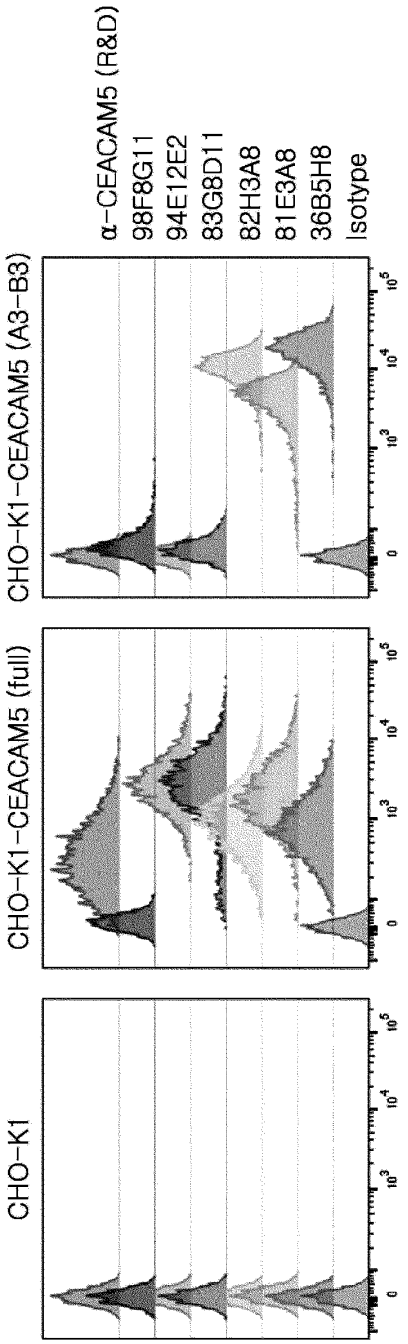
[도4b]

Subclones

Clone list	ELISA		
	CEACAM5 (full-length) (+)	CEACAM1 (-)	CEACAM6 (-)
3C12B10	0.874	0.104	0.056
12C12F7	1.331	0.064	0.067
24H7F2	1.437	0.116	0.161
27F9D1	1.42	0.076	0.055
27F9H5	1.41	0.127	0.071
36C7F6	1.444	0.087	0.057
36C7H8	1.318	0.075	0.07
36B5H8	1.66	0.135	0.058
49F12H9	1.664	0.094	0.05
27A10(E5)	0.514	0.126	0.067
NC	0.074	0.133	0.058
PC	1.766	1.637	1.560

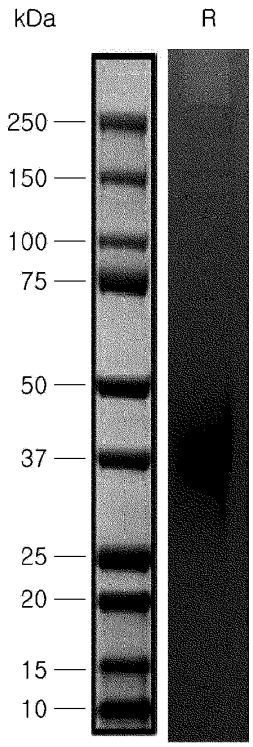
Clone list	ELISA		
	CEACAM5 (full-length) (+)	CEACAM1 (-)	CEACAM6 (-)
81E3A8	1.928	0.09	0.052
81E3E6	2.105	0.054	0.052
83G8D11	1.957	0.062	0.064
83G8H7	1.872	0.055	0.053
84B11C10	1.981	0.051	0.056
84B11E3	1.391	0.051	0.046
86E8F7	1.981	0.055	0.049
86E8G4	1.811	0.055	0.048
94E12E2	2.05	0.054	0.045
94E12G3	1.441	0.059	0.051
82H3A10	1.85	0.061	0.053
82H3C12	1.762	0.056	0.056
98F8G3	0.892	0.056	0.051
98F8G11	1.781	0.063	0.06

[도5]


CHO-K1
CHO-K1-CEACAM5 (full)
CHO-K1-CEACAM5 (A3-B3)
α-CEACAM5 (R&D)
98F8G11
94E12E2
83G8D11
82H3A8
81E3A8
36B5H8
Isotype

[도6]

CEACAM5 A3-B3 domain (496-685)



[도7]

Immunized mouse serum antibody titer (Cohort II)

- ELISA antigen : CEACAM5 (496-685aa)
- Coating Concentration : 0.5ug/ml, 100ul/well
- Coating buffer : Phosphate buffered saline

Serum Dilution Factor	1:1,000	1:2,000	1:4,000	1:8,000	1:16,000	1:32,000	1:64,000	1:128,000	1:256,000	1:512,000	Blank	Immune Titer	Coating
Mouse ID	1	2	3	4	5	6	7	8	9	10	11		
5487	2.303	2.191	2.159	1.968	1.683	1.343	1.019	0.668	0.429	0.267	0.045	1:512,000	A
5488	2.401	2.293	2.147	2.037	1.739	1.399	0.912	0.608	0.364	0.217	0.044	1:512,000	A
5489	2.439	2.166	2.067	1.970	1.651	1.354	0.947	0.594	0.356	0.201	0.043	1:512,000	A
5490	2.362	2.321	2.060	1.883	1.542	1.128	0.768	0.475	0.269	0.168	0.048	1:512,000	A
5491	2.362	2.396	2.212	2.111	1.857	1.649	1.121	0.784	0.473	0.291	0.051	1:512,000	A

[도8a]

Parental clones

Clone List	Protein binding (ELISA)			Cell binding (FACS)	
	CEACAM5 (496-684aa)(+)	CEACAM1 (-)	CEACAM6 (-)	CHO-K1/ CEACAM 5 (+)	Ratio
89-C6	0.591	0.065	0.066	1040	30.588
89-F4	0.164	0.063	0.057	913	26.853
89-F3	0.596	0.063	0.057	331	9.735
40-E1	1.973	0.075	0.057	607	8.940
32-H2	2.116	0.065	0.056	579	8.527
34-H3	1.997	0.071	0.058	512	7.541
97-D2	1.291	0.062	0.051	240	7.059
33-F3	1.961	0.061	0.049	447	6.583
39-H4	1.626	0.072	0.057	397	5.847
45-A2	2.037	0.072	0.057	346	5.096
61-C12	1.008	0.064	0.054	158	4.647
38-E5	1.831	0.064	0.053	312	4.595
33-F2	1.914	0.060	0.052	300	4.418
21-G6	1.720	0.064	0.056	292	4.300
13-F5	1.998	0.061	0.051	287	4.227

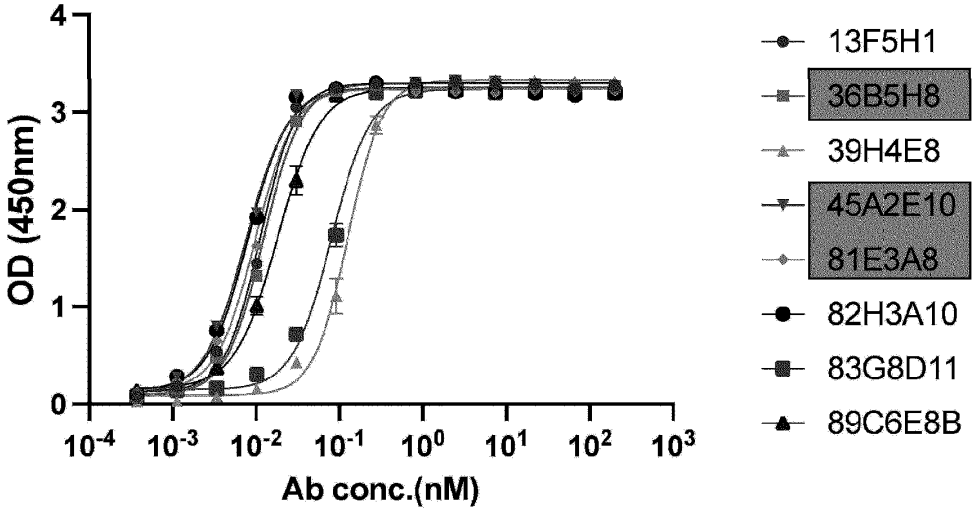
[도8b]

A. 1st Screening
B. (Cohort II)

Subclones

Cell binding (FACS)		
Clone list	CHO-K1/ CEACAM5 (+)	Ratio
13F5H1	1969	10.70109
32H2A10	2303	12.5163
32H2G12	2032	11.04348
32H2H5	2410	13.09783
33F2C2	2370	12.88043
33F2D9	2607	14.16848
33F3A8	3175	17.25543
38E5B11	1903	10.34239
38E5E12	1814	9.858696
38E5H4	1830	9.945652
39H4E8	1739	9.451087
40E1A9	1988	10.80435
40E1E7	1800	9.782609
40E1H3	1880	10.21739
45A2E10	2363	12.84239
45A2F11	2206	11.98913
45A2F12	2456	13.34783
61C12E2	2346	12.75
61C12F11	2049	11.13587
61C12G11	2634	14.31522
21G6A12A4	2008	16.3252
21G6A12B4	2065	16.78862
21G6A12G2	1976	16.06504
89C6E8B6	1331	10.82114
89C6E8C8	1313	10.6748
89C6E8F11	1322	10.74797

[도9a]

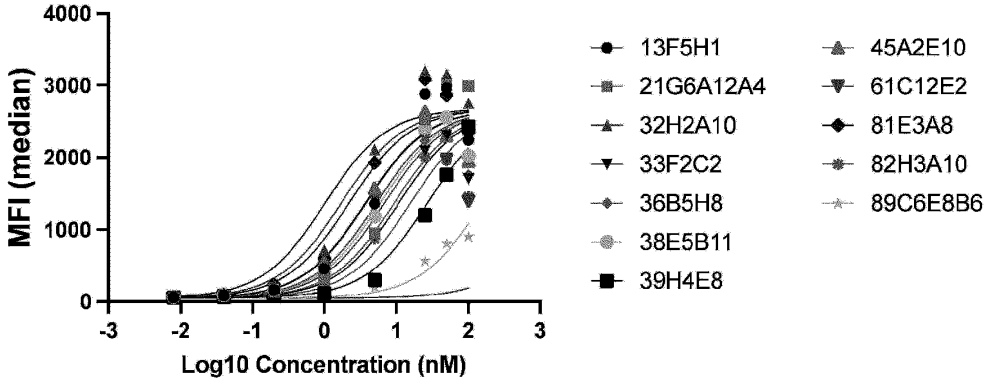


[도9b]

Clone ID	EC50 (nM)
13F5H1	0.011
36B5H8	0.012
39H4E8	0.126
45A2E10	0.008
81E3A8	0.010
82H3A10	0.008
83G8D11	0.080
89C6E8B	0.018

[도10a]

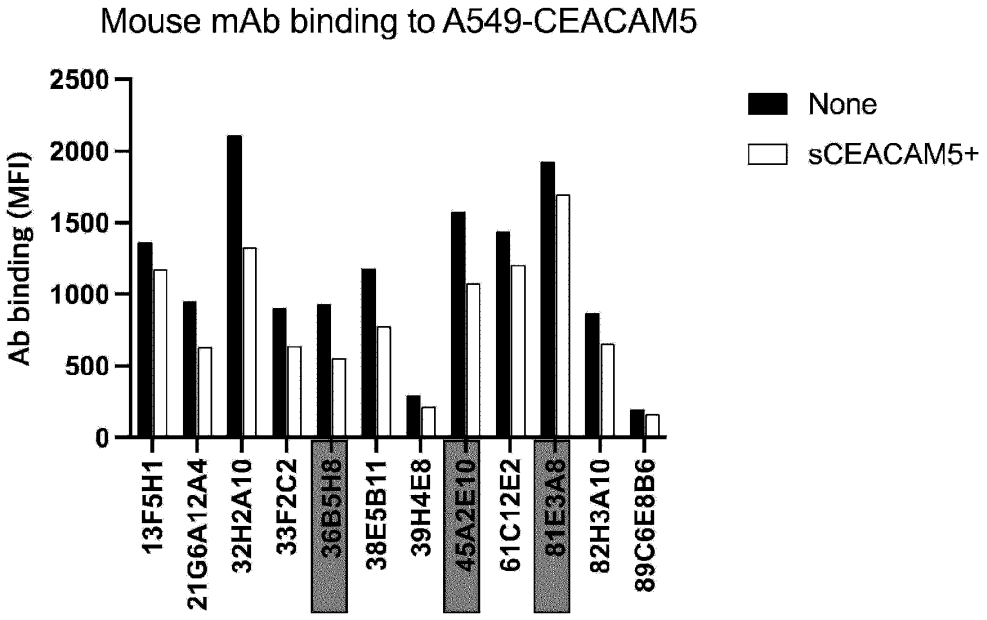
Binding activity of purified mouse monoclonal antibodies obtained from Cohort I and Cohort II



[도10b]

Clone List	EC50 (nM)
13F5H1	3.658
21G6A12A4	5.246
32H2A10	1.695
33F2C2	10.73
36B5H8	9.263
38E5B11	5.917
39H4E8	26.87
45A2E10	3.758
61C12E2	6.779
81E3A8	2.183
82H3A10	15.6
89C6E8B6	155.1

[도11a]



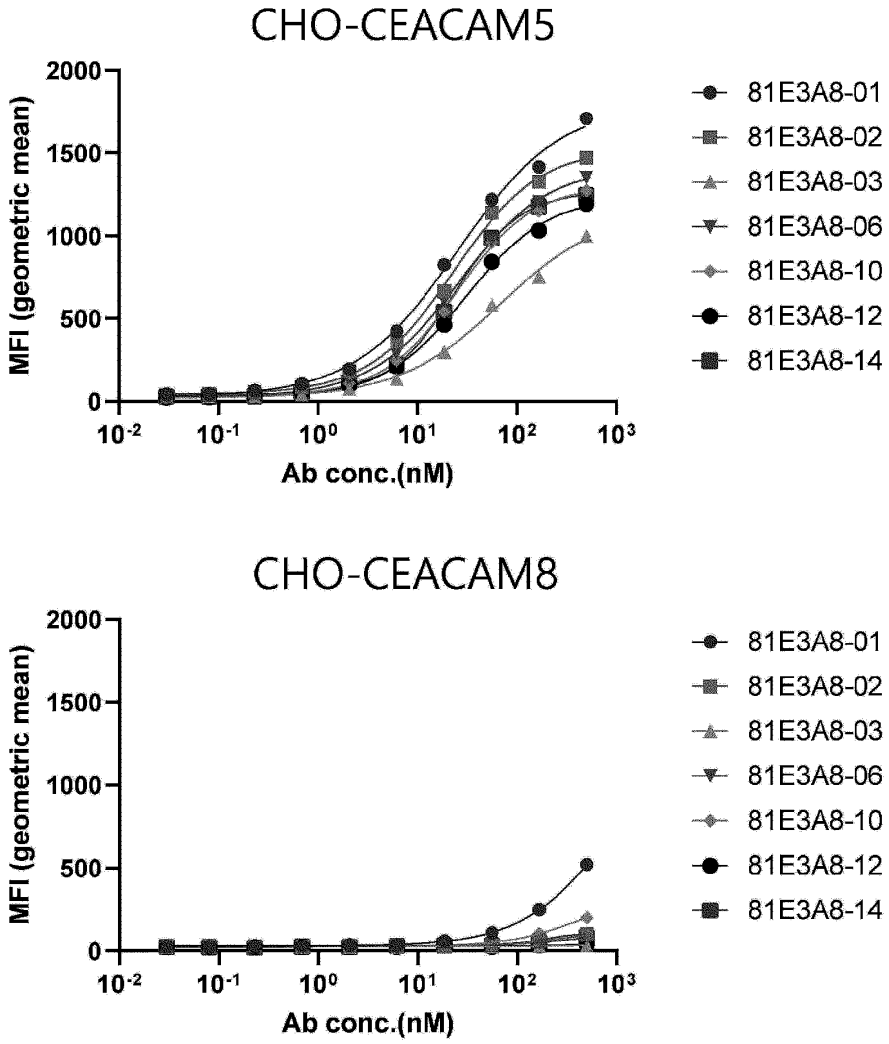
[도11b]

Clone ID	None	sCEACAM5+	% Change in Ab Binding
13F5H1	1363	1177	13.65
21G6A12A4	952	630	33.82
32H2A10	2111	1330	37.00
33F2C2	908	638	29.74
36B5H8	933	556	40.41
38E5B11	1181	779	34.04
39H4E8	299	216	27.76
45A2E10	1576	1080	31.47
61C12E2	1442	1206	16.37
81E3A8	1928	1699	11.88
82H3A10	871	660	24.23
89C6E8B6	196	165	15.82

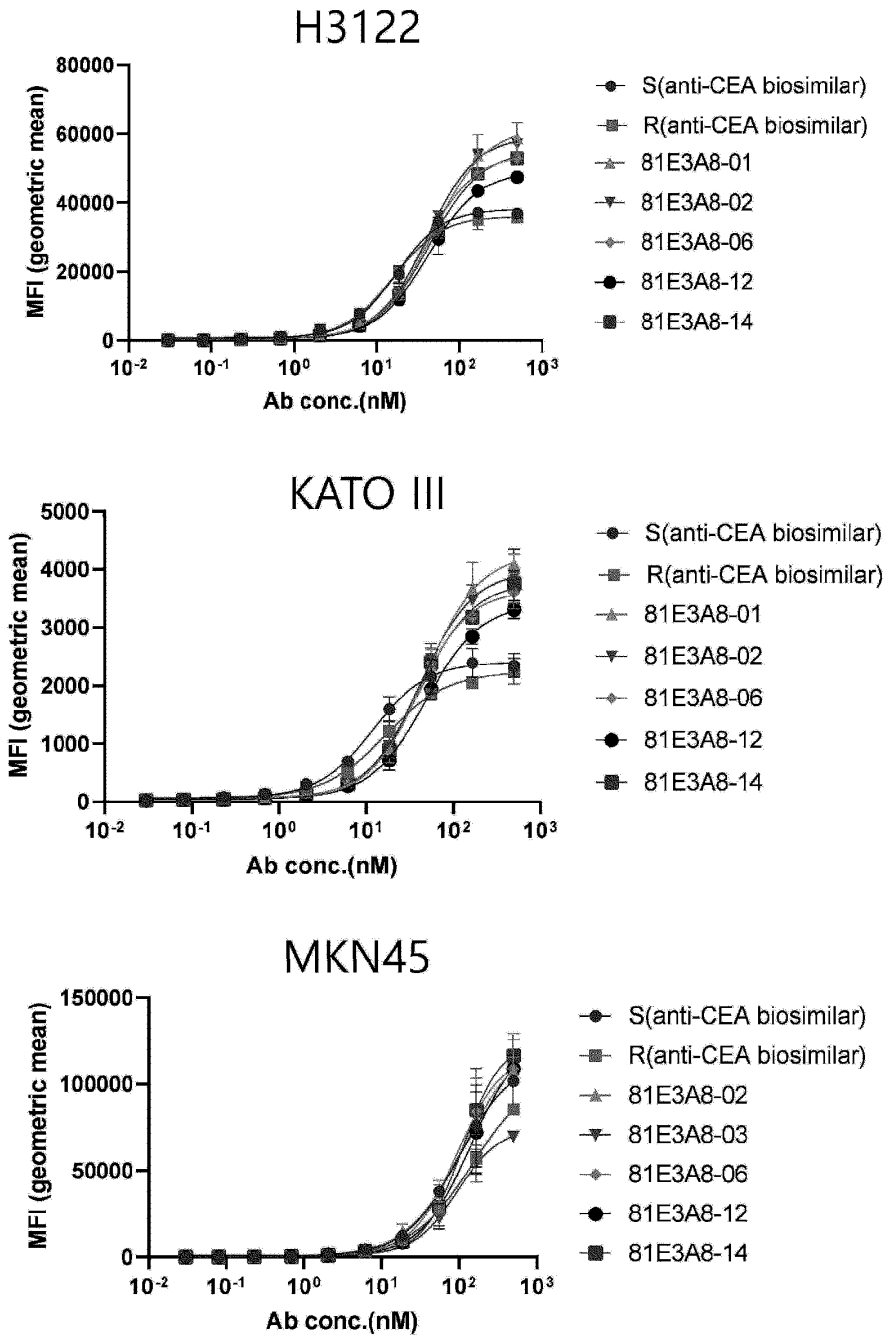
[도12a]

Ligand	Capture Conc.	Analyte 1 Solution	Analyte Conc.	1:1 binding ka (1/Ms)	kd (1/s)	KD (M)
81E3A8-01 (parental antibody)	0.5μg/ml	CEACAM5	1.5625-200nM(2-fold)	1.79E+05	1.08E-04	6.01E-10
81E3A8-02	0.5μg/ml	CEACAM5	1.5625-200nM(2-fold)	1.21E+05	7.75E-05	6.41E-10
81E3A8-06	0.5μg/ml	CEACAM5	1.5625-200nM(2-fold)	9.57E+04	7.36E-05	7.69E-10
81E3A8-10	0.5μg/ml	CEACAM5	1.5625-200nM(2-fold)	1.48E+05	9.74E-05	6.58E-10
81E3A8-12	0.5μg/ml	CEACAM5	1.5625-200nM(2-fold)	1.06E+05	1.72E-04	1.62E-09
81E3A8-14	0.5μg/ml	CEACAM5	1.5625-200nM(2-fold)	1.13E+05	9.23E-05	8.19E-10

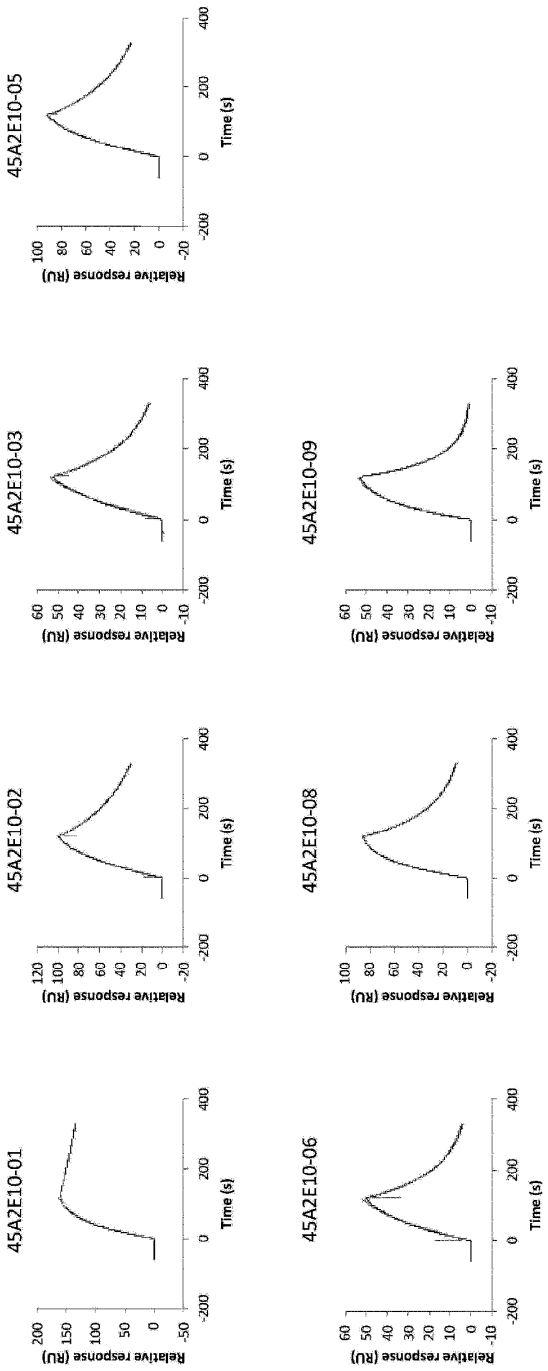
[도12b]


CHO-CEACAM5
MFI (geometric mean)
2000
1500
1000
500
0
10-2
10-1
100
101
102
103
Ab conc.(nM)
81E3A8-01
81E3A8-02
81E3A8-03
81E3A8-06
81E3A8-10
81E3A8-12
81E3A8-14
CHO-CEACAM8
MFI (geometric mean)
2000
1500
1000
500
0
10-2
10-1
100
101
102
103
Ab conc.(nM)
81E3A8-01
81E3A8-02
81E3A8-03
81E3A8-06
81E3A8-10
81E3A8-12
81E3A8-14

[도12c]


H3122
MFI (geometric mean)
80000
60000
40000
20000
0
10-2
10-1
100
101
102
103
Ab conc.(nM)
S(anti-CEA biosimilar)
R(anti-CEA biosimilar)
81E3A8-01
81E3A8-02
81E3A8-06
81E3A8-12
81E3A8-14
KATO III
MFI (geometric mean)
5000
4000
3000
2000
1000
0
10-2
10-1
100
101
102
103
Ab conc.(nM)
S(anti-CEA biosimilar)
R(anti-CEA biosimilar)
81E3A8-01
81E3A8-02
81E3A8-06
81E3A8-12
81E3A8-14
MKN45
MFI (geometric mean)
150000
100000
50000
0
10-2
10-1
100
101
102
103
Ab conc.(nM)
S(anti-CEA biosimilar)
R(anti-CEA biosimilar)
81E3A8-02
81E3A8-03
81E3A8-06
81E3A8-12
81E3A8-14

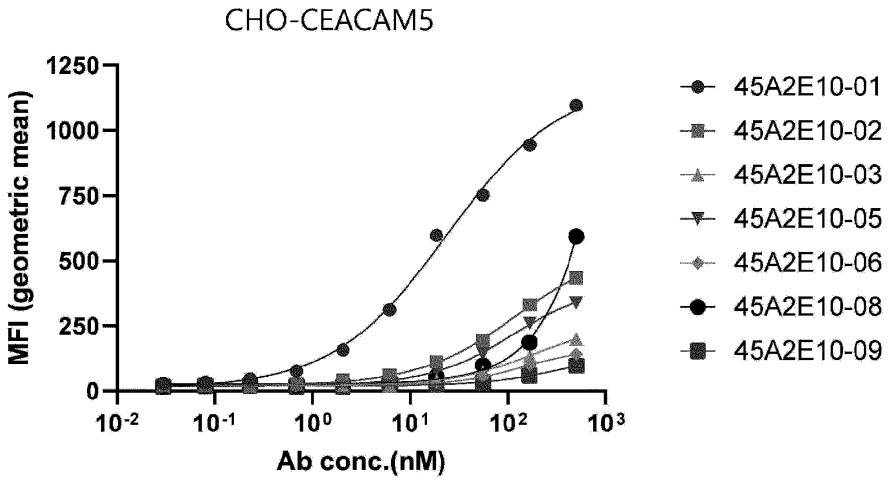
[도13a]


45A2E10-05
45A2E10-03
45A2E10-09
45A2E10-02
45A2E10-08
45A2E10-01
45A2E10-06
Relative response (RU)
Time (s)

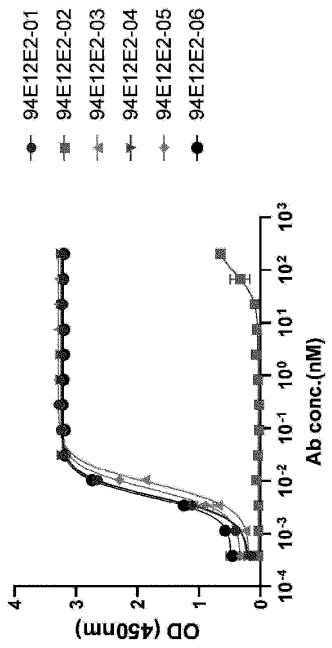
[도13b]

Ligand	Capture Conc.	Analyte 1 Solution	Analyte Conc.	1:1 binding ka (1/Ms)	kd (1/s)	KD (M)
45A2E10-01 (parental antibody)	1μg/ml	CEACAM5	200nM	1.53E+05	9.78E-04	6.41E-09
45A2E10-02	1μg/ml	CEACAM5	200nM	1.91E+05	1.74E-02	9.10E-08
45A2E10-03	1μg/ml	CEACAM5	200nM	7.78E+03	3.83E-02	4.92E-06
45A2E10-05	1μg/ml	CEACAM5	200nM	1.79E+05	2.30E-02	1.29E-07
45A2E10-06	1μg/ml	CEACAM5	200nM	5.71E+04	7.02E-02	1.23E-06
45A2E10-08	1μg/ml	CEACAM5	200nM	1.78E+05	3.13E-02	1.76E-07
45A2E10-09	1μg/ml	CEACAM5	200nM	1.25E+04	7.17E-02	5.72E-06

[도14]

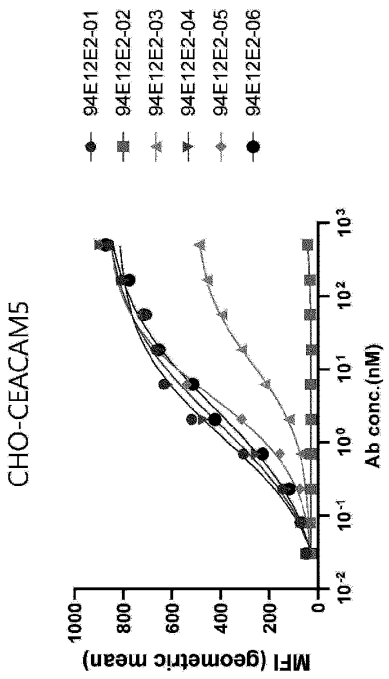

CHO-CEACAM5
MFI (geometric mean)
1250
1000
750
500
250
0
10-2
10-1
100
101
102
103
Ab conc.(nM)
45A2E10-01
45A2E10-02
45A2E10-03
45A2E10-05
45A2E10-06
45A2E10-08
45A2E10-09

[도15]



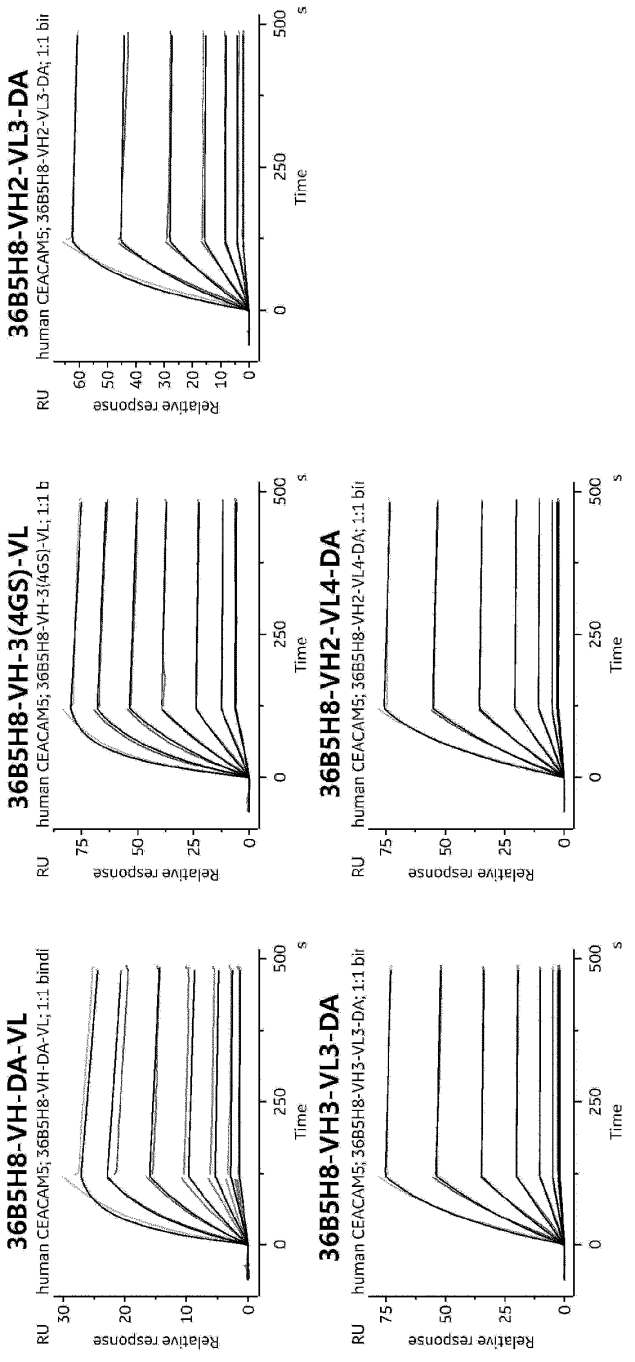
EC50 (nM)	94E12E2-01	94E12E2-02	94E12E2-03	94E12E2-04	94E12E2-05	94E12E2-06
hCEACAM5	0.005022	ND	0.008755	0.005146	0.006937	0.005055

[도16]



EC50 (nM)	94E12E2-01	94E12E2-02	94E12E2-03	94E12E2-04	94E12E2-05	94E12E2-06
hCEACAM5	1.152	ND	11.58	1.883	4.289	2.948

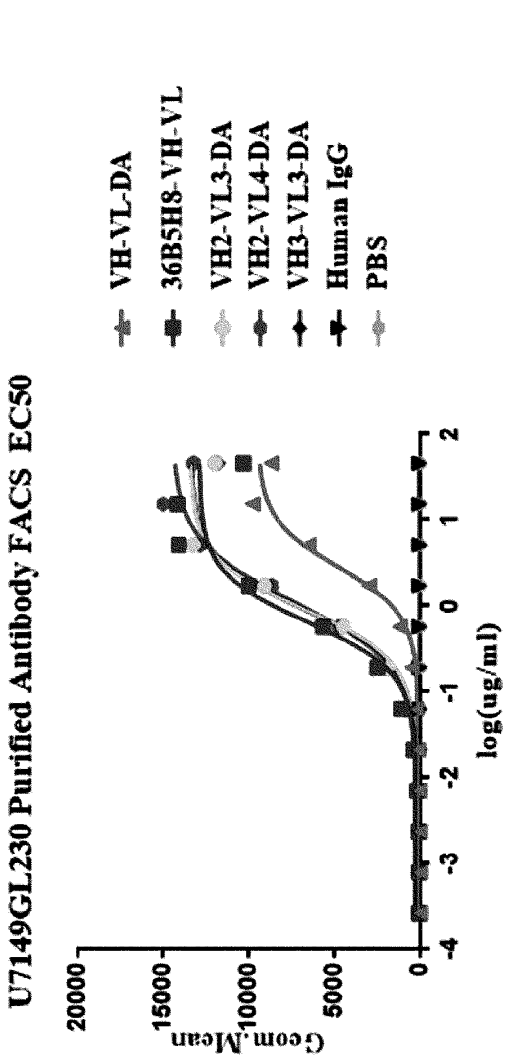
[도17a]


36B5H8-VH-DA-VL
human CEACAM5; 36B5H8-VH-DA-VL; 1:1 bindi
36B5H8-VH-3(4GS)-VL
human CEACAM5; 36B5H8-VH-3(4GS)-VL; 1:1 b
36B5H8-VH2-VL3-DA
human CEACAM5; 36B5H8-VH2-VL3-DA; 1:1 bi
36B5H8-VH3-VL3-DA
human CEACAM5; 36B5H8-VH3-VL3-DA; 1:1 bi
36B5H8-VH2-VL4-DA
human CEACAM5; 36B5H8-VH2-VL4-DA; 1:1 bi
RU
Relative response
Time
s

[도17b]

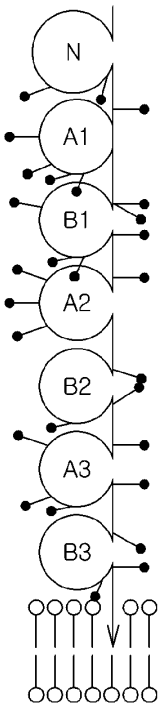
Ligand	Analyte	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)
36B5H8-VH-DA-VL	human CEACAM5	3.56E+04	2.84E-04	8.00E-09	28.3	4.26E-01
36B5H8-VH-3(4GS)-VL	human CEACAM5	3.69E+04	1.72E-04	4.67E-09	76	5.58E-01
36B5H8-VH2-VL3-DA	human CEACAM5	2.02E+04	7.81E-05	3.87E-09	73.3	5.30E-01
36B5H8-VH2-VL4-DA	human CEACAM5	1.90E+04	1.09E-04	5.76E-09	85	1.31E-01
36B5H8-VH3-VL3-DA	human CEACAM5	1.58E+04	9.10E-05	5.77E-09	86.1	2.04E-01

[도18]



	VH-VL-DA	36B5H8-VH-VL	VH2-VL3-DA	VH2-VL4-DA	VH3-VL3-DA	Human IgG	PBS
EC50	2.772	0.6333	0.9118	1.076	0.8843	~ 1.108e+006	~ 0.4445

[도19a]



[도19b]

