Figure 1

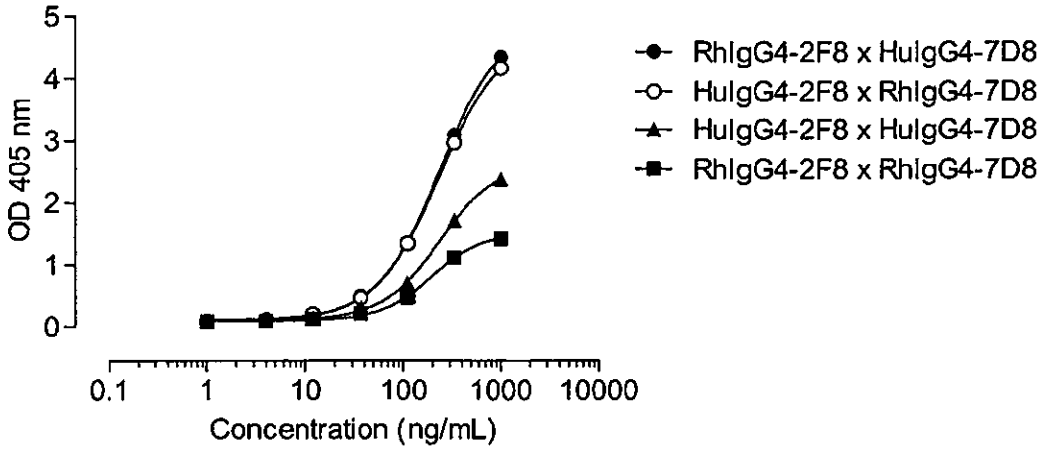

OD 405 nm
5
4
3
2
1
0
0.1
1
10
100
1000
10000
Concentration (ng/mL)
RhIgG4-2F8 x HuIgG4-7D8
HuIgG4-2F8 x RhIgG4-7D8
HuIgG4-2F8 x HuIgG4-7D8
RhIgG4-2F8 x RhIgG4-7D8

Figure 2

Species (common name)	Isotype	Amino-acid position*																				
		Core-hinge					CH3-CH3 interface															
		226	227	228	229	230	347	349	350	351	366	368	370	392	394	395	397	398	399	405	407	409
Homo sapiens	IgG1	C	P	P	C	P	Q	Y	T	L	T	L	K	K	T	P	V	L	D	F	Y	K
	IgG2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	M	-	-	-	-	-
	IgG3	-	-	R	-	-	-	-	-	-	-	-	-	N	-	-	M	-	-	-	-	-
	IgG4	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R
Macaca mulatta	IgG1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(Rhesus Monkey)	IgG2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	IgG3	-	-	-	-	-	-	-	I	-	-	-	T	-	-	-	-	-	-	-	-	-
	IgG4 (In)**	-	-	-	-	-	-	-	I	-	-	-	T	-	-	-	-	-	-	L	-	-
	IgG4 (Ch)***	-	-	A	-	-	-	-	I	-	-	-	T	-	-	-	-	-	-	L	-	-

*EU numbering; **In, Indian; ***Ch, Chinese

Figure 3

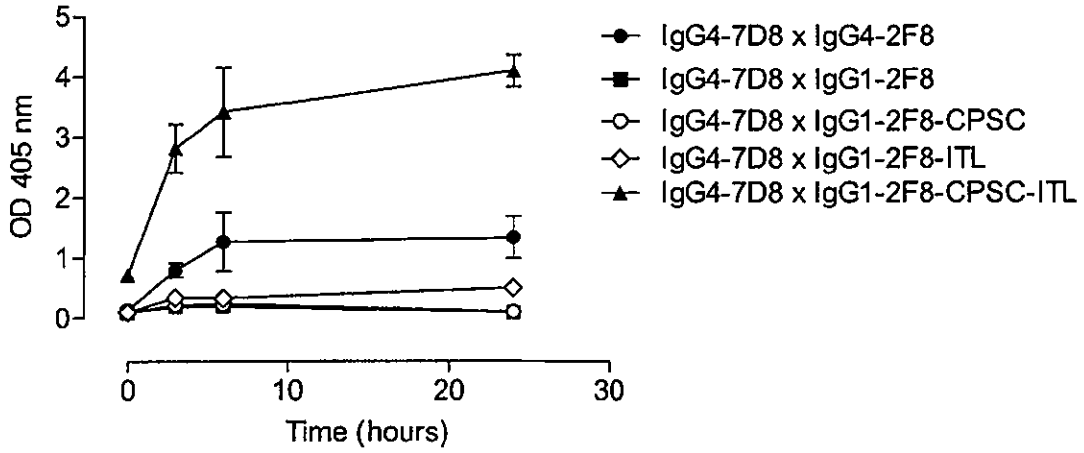

OD 405 nm
5
4
3
2
1
0
0
10
20
30
Time (hours)
IgG4-7D8 x IgG4-2F8
IgG4-7D8 x IgG1-2F8
IgG4-7D8 x IgG1-2F8-CPSC
IgG4-7D8 x IgG1-2F8-ITL
IgG4-7D8 x IgG1-2F8-CPSC-ITL

Figure 4

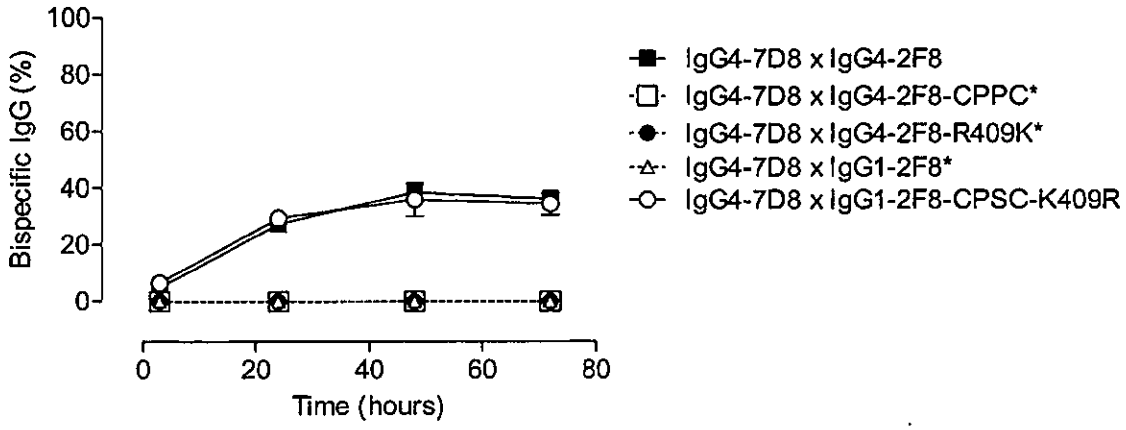

Bispecific IgG (%)
100
80
60
40
20
0
0
20
40
60
80
Time (hours)
IgG4-7D8 x IgG4-2F8
IgG4-7D8 x IgG4-2F8-CPPC*
IgG4-7D8 x IgG4-2F8-R409K*
IgG4-7D8 x IgG1-2F8*
IgG4-7D8 x IgG1-2F8-CPSC-K409R

Figure 5

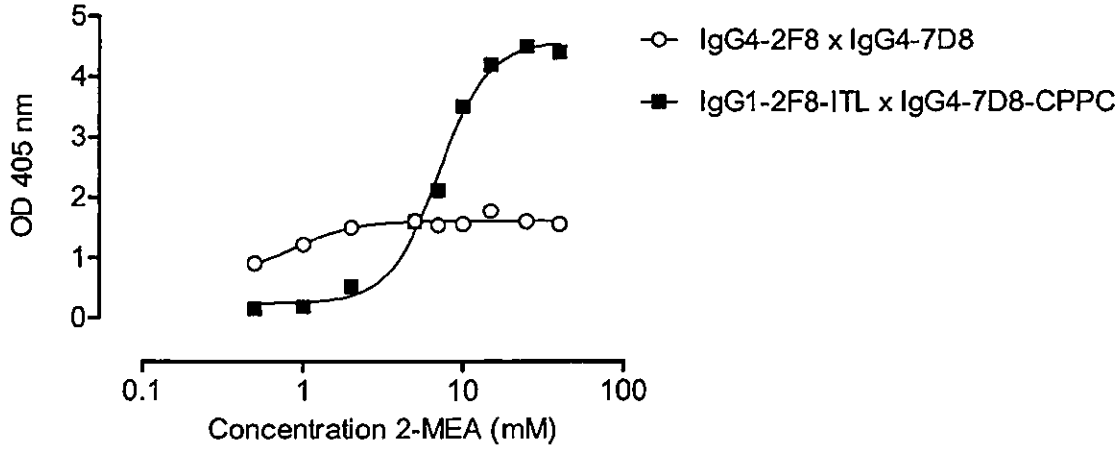
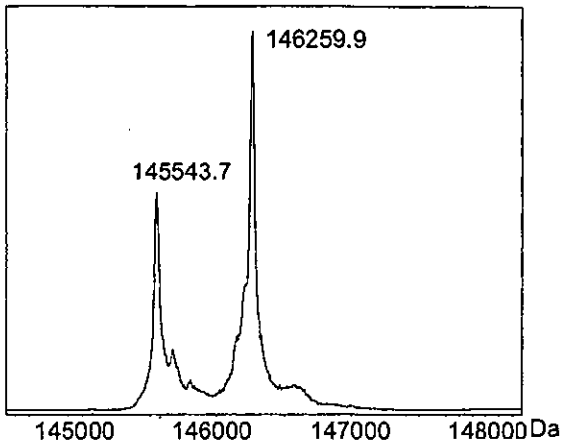

OD 405 nm
5
4
3
2
1
0
0.1
1
10
100
Concentration 2-MEA (mM)
IgG4-2F8 x IgG4-7D8
IgG1-2F8-ITL x IgG4-7D8-CPPC

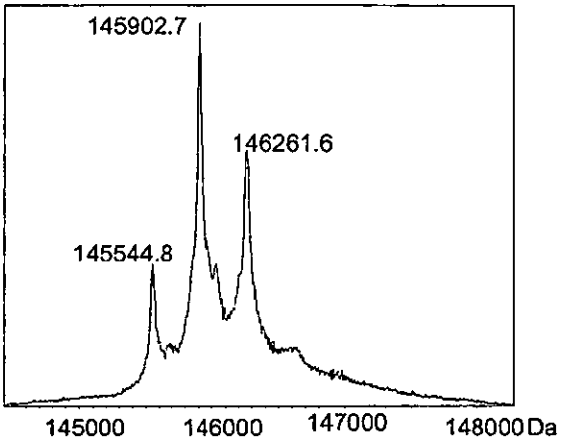
Figure 6

A

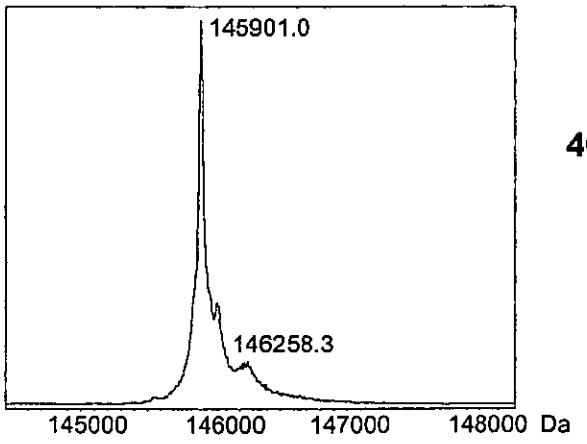
IgG1-2F8-ITL X IgG4-7D8-CPPC



0 mM 2-MEA



7 mM 2-MEA



40 mM 2-MEA

Figure 6 (continued)

B

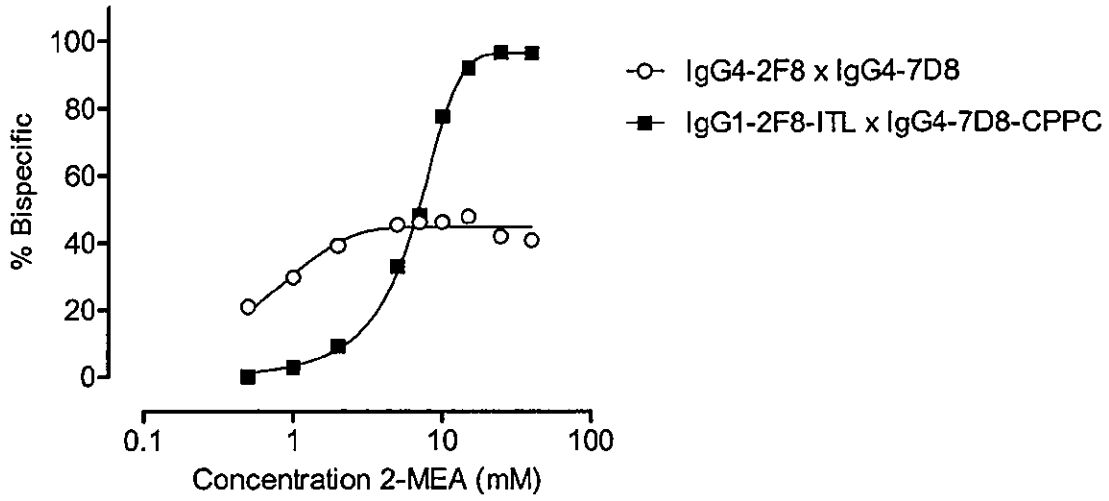
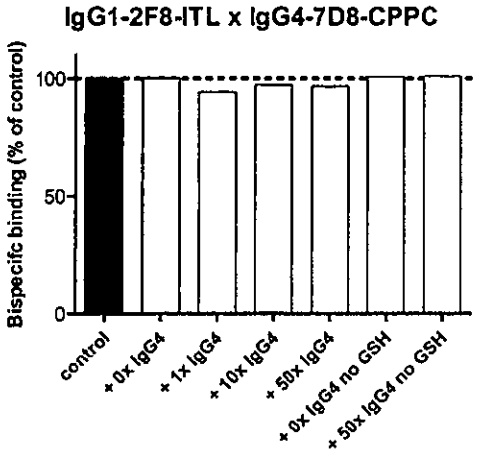


Figure 7

A


IgG1-2F8-ITL x IgG4-7D8-CPPC
Bispecifc binding (% of control)
100
50
0
control
+ 0x IgG4
+ 1x IgG4
+ 10x IgG4
+ 50x IgG4
+ 0x IgG4 no GSH
+ 50x IgG4 no GSH

B

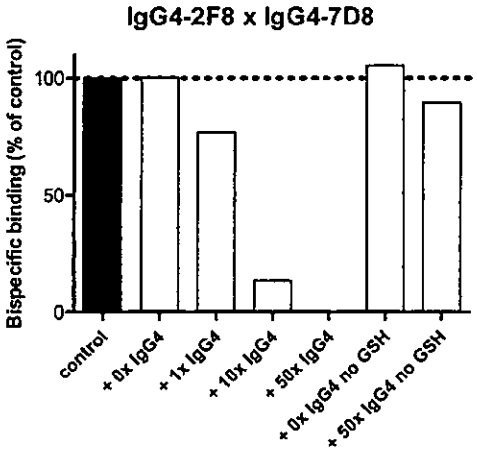
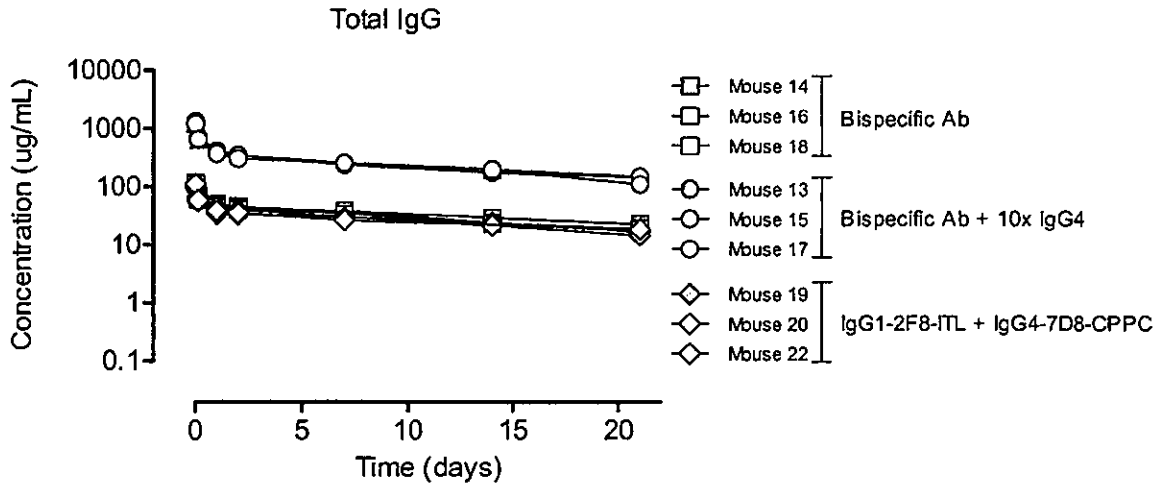

IgG4-2F8 x IgG4-7D8
Bispecific binding (% of control)
100
50
0
control
+ 0x IgG4
+ 1x IgG4
+ 10x IgG4
+ 50x IgG4
+ 0x IgG4 no GSH
+ 50x IgG4 no GSH

Figure 8

A


Total IgG
Concentration (ug/mL)
10000
1000
100
10
1
0.1
0
5
10
15
20
Time (days)
Mouse 14
Mouse 16
Mouse 18
Bispecific Ab
Mouse 13
Mouse 15
Mouse 17
Bispecific Ab + 10x IgG4
Mouse 19
Mouse 20
Mouse 22
IgG1-2F8-ITL + IgG4-7D8-CPPC

B

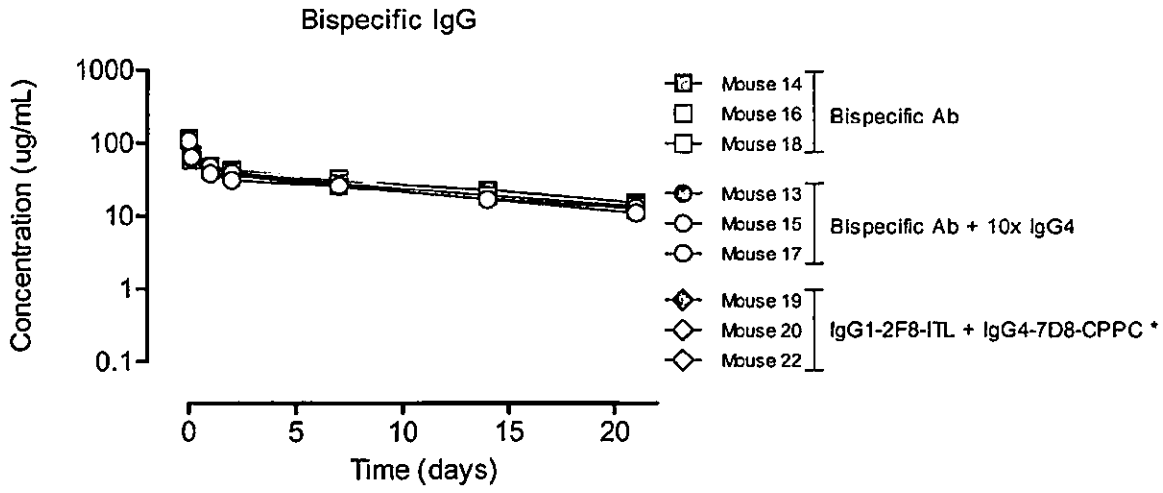

Bispecific IgG
Concentration (ug/mL)
1000
100
10
1
0.1
0
5
10
15
20
Time (days)
Mouse 14
Mouse 16
Mouse 18
Bispecific Ab
Mouse 13
Mouse 15
Mouse 17
Bispecific Ab + 10x IgG4
Mouse 19
Mouse 20
Mouse 22
IgG1-2F8-ITL + IgG4-7D8-CPPC *

Figure 9A

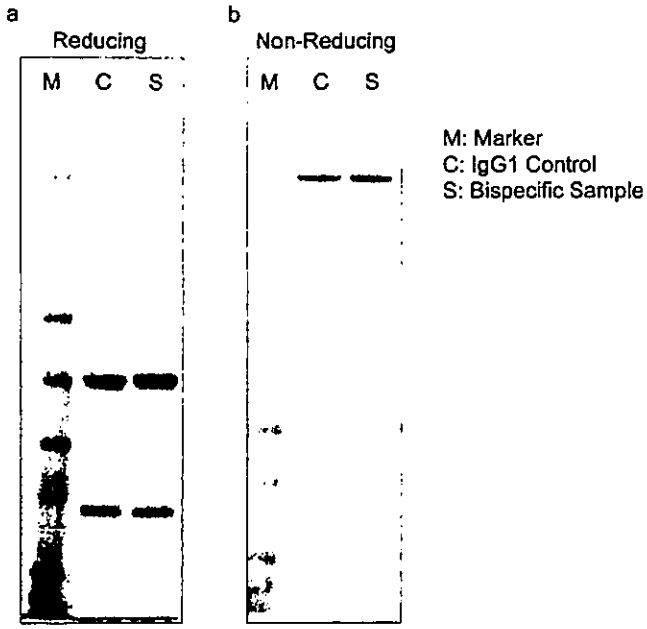
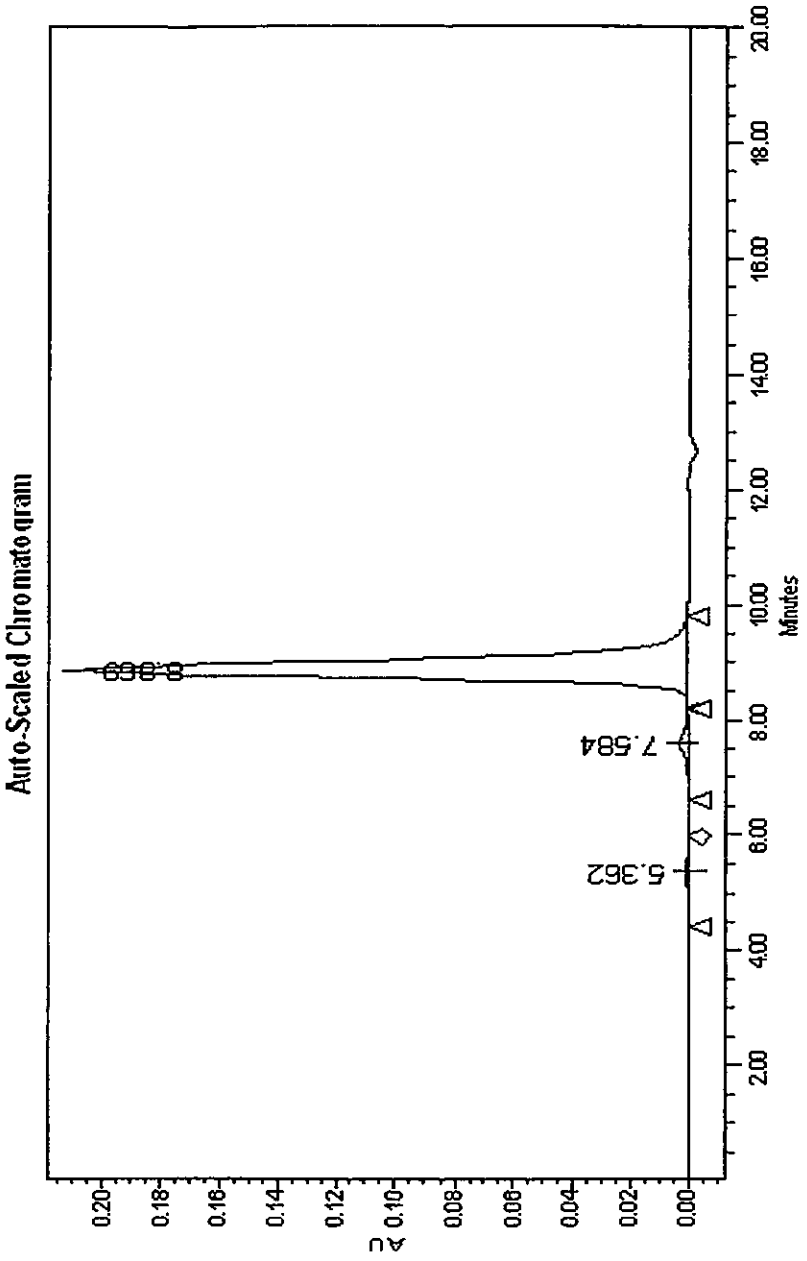

a
Reducing
M C S
b
Non-Reducing
M C S
M: Marker
C: IgG1 Control
S: Bispecific Sample

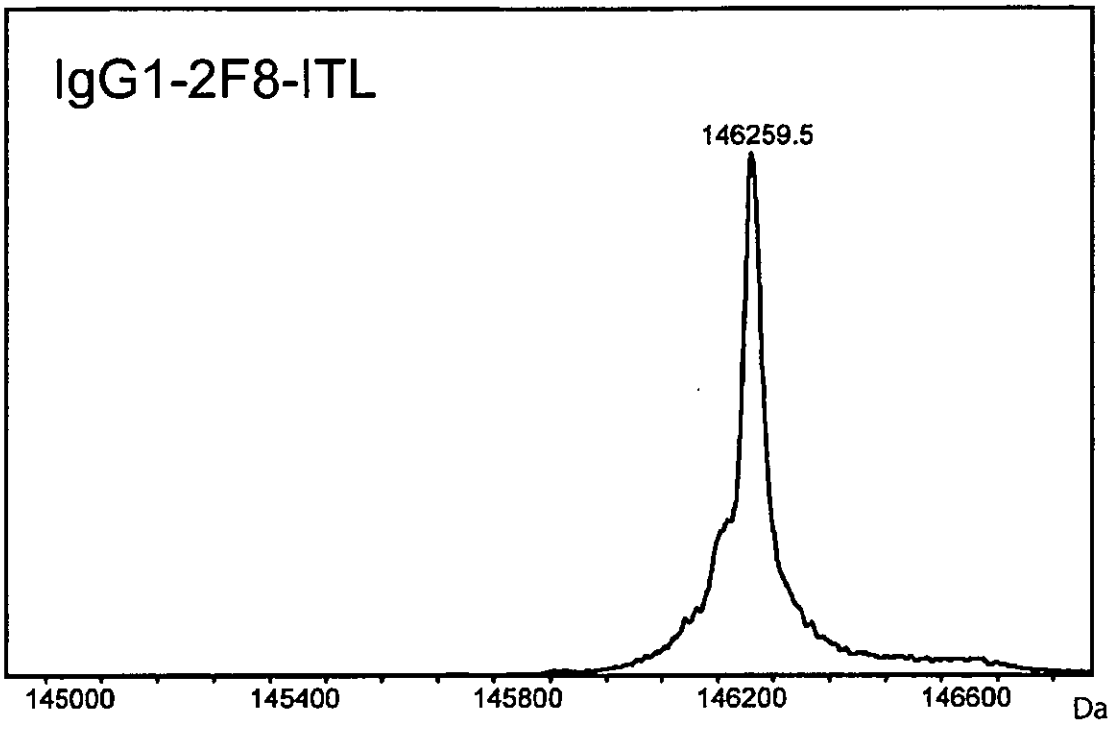
Figure 9B



Peak results:

	RT (min)	Area	Height	% Height
1	5.362	18309	448	0.21
2	7.584	82197	2876	1.36
3	8.869	4431069	207416	98.42

Figure 9C


IgG1-2F8-ITL
146259.5
145000
145400
145800
146200
146600
Da

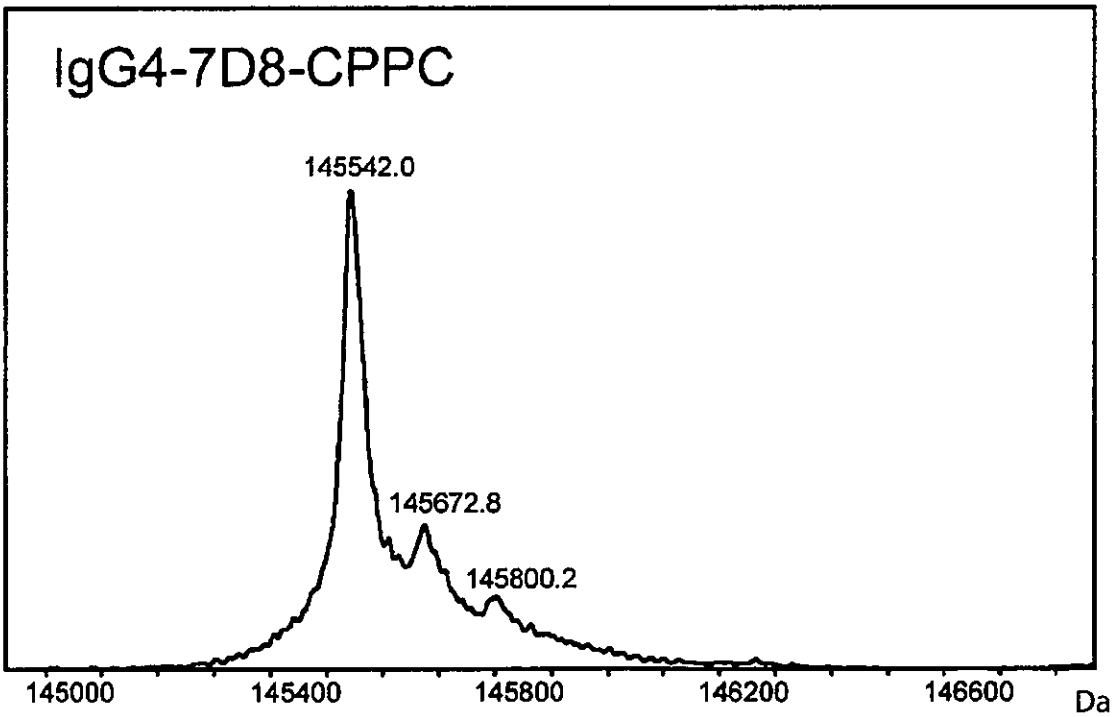
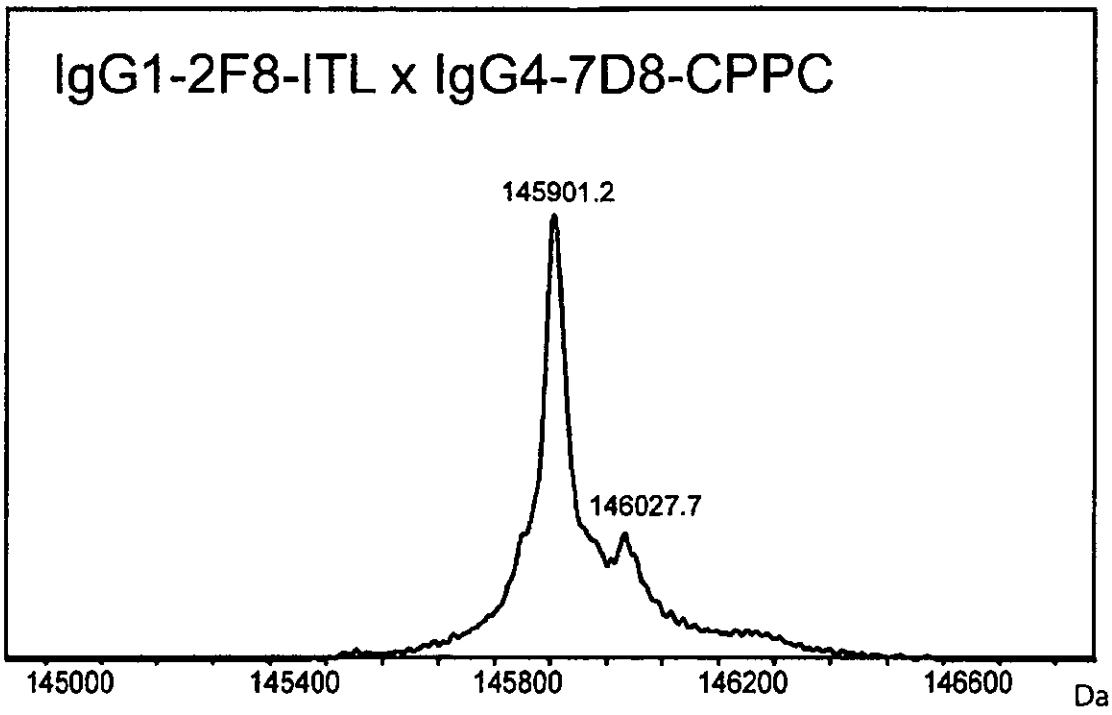

IgG4-7D8-CPPC
145542.0
145672.8
145800.2
145000
145400
145800
146200
146600
Da

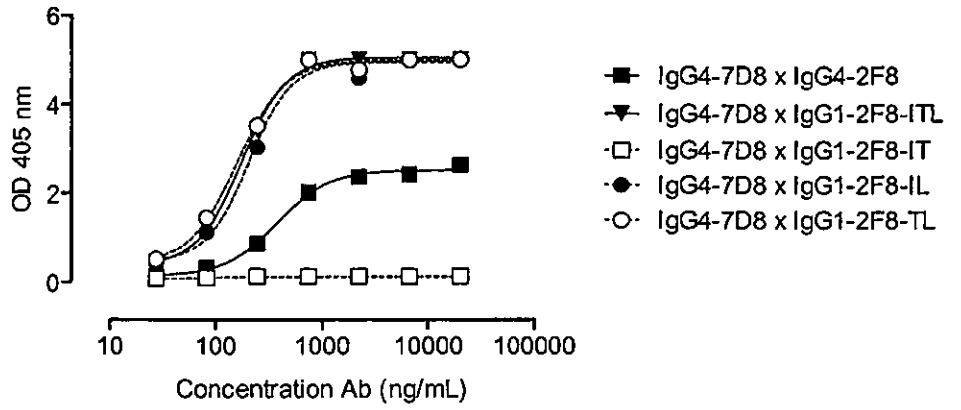
Figure 9C (continued)



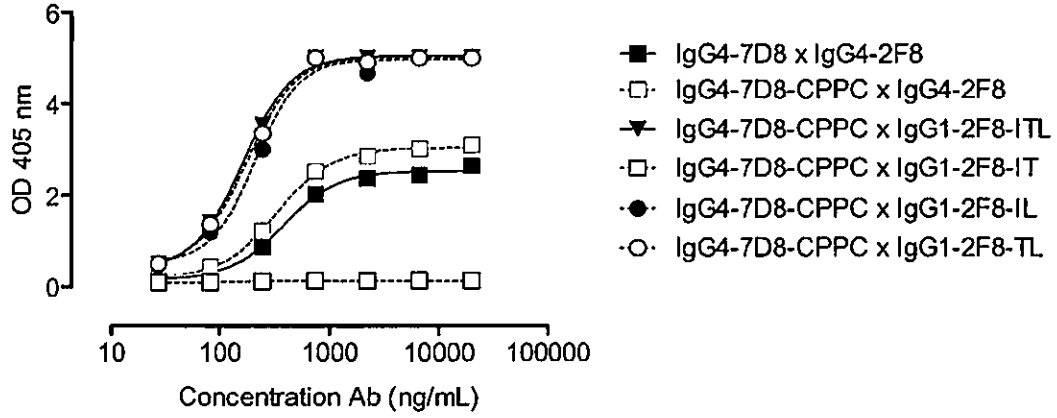
	Measured	Measured	Measured
IgG1-2F8-ITL	146259.5		
IgG4-7D8-CPPC	145542.0	145672.8	145800.2
IgG1-2F8-ITL x IgG4-7D8-	145901.2	146027.7	

Figure 10

(A)



(B)



(C)

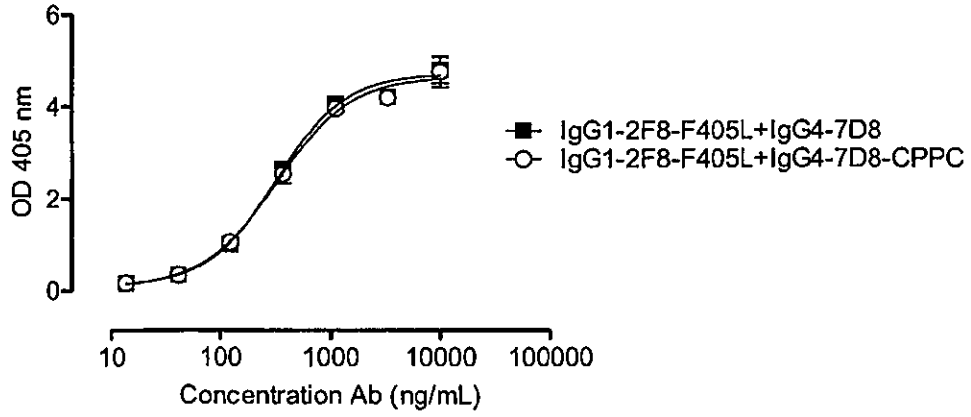


Figure 11

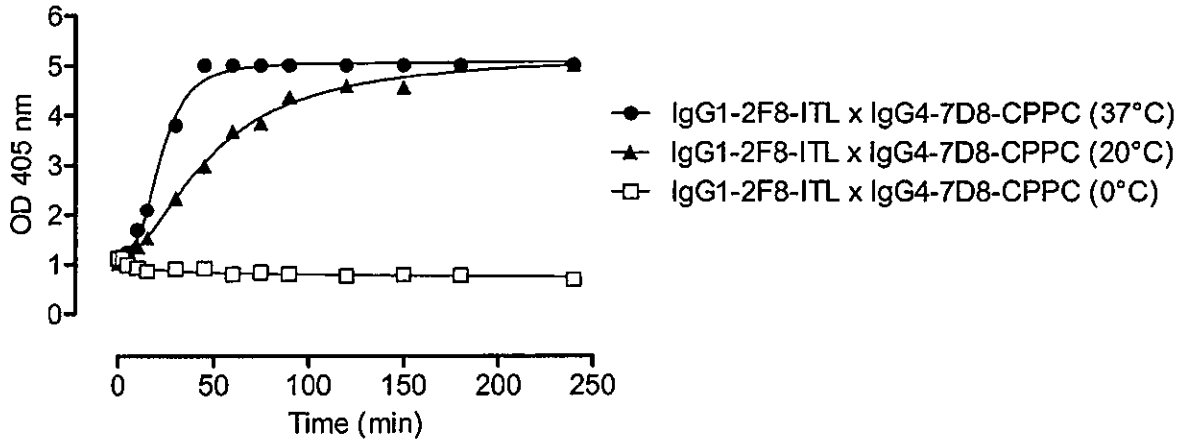

OD 405 nm
0
1
2
3
4
5
6
0
50
100
150
200
250
Time (min)
IgG1-2F8-ITL x IgG4-7D8-CPPC (37°C)
IgG1-2F8-ITL x IgG4-7D8-CPPC (20°C)
IgG1-2F8-ITL x IgG4-7D8-CPPC (0°C)

Figure 12

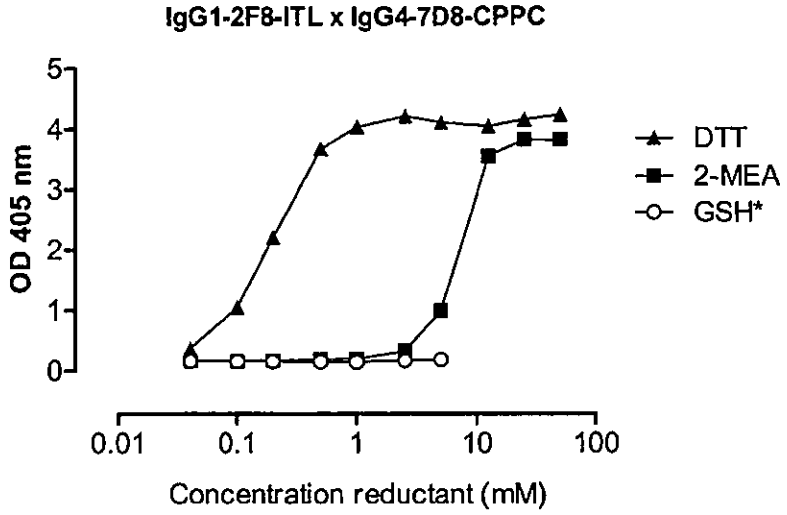
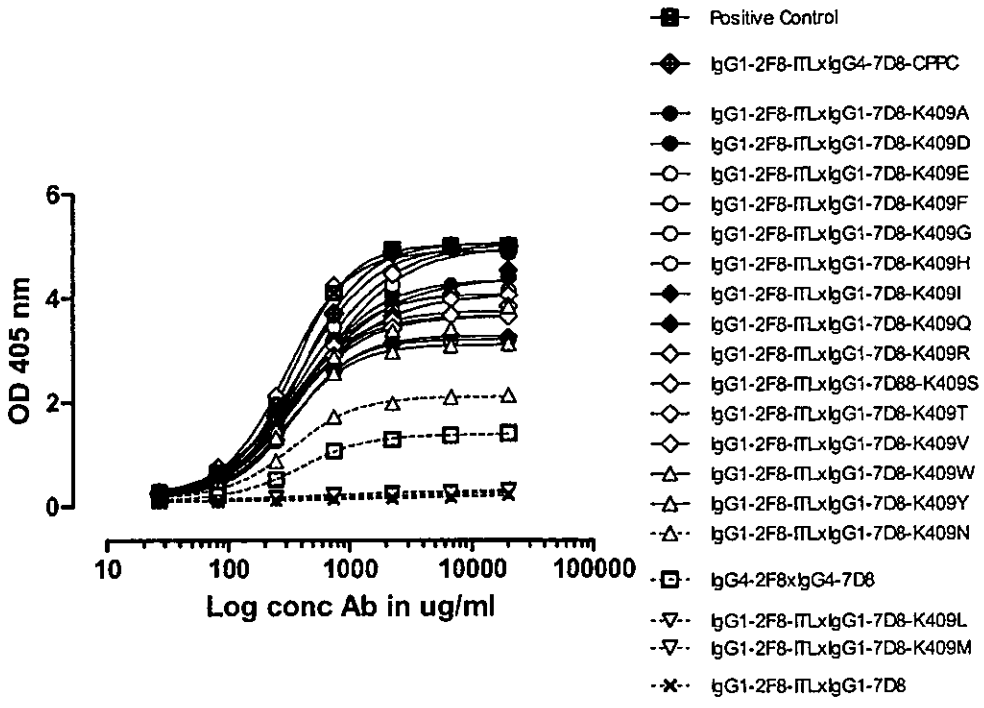

IgG1-2F8-ITL x IgG4-7D8-CPPC
OD 405 nm
5
4
3
2
1
0
0.01
0.1
1
10
100
Concentration reductant (mM)
DTT
2-MEA
GSH*

Figure 13

A


OD 405 nm
0
2
4
6
10
100
1000
10000
100000
Log conc Ab in ug/ml
Positive Control
IgG1-2F8-ITLxIgG4-7D8-CPPC
IgG1-2F8-ITLxIgG1-7D8-K409A
IgG1-2F8-ITLxIgG1-7D8-K409D
IgG1-2F8-ITLxIgG1-7D8-K409E
IgG1-2F8-ITLxIgG1-7D8-K409F
IgG1-2F8-ITLxIgG1-7D8-K409G
IgG1-2F8-ITLxIgG1-7D8-K409H
IgG1-2F8-ITLxIgG1-7D8-K409I
IgG1-2F8-ITLxIgG1-7D8-K409Q
IgG1-2F8-ITLxIgG1-7D8-K409R
IgG1-2F8-ITLxIgG1-7D88-K409S
IgG1-2F8-ITLxIgG1-7D8-K409T
IgG1-2F8-ITLxIgG1-7D8-K409V
IgG1-2F8-ITLxIgG1-7D8-K409W
IgG1-2F8-ITLxIgG1-7D8-K409Y
IgG1-2F8-ITLxIgG1-7D8-K409N
IgG4-2F8xIgG4-7D8
IgG1-2F8-ITLxIgG1-7D8-K409L
IgG1-2F8-ITLxIgG1-7D8-K409M
IgG1-2F8-ITLxIgG1-7D8

B

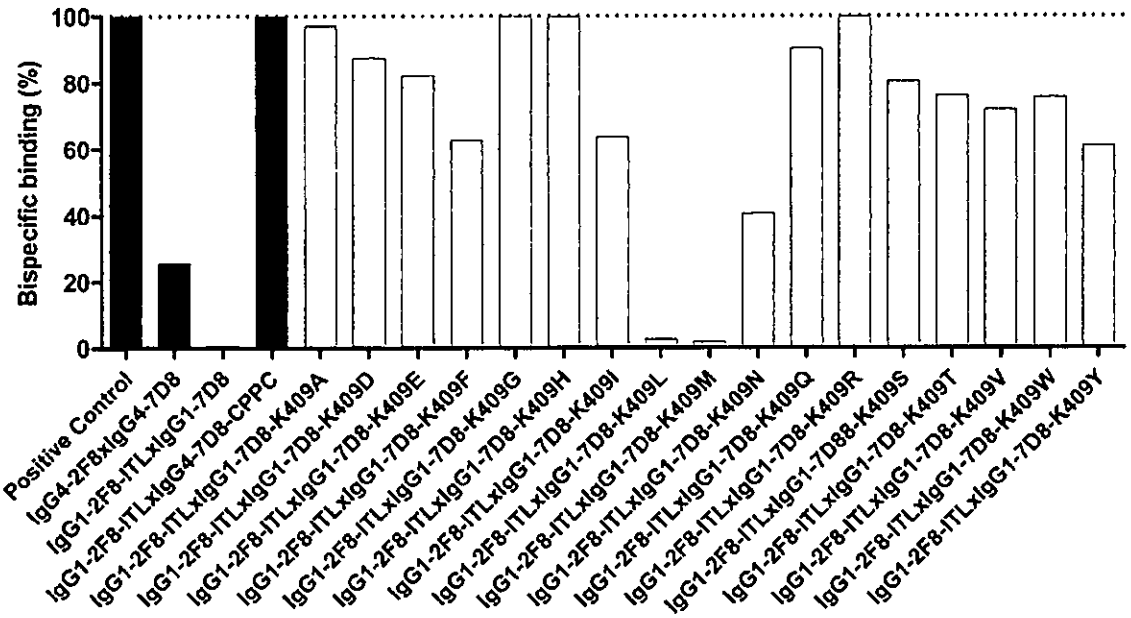

Bispecific binding (%)
0
20
40
60
80
100
Positive Control
IgG4-2F8xIgG4-7D8
IgG1-2F8-ITLxIgG1-7D8
IgG1-2F8-ITLxIgG4-7D8-CPPC
IgG1-2F8-ITLxIgG1-7D8-K409A
IgG1-2F8-ITLxIgG1-7D8-K409D
IgG1-2F8-ITLxIgG1-7D8-K409E
IgG1-2F8-ITLxIgG1-7D8-K409F
IgG1-2F8-ITLxIgG1-7D8-K409G
IgG1-2F8-ITLxIgG1-7D8-K409H
IgG1-2F8-ITLxIgG1-7D8-K409I
IgG1-2F8-ITLxIgG1-7D8-K409L
IgG1-2F8-ITLxIgG1-7D8-K409M
IgG1-2F8-ITLxIgG1-7D8-K409N
IgG1-2F8-ITLxIgG1-7D8-K409Q
IgG1-2F8-ITLxIgG1-7D8-K409R
IgG1-2F8-ITLxIgG1-7D88-K409S
IgG1-2F8-ITLxIgG1-7D8-K409T
IgG1-2F8-ITLxIgG1-7D8-K409V
IgG1-2F8-ITLxIgG1-7D8-K409W
IgG1-2F8-ITLxIgG1-7D8-K409Y

Figure 14

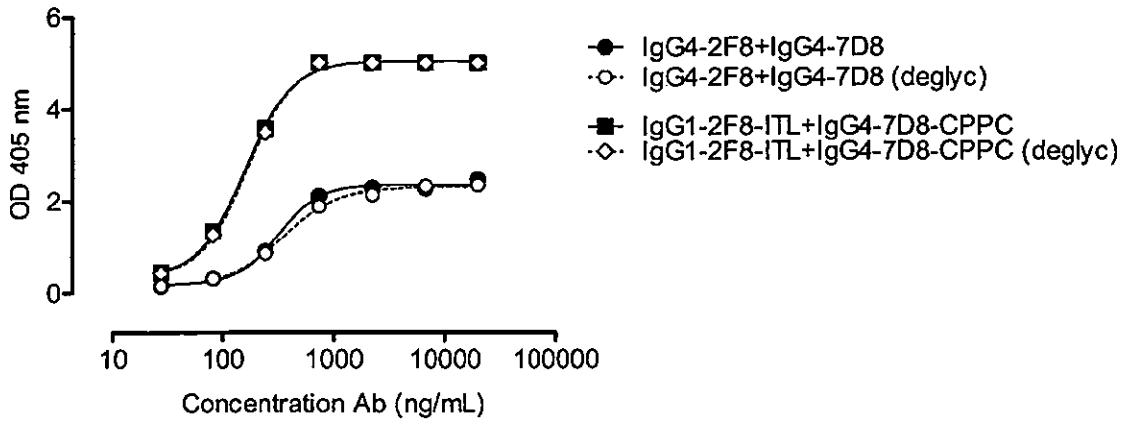
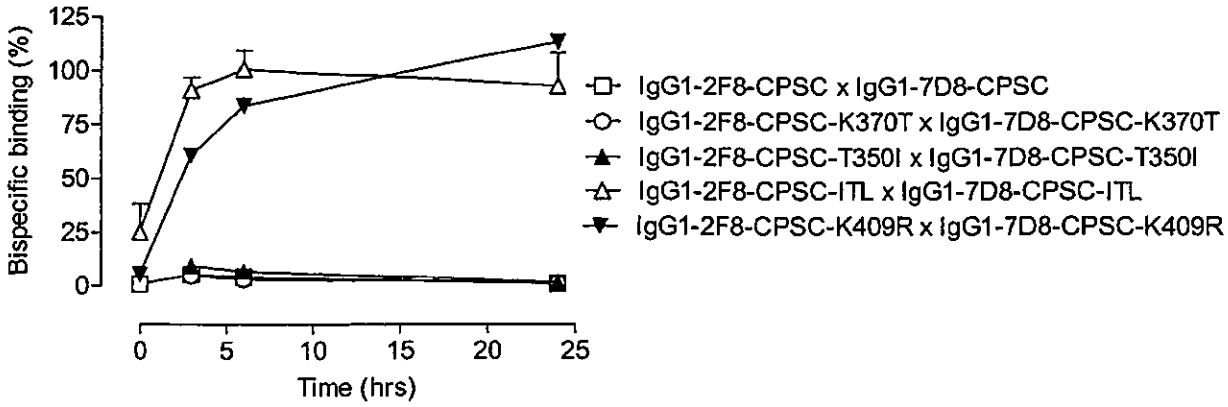
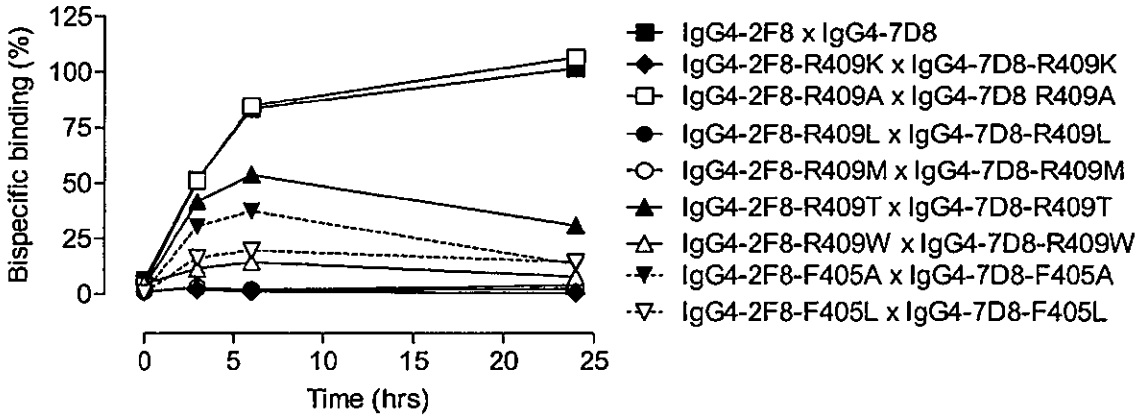

6
4
2
0
OD 405 nm
10
100
1000
10000
100000
Concentration Ab (ng/mL)
IgG4-2F8+IgG4-7D8
IgG4-2F8+IgG4-7D8 (deglyc)
IgG1-2F8-ITL+IgG4-7D8-CPPC
IgG1-2F8-ITL+IgG4-7D8-CPPC (deglyc)

Figure 15

A



B



C

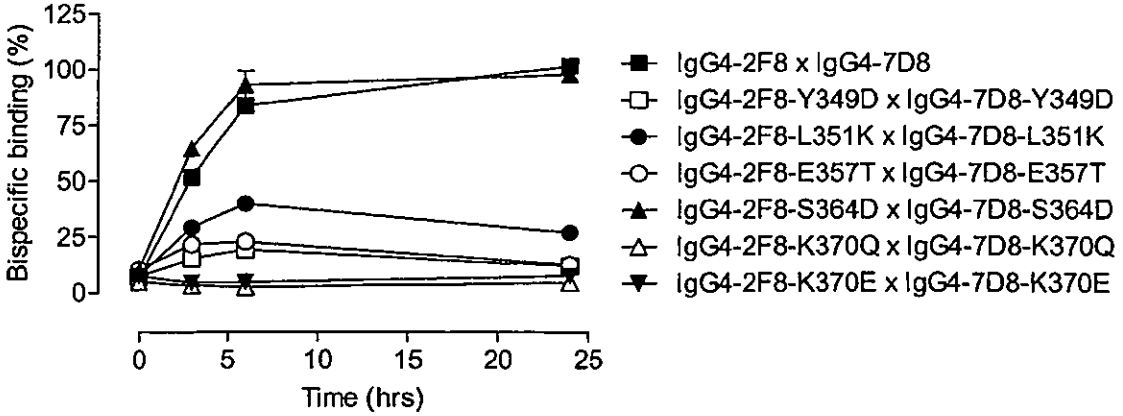
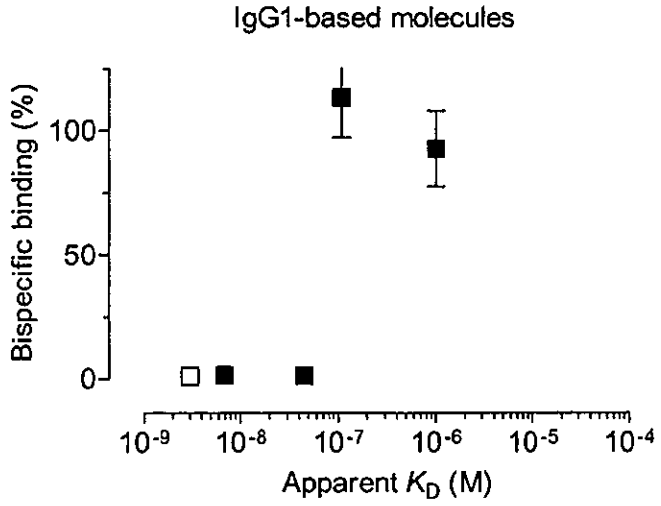


Figure 15 (continued)

D



E

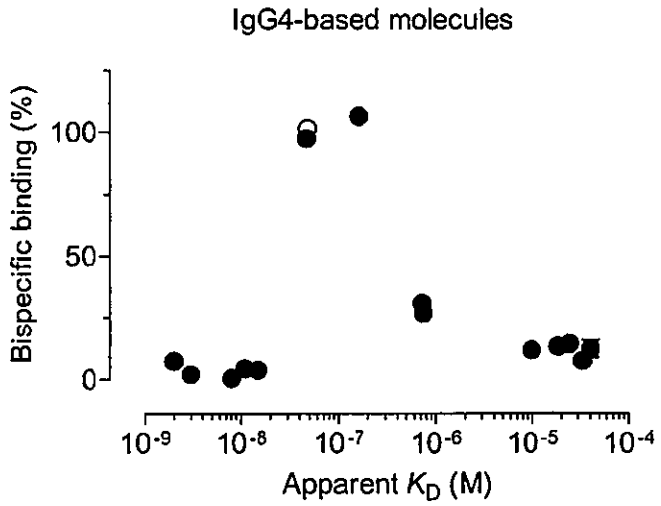


Figure 16

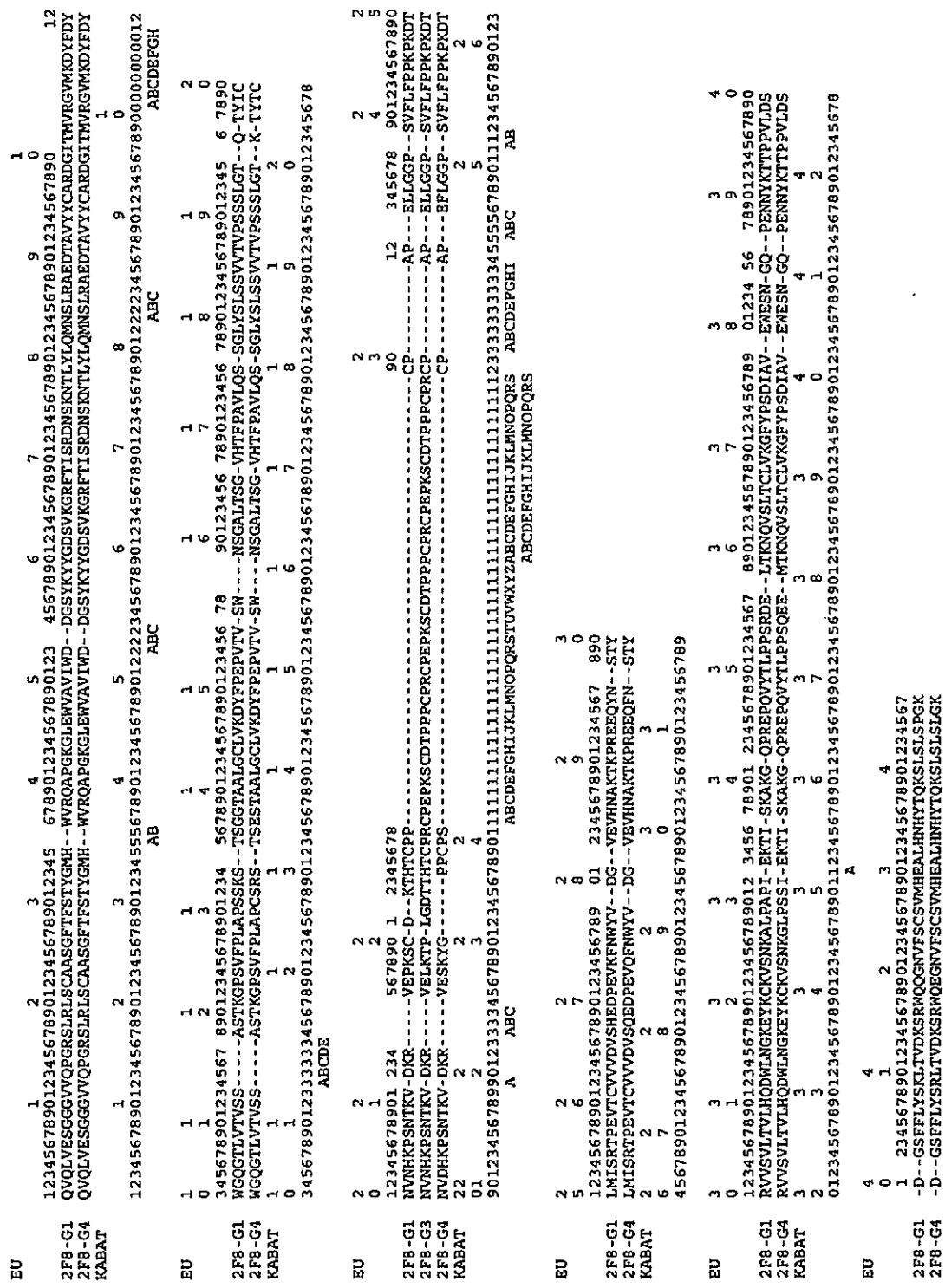


Figure 17

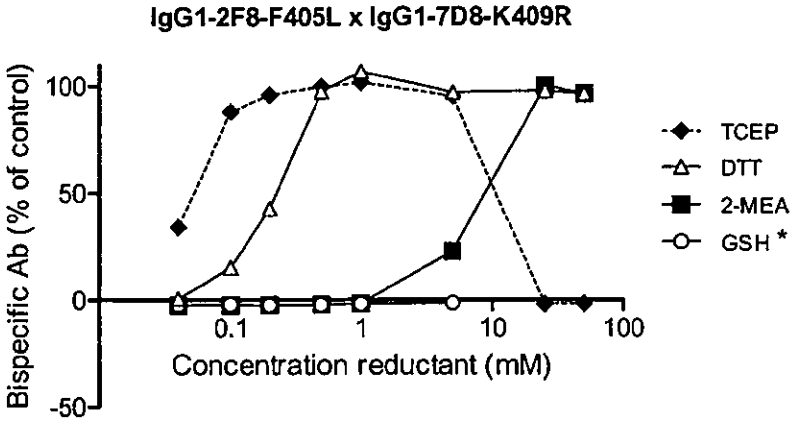
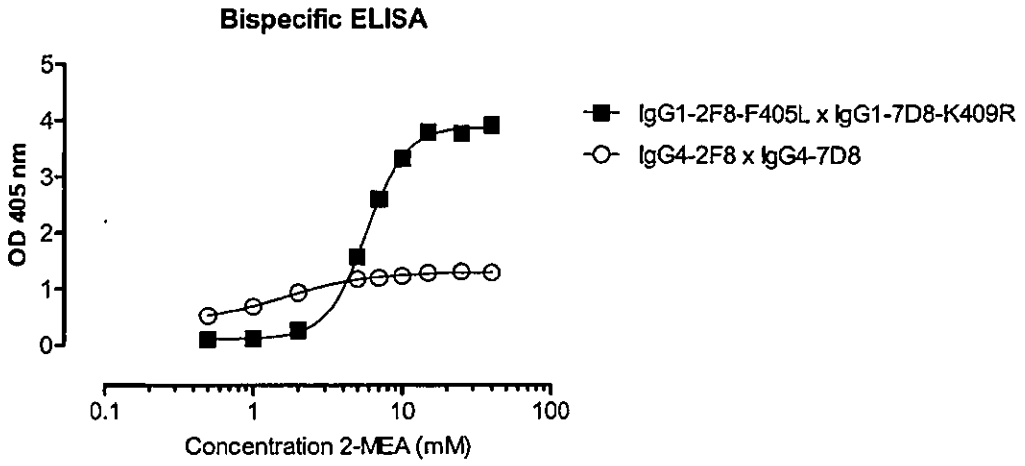

IgG1-2F8-F405L x IgG1-7D8-K409R
Bispecific Ab (% of control)
100
50
0
-50
0.1
1
10
100
Concentration reductant (mM)
TCEP
DTT
2-MEA
GSH *

Figure 18

A


Bispecific ELISA
OD 405 nm
0
1
2
3
4
5
0.1
1
10
100
Concentration 2-MEA (mM)
IgG1-2F8-F405L x IgG1-7D8-K409R
IgG4-2F8 x IgG4-7D8

B

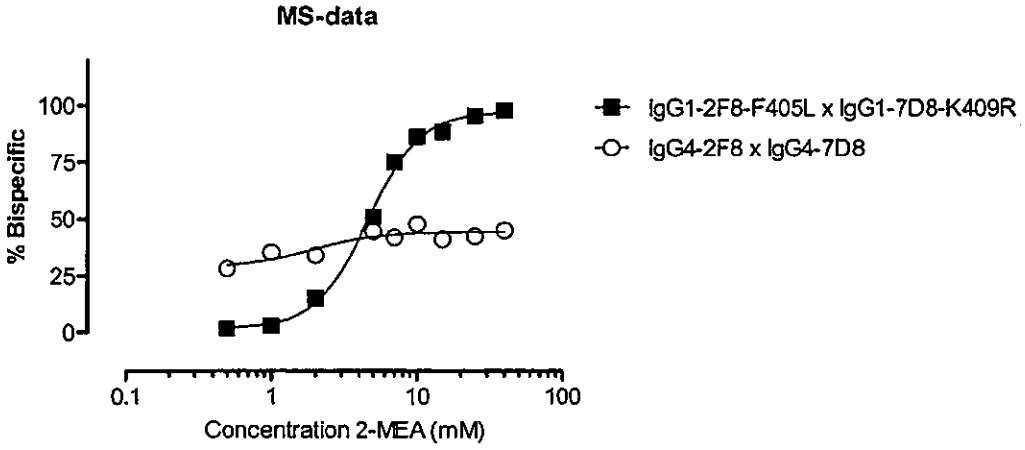

MS-data
% Bispecific
0
25
50
75
100
0.1
1
10
100
Concentration 2-MEA (mM)
IgG1-2F8-F405L x IgG1-7D8-K409R
IgG4-2F8 x IgG4-7D8

Figure 19

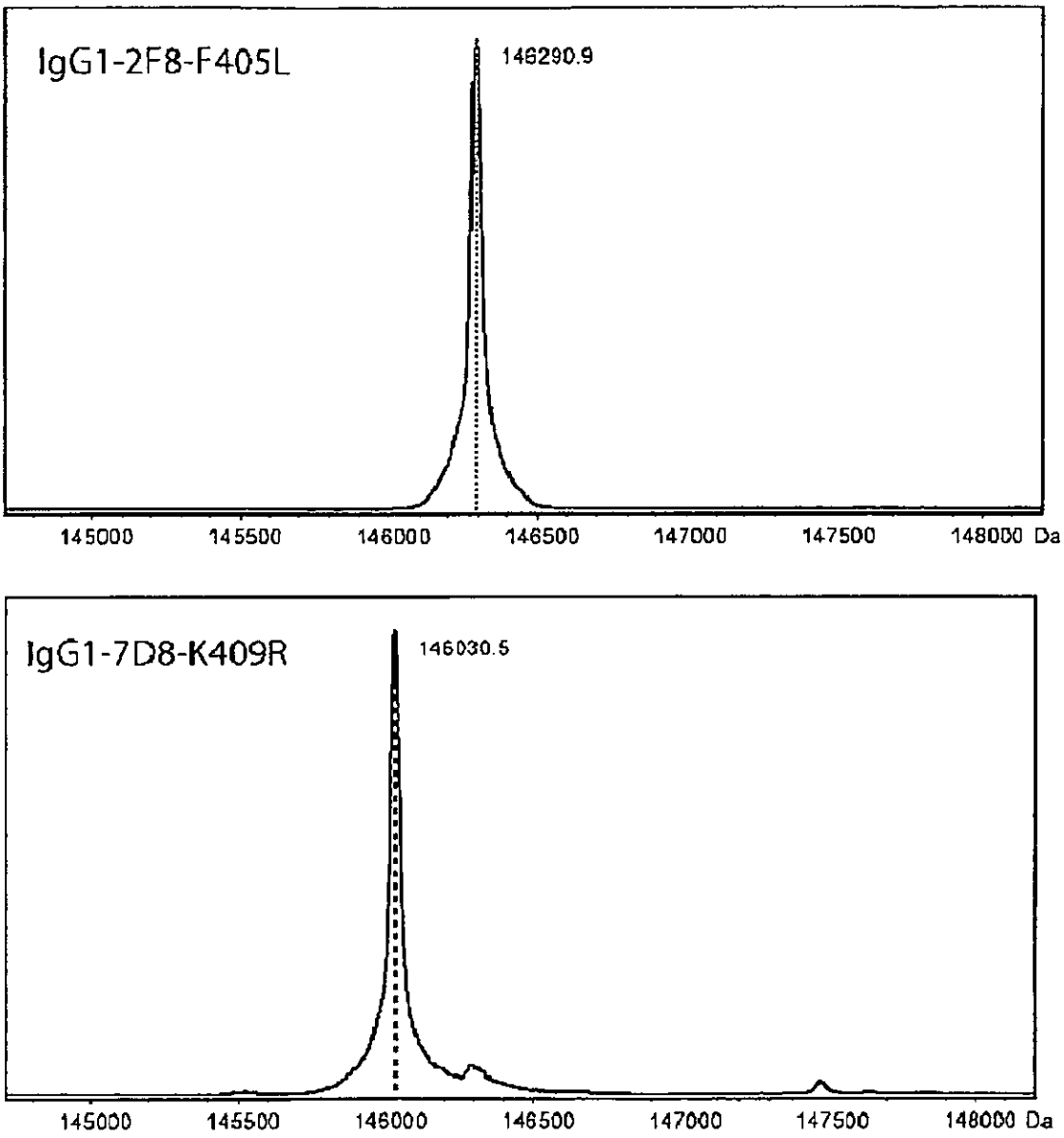
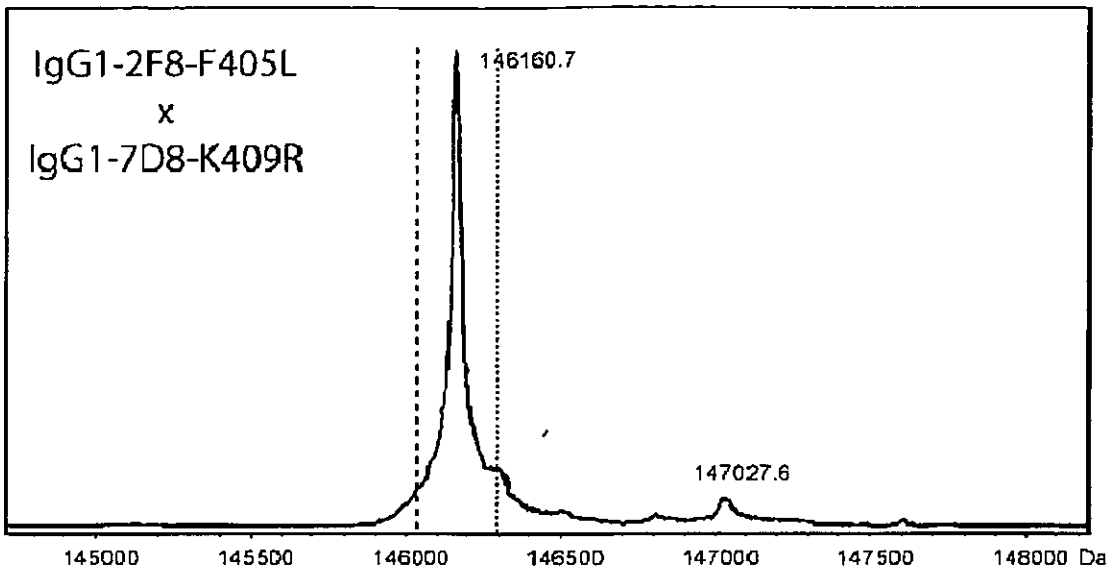

IgG1-2F8-F405L
146290.9
145000
145500
146000
146500
147000
147500
148000 Da
IgG1-7D8-K409R
146030.5
145000
145500
146000
146500
147000
147500
148000 Da

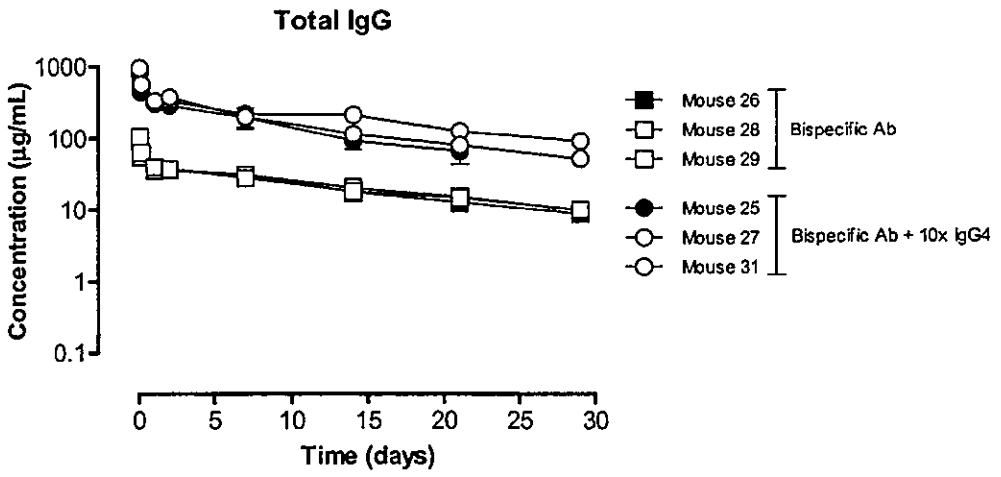
Figure 19 (continued)



	Measured MW
IgG1-2F8-F405L	**146,290.9**
IgG1-7D8-K409R	**146,030.5**
IgG1-2F8-F405L x IgG1-7D8-K409R	**146,160.7**

Figure 20

A


Total IgG
Concentration (µg/mL)
1000
100
10
1
0.1
0
5
10
15
20
25
30
Time (days)
Mouse 26
Mouse 28
Mouse 29
Bispecific Ab
Mouse 25
Mouse 27
Mouse 31
Bispecific Ab + 10x IgG4

B

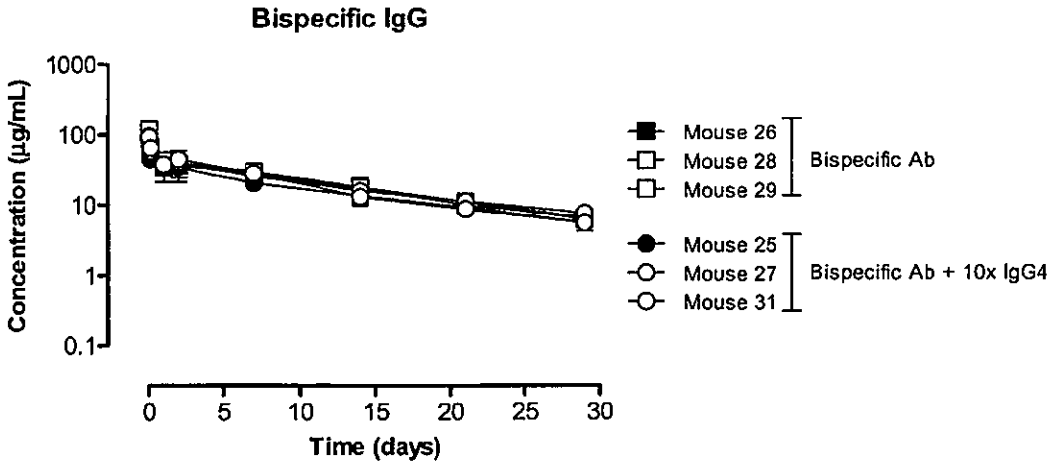
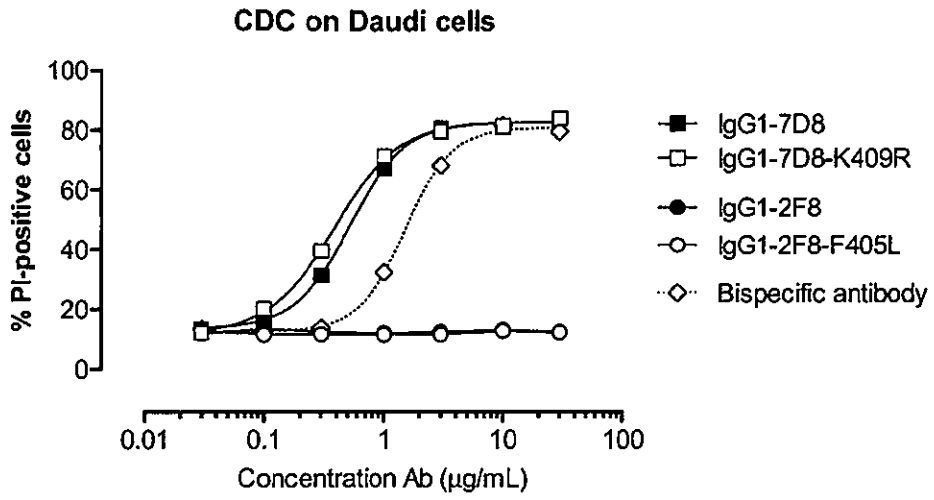

Bispecific IgG
Concentration (µg/mL)
1000
100
10
1
0.1
0
5
10
15
20
25
30
Time (days)
Mouse 26
Mouse 28
Mouse 29
Bispecific Ab
Mouse 25
Mouse 27
Mouse 31
Bispecific Ab + 10x IgG4

Figure 21

A


CDC on Daudi cells
% PI-positive cells
100
80
60
40
20
0
0.01
0.1
1
10
100
Concentration Ab (µg/mL)
IgG1-7D8
IgG1-7D8-K409R
IgG1-2F8
IgG1-2F8-F405L
Bispecific antibody

B

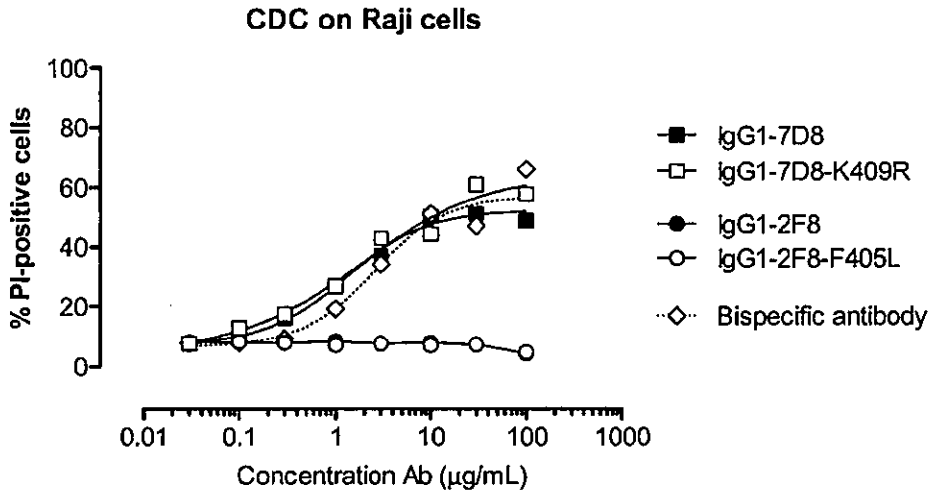

CDC on Raji cells
% PI-positive cells
100
80
60
40
20
0
0.01
0.1
1
10
100
1000
Concentration Ab (µg/mL)
IgG1-7D8
IgG1-7D8-K409R
IgG1-2F8
IgG1-2F8-F405L
Bispecific antibody

Figure 22

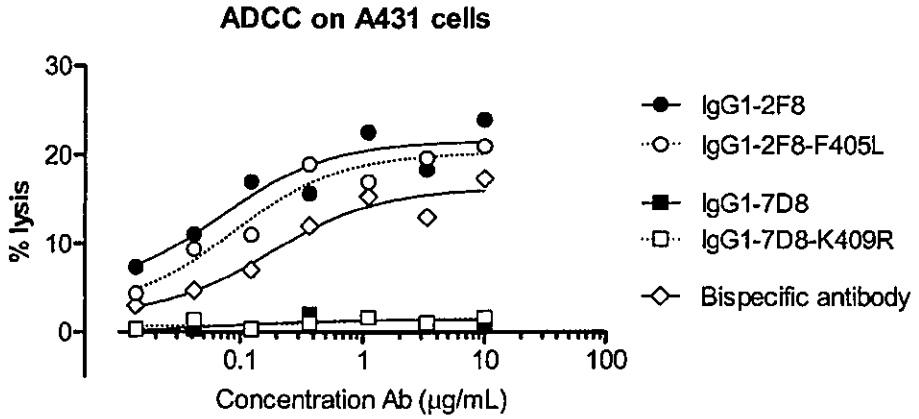
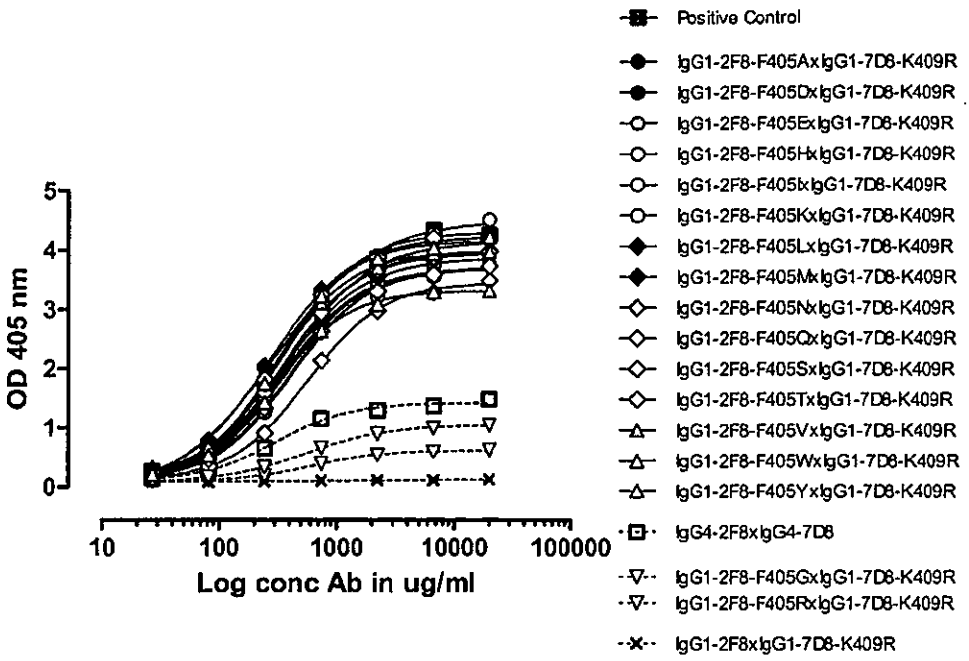
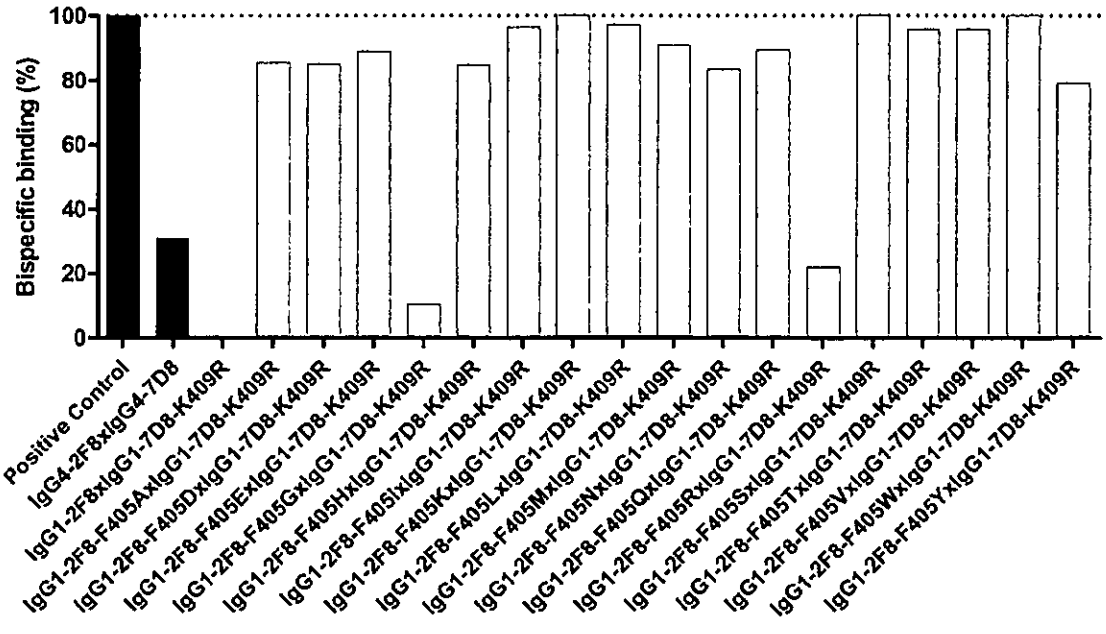

ADCC on A431 cells
% lysis
30
20
10
0
0.1
1
10
100
Concentration Ab (µg/mL)
IgG1-2F8
IgG1-2F8-F405L
IgG1-7D8
IgG1-7D8-K409R
Bispecific antibody

Figure 23

A



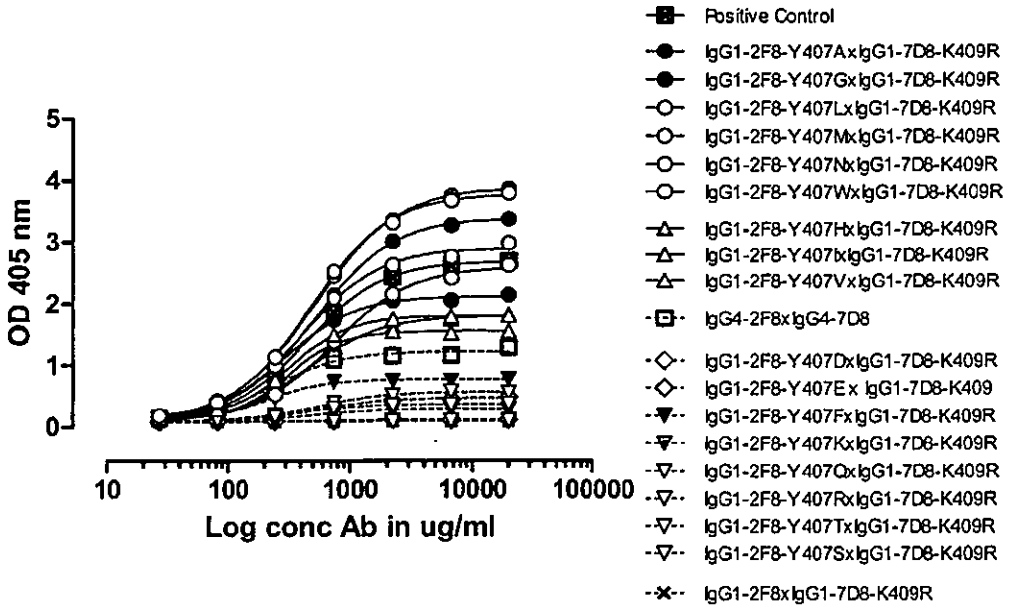
B



B

Figure 24

A


OD 405 nm
0
1
2
3
4
5
10
100
1000
10000
100000
Log conc Ab in ug/ml
Positive Control
IgG1-2F8-Y407AxIgG1-7D8-K409R
IgG1-2F8-Y407GxIgG1-7D8-K409R
IgG1-2F8-Y407LxIgG1-7D8-K409R
IgG1-2F8-Y407MxIgG1-7D8-K409R
IgG1-2F8-Y407NxIgG1-7D8-K409R
IgG1-2F8-Y407WxIgG1-7D8-K409R
IgG1-2F8-Y407HxIgG1-7D8-K409R
IgG1-2F8-Y407IxIgG1-7D8-K409R
IgG1-2F8-Y407VxIgG1-7D8-K409R
IgG4-2F8xIgG4-7D8
IgG1-2F8-Y407DxIgG1-7D8-K409R
IgG1-2F8-Y407Ex IgG1-7D8-K409
IgG1-2F8-Y407FxIgG1-7D8-K409R
IgG1-2F8-Y407KxIgG1-7D8-K409R
IgG1-2F8-Y407QxIgG1-7D8-K409R
IgG1-2F8-Y407RxIgG1-7D8-K409R
IgG1-2F8-Y407TxIgG1-7D8-K409R
IgG1-2F8-Y407SxIgG1-7D8-K409R
IgG1-2F8xIgG1-7D8-K409R

B

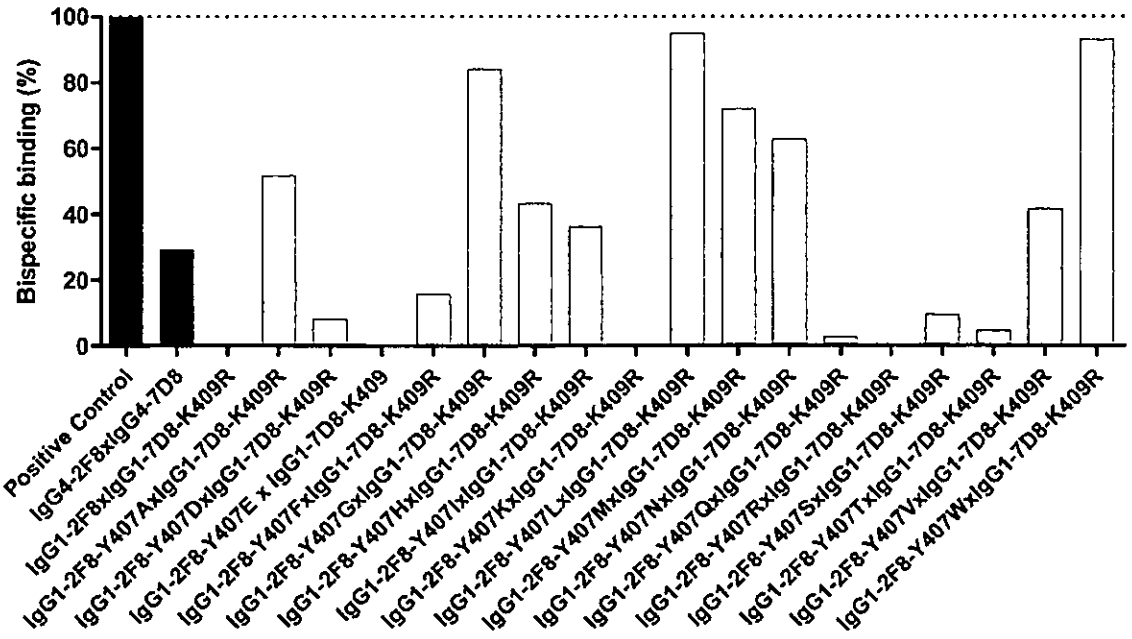

Bispecific binding (%)
0
20
40
60
80
100
Positive Control
IgG4-2F8xIgG4-7D8
IgG1-2F8xIgG1-7D8-K409R
IgG1-2F8-Y407AxIgG1-7D8-K409R
IgG1-2F8-Y407DxIgG1-7D8-K409R
IgG1-2F8-Y407E x IgG1-7D8-K409
IgG1-2F8-Y407FxIgG1-7D8-K409R
IgG1-2F8-Y407GxIgG1-7D8-K409R
IgG1-2F8-Y407HxIgG1-7D8-K409R
IgG1-2F8-Y407IxIgG1-7D8-K409R
IgG1-2F8-Y407KxIgG1-7D8-K409R
IgG1-2F8-Y407LxIgG1-7D8-K409R
IgG1-2F8-Y407MxIgG1-7D8-K409R
IgG1-2F8-Y407NxIgG1-7D8-K409R
IgG1-2F8-Y407QxIgG1-7D8-K409R
IgG1-2F8-Y407RxIgG1-7D8-K409R
IgG1-2F8-Y407SxIgG1-7D8-K409R
IgG1-2F8-Y407TxIgG1-7D8-K409R
IgG1-2F8-Y407VxIgG1-7D8-K409R
IgG1-2F8-Y407WxIgG1-7D8-K409R

Figure 25

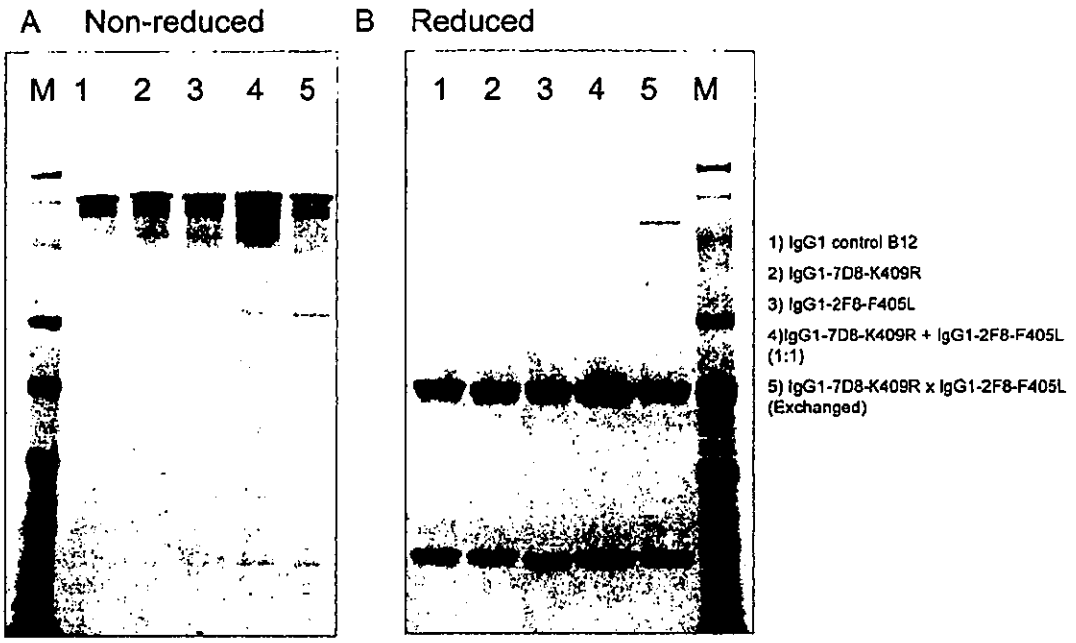

A Non-reduced
M 1 2 3 4 5
B Reduced
1 2 3 4 5 M
1) IgG1 control B12
2) IgG1-7D8-K409R
3) IgG1-2F8-F405L
4)IgG1-7D8-K409R + IgG1-2F8-F405L (1:1)
5) IgG1-7D8-K409R x IgG1-2F8-F405L (Exchanged)

Figure 26

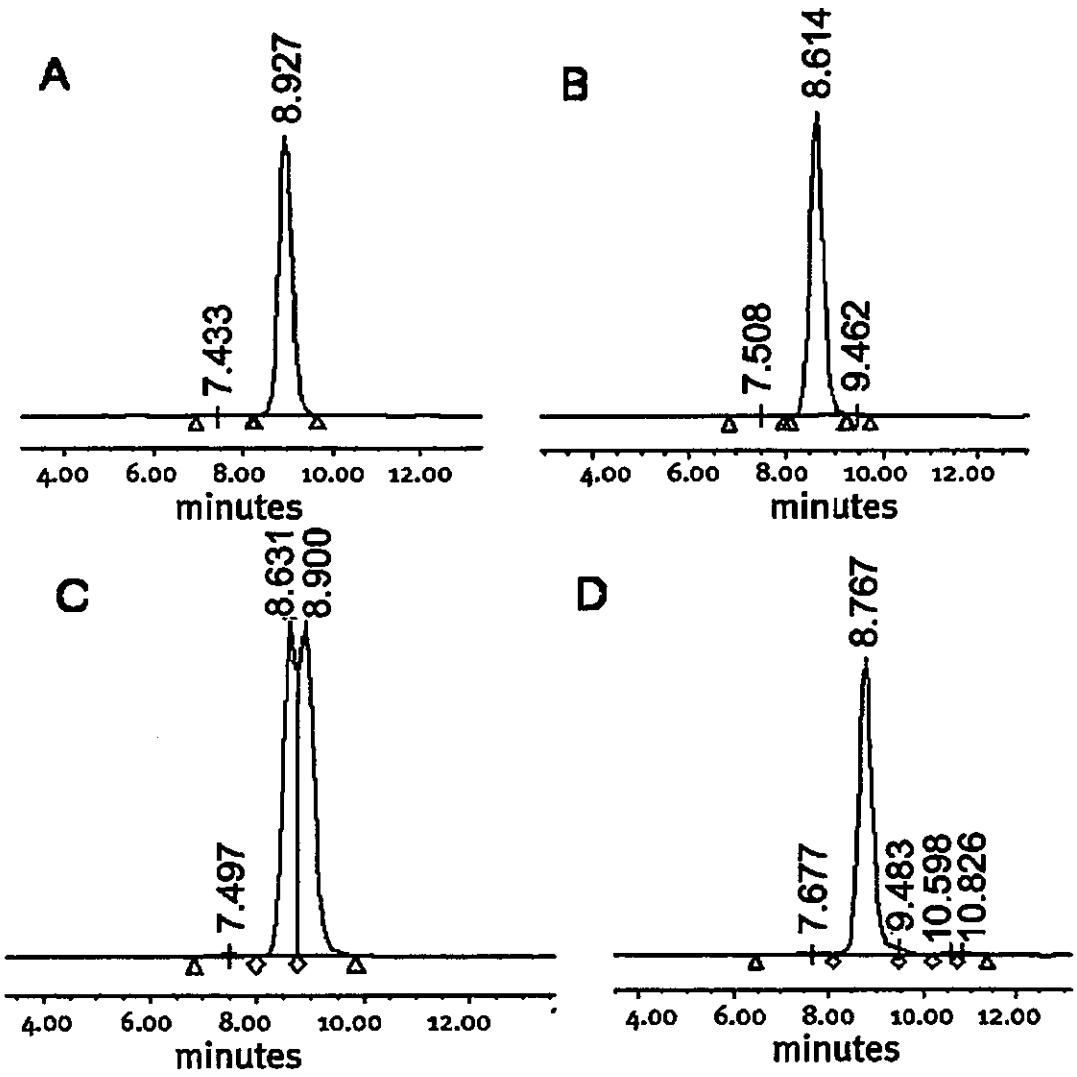

A
8.927
7.433
4.00 6.00 8.00 10.00 12.00
minutes
B
8.614
7.508
9.462
4.00 6.00 8.00 10.00 12.00
minutes
C
8.631
8.900
7.497
4.00 6.00 8.00 10.00 12.00
minutes
D
8.767
7.677
9.483
10.598
10.826
4.00 6.00 8.00 10.00 12.00
minutes

Figure 27

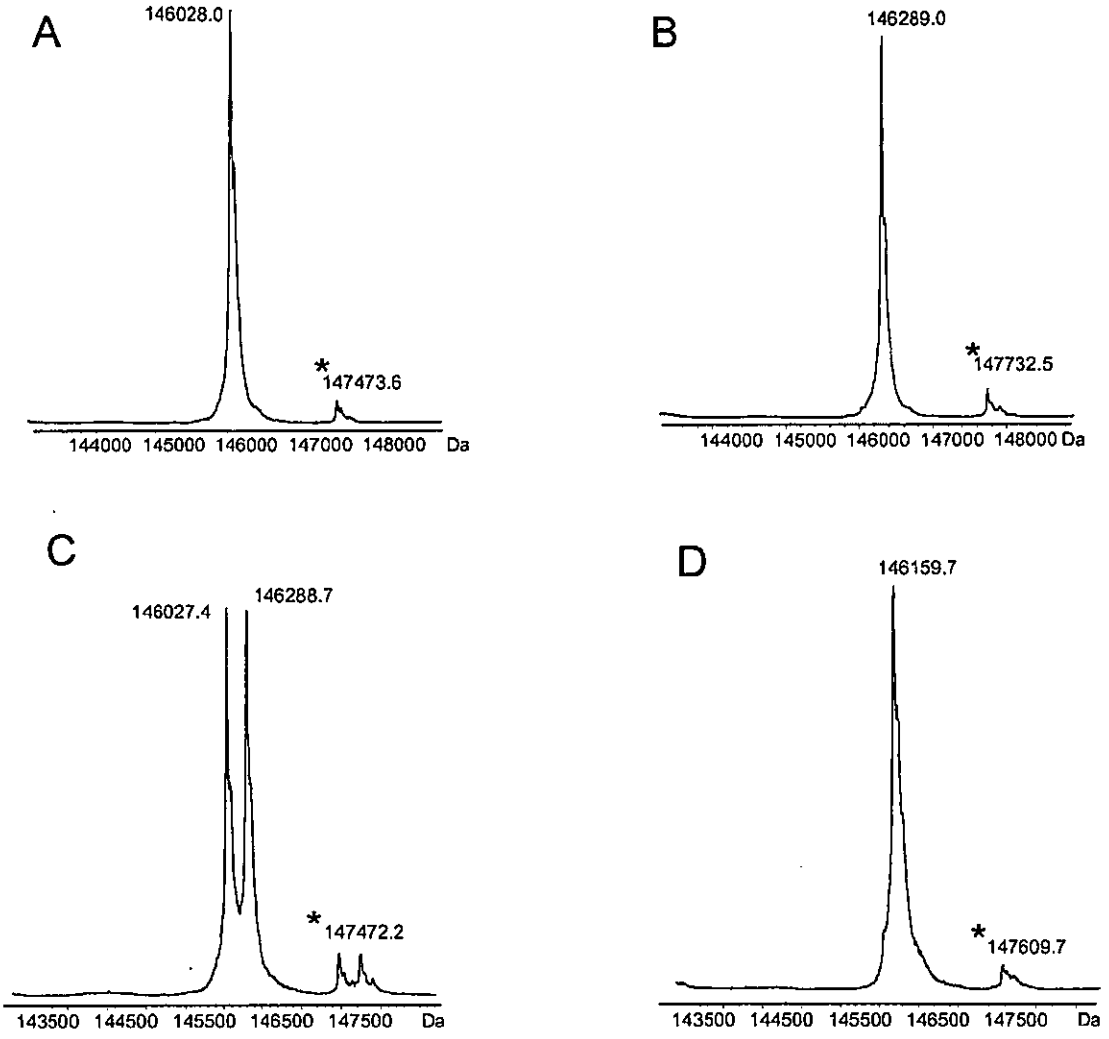
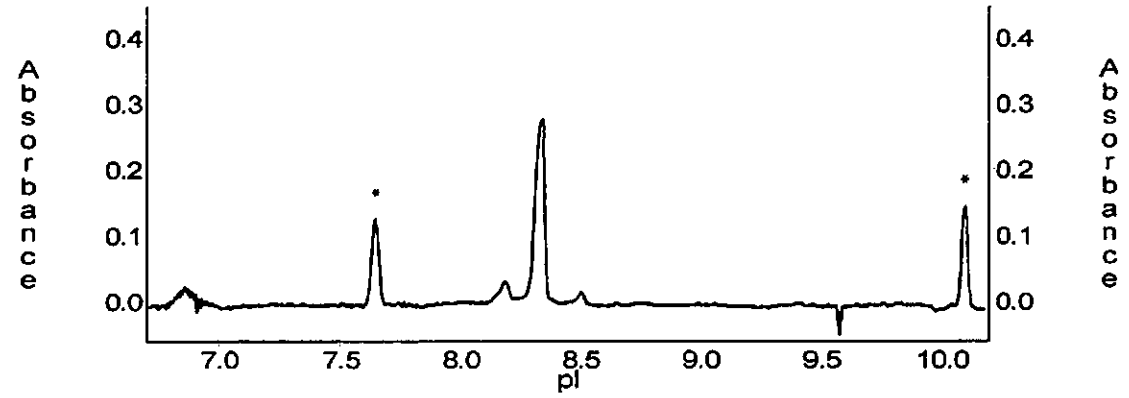
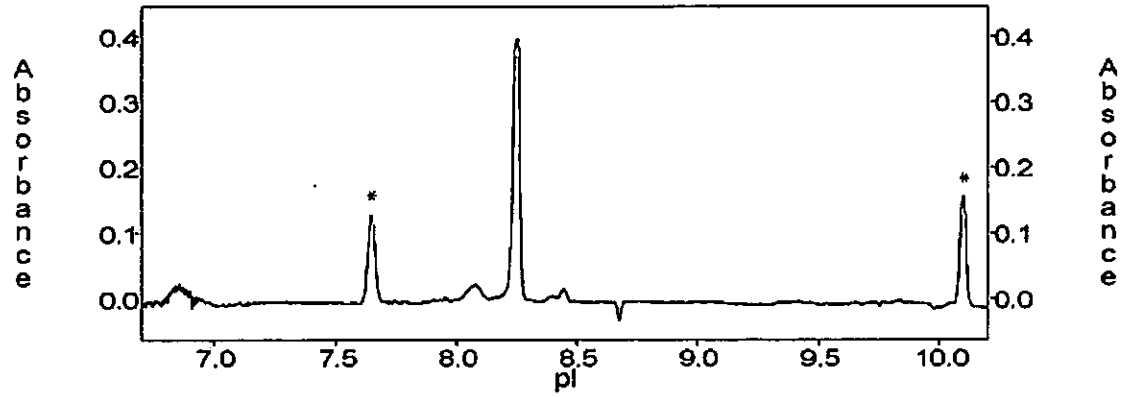

A
146028.0
*147473.6
144000 145000 146000 147000 148000 Da
B
146289.0
*147732.5
144000 145000 146000 147000 148000 Da
C
146027.4
146288.7
*147472.2
143500 144500 145500 146500 147500 Da
D
146159.7
*147609.7
143500 144500 145500 146500 147500 Da

Figure 28

A



B



C

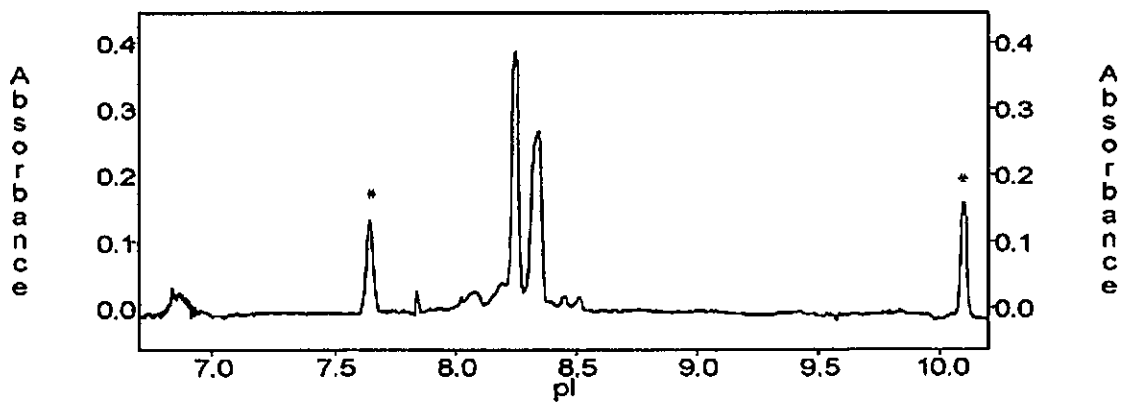


Figure 28 (continued)

D

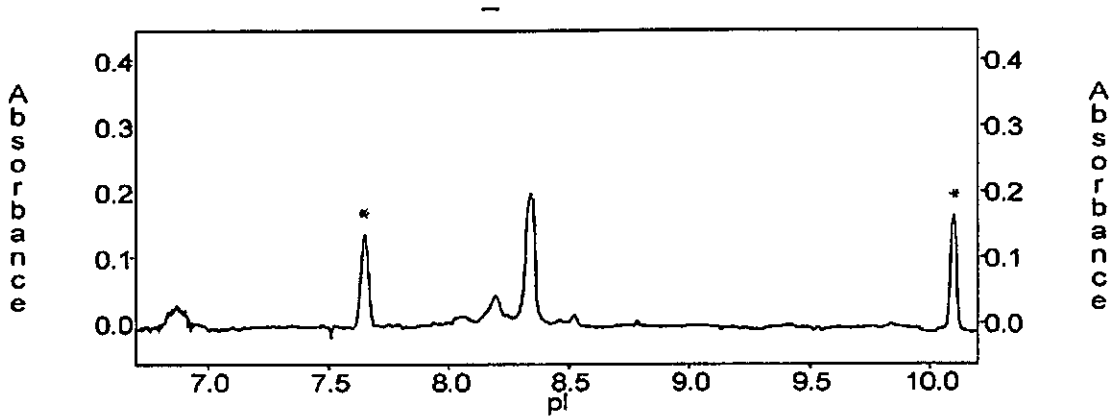


Figure 29

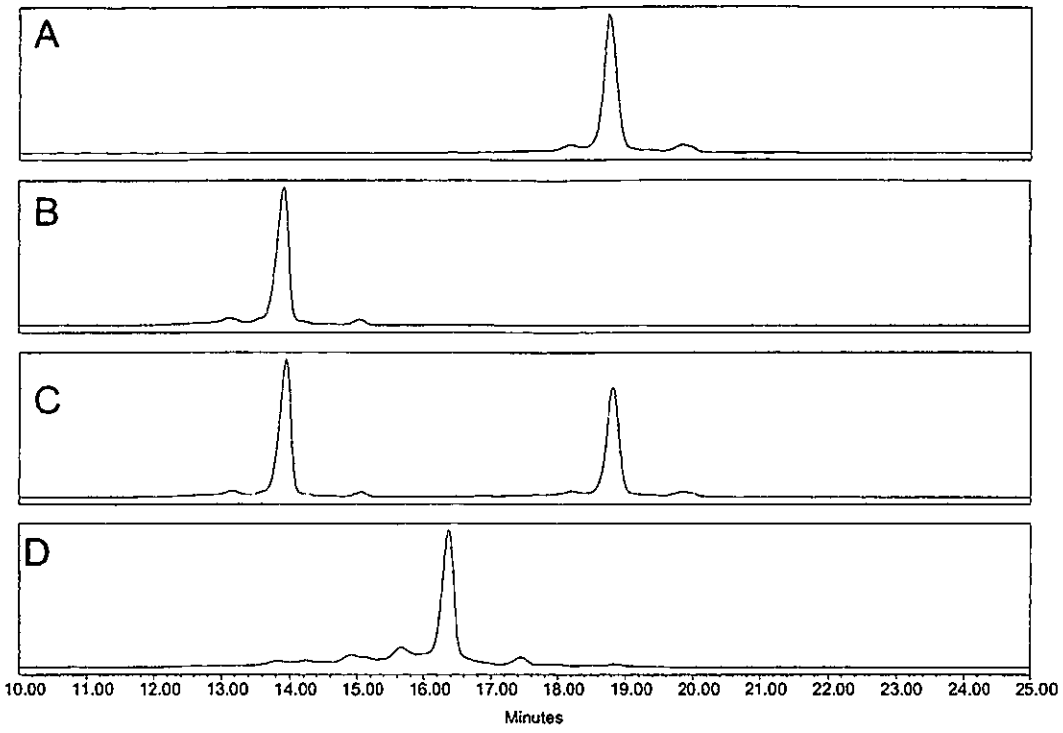

A
B
C
D
10.00 11.00 12.00 13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00 21.00 22.00 23.00 24.00 25.00
Minutes

Figure 30

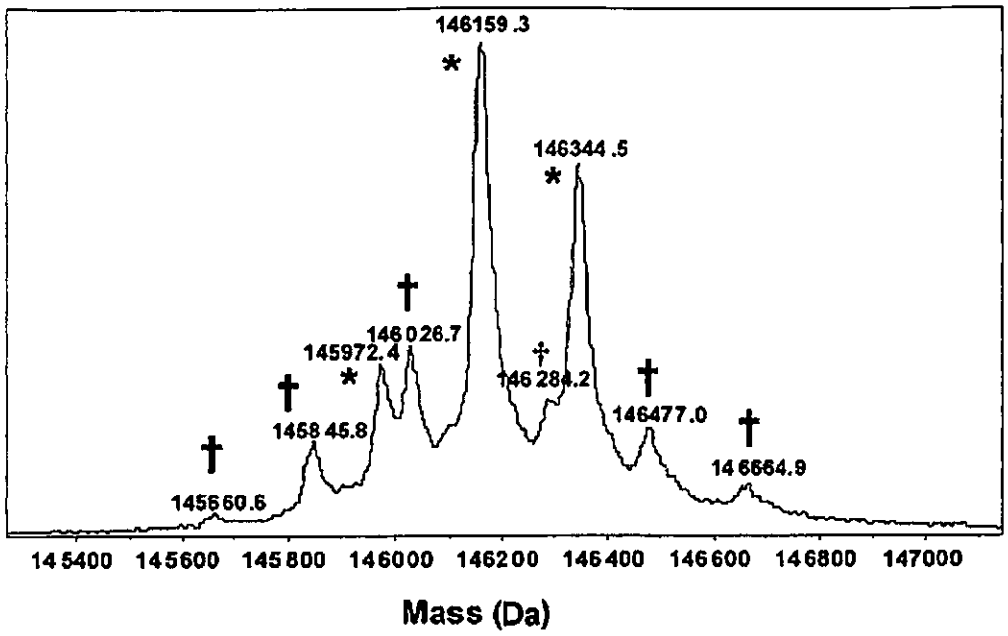

146159.3
146344.5
146026.7
145972.4
146284.2
146845.8
146477.0
146664.9
145560.6
145400
145600
145800
146000
146200
146400
146600
146800
147000
Mass (Da)

Figure 31

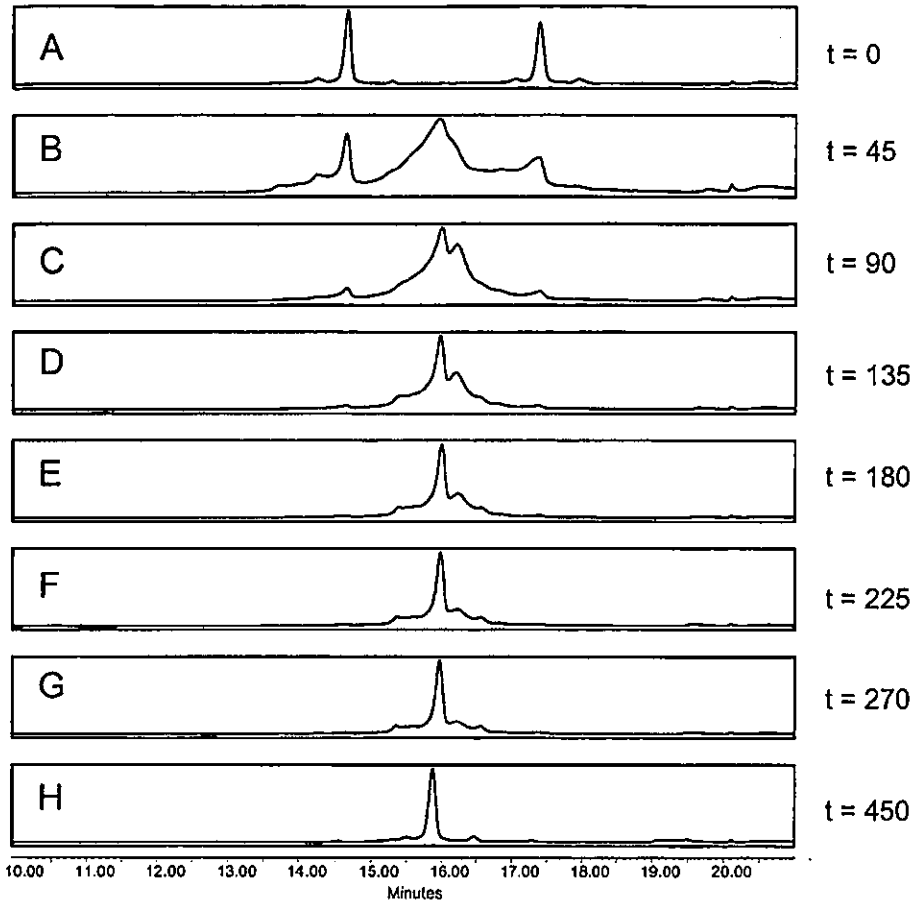

A
B
C
D
E
F
G
H
t = 0
t = 45
t = 90
t = 135
t = 180
t = 225
t = 270
t = 450
10.00
11.00
12.00
13.00
14.00
15.00
16.00
17.00
18.00
19.00
20.00
Minutes

Figure 32

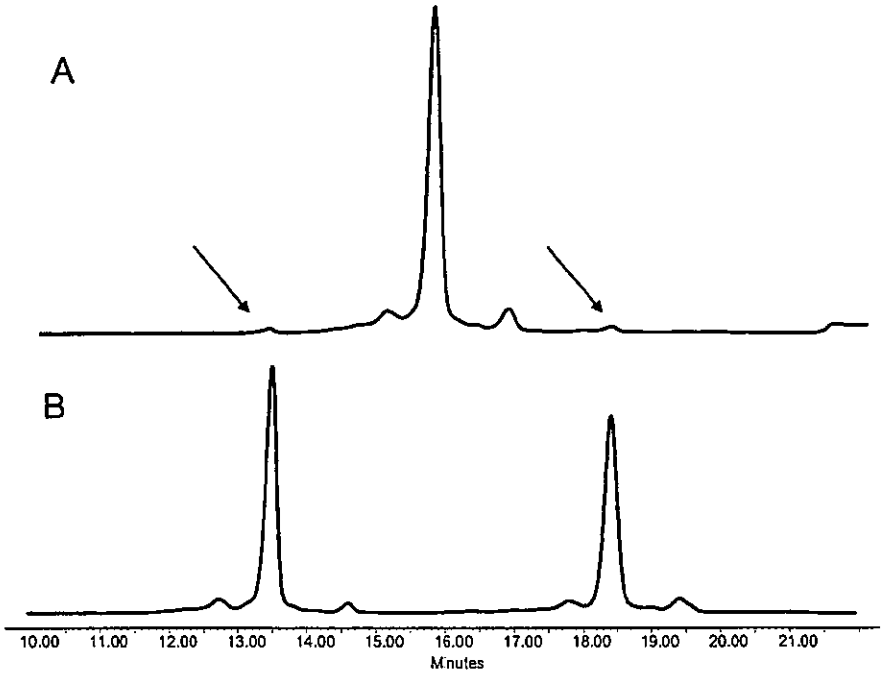
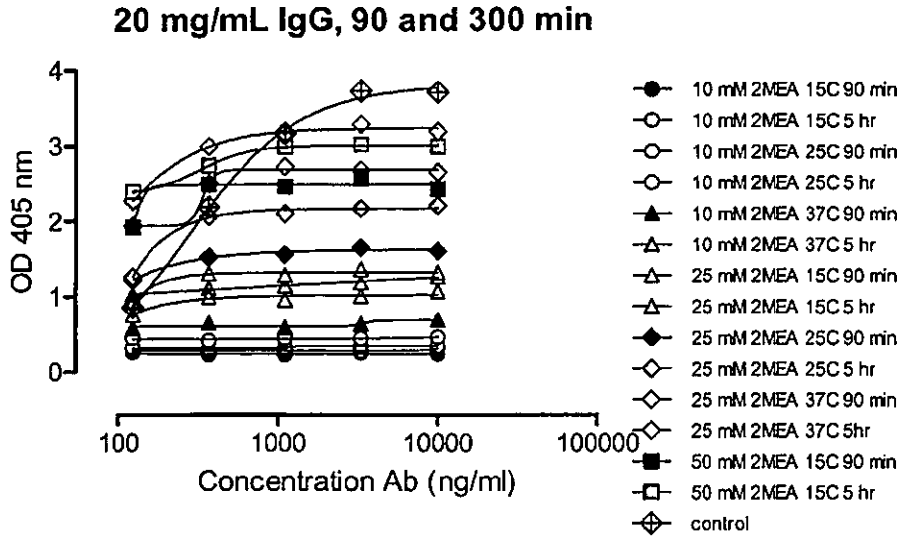

A
B
10.00
11.00
12.00
13.00
14.00
15.00
16.00
17.00
18.00
19.00
20.00
21.00
Minutes

Figure 33

A


20 mg/mL IgG, 90 and 300 min
OD 405 nm
0
1
2
3
4
100
1000
10000
100000
Concentration Ab (ng/ml)
10 mM 2MEA 15C 90 min
10 mM 2MEA 15C 5 hr
10 mM 2MEA 25C 90 min
10 mM 2MEA 25C 5 hr
10 mM 2MEA 37C 90 min
10 mM 2MEA 37C 5 hr
25 mM 2MEA 15C 90 min
25 mM 2MEA 15C 5 hr
25 mM 2MEA 25C 90 min
25 mM 2MEA 25C 5 hr
25 mM 2MEA 37C 90 min
25 mM 2MEA 37C 5hr
50 mM 2MEA 15C 90 min
50 mM 2MEA 15C 5 hr
control

B

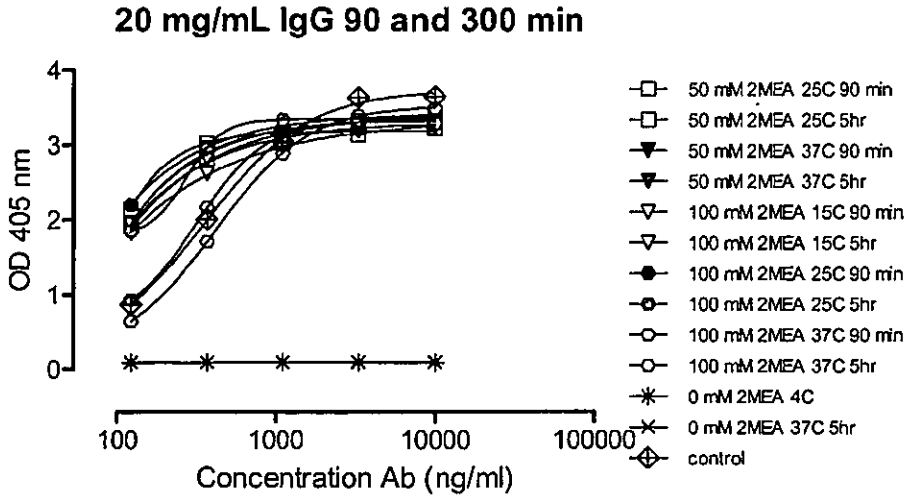
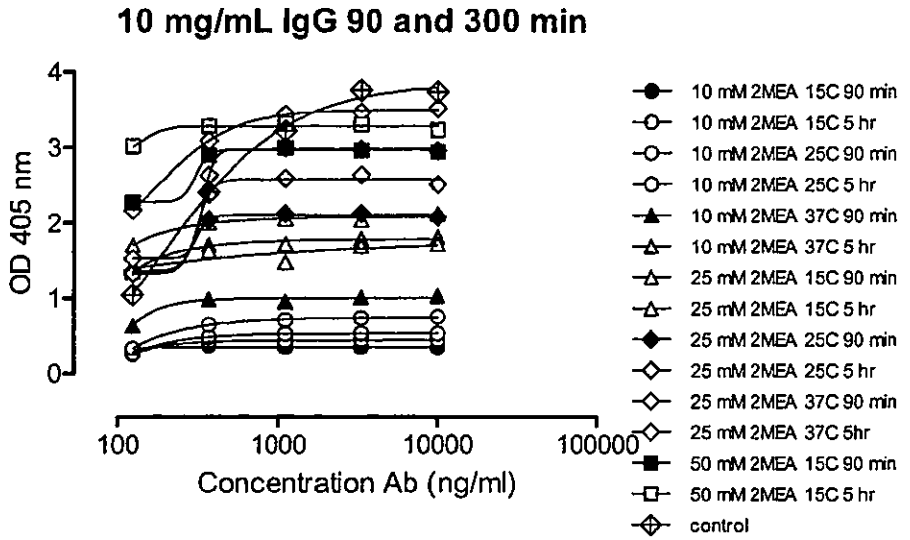

20 mg/mL IgG 90 and 300 min
OD 405 nm
0
1
2
3
4
100
1000
10000
100000
Concentration Ab (ng/ml)
50 mM 2MEA 25C 90 min
50 mM 2MEA 25C 5hr
50 mM 2MEA 37C 90 min
50 mM 2MEA 37C 5hr
100 mM 2MEA 15C 90 min
100 mM 2MEA 15C 5hr
100 mM 2MEA 25C 90 min
100 mM 2MEA 25C 5hr
100 mM 2MEA 37C 90 min
100 mM 2MEA 37C 5hr
0 mM 2MEA 4C
0 mM 2MEA 37C 5hr
control

Figure 33 (continued)

C



D

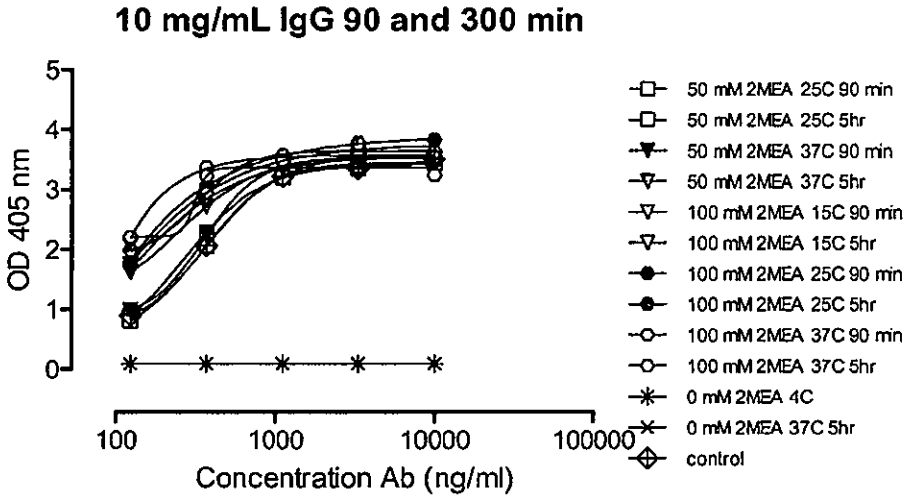
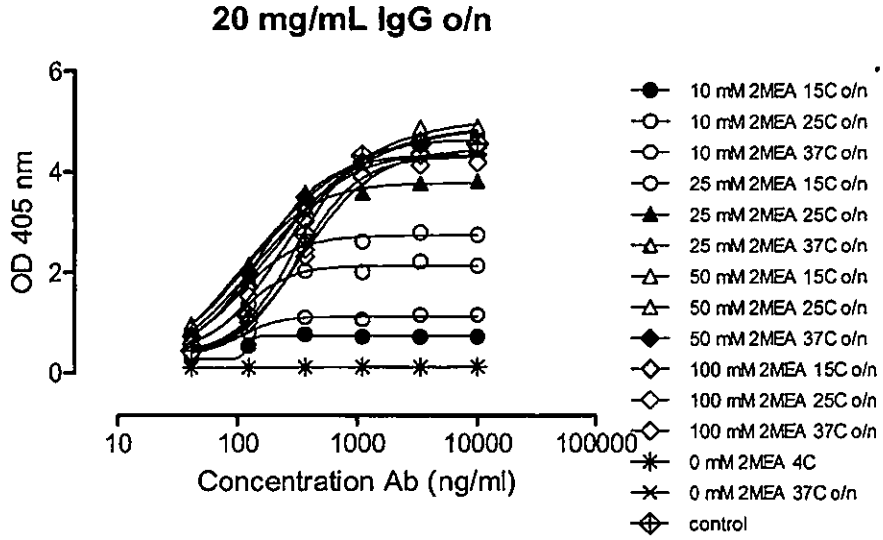


Figure 33 (continued)

E



F

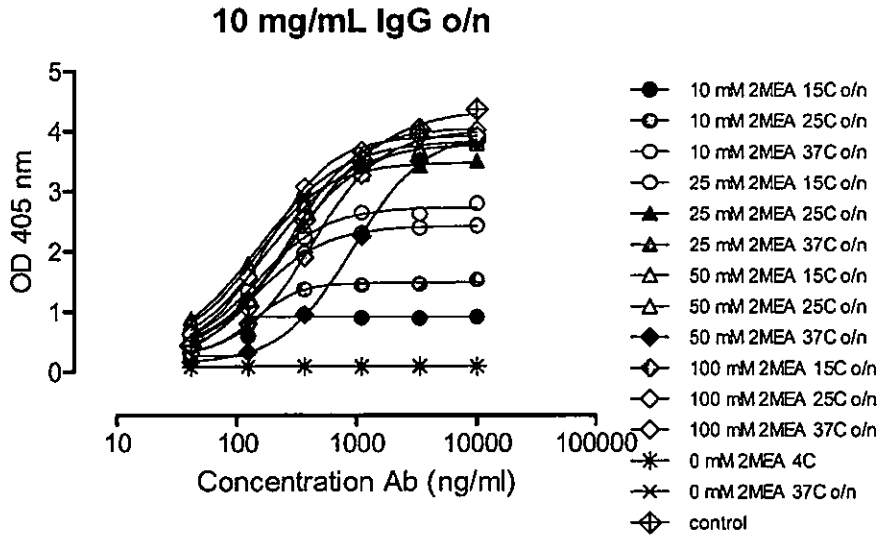
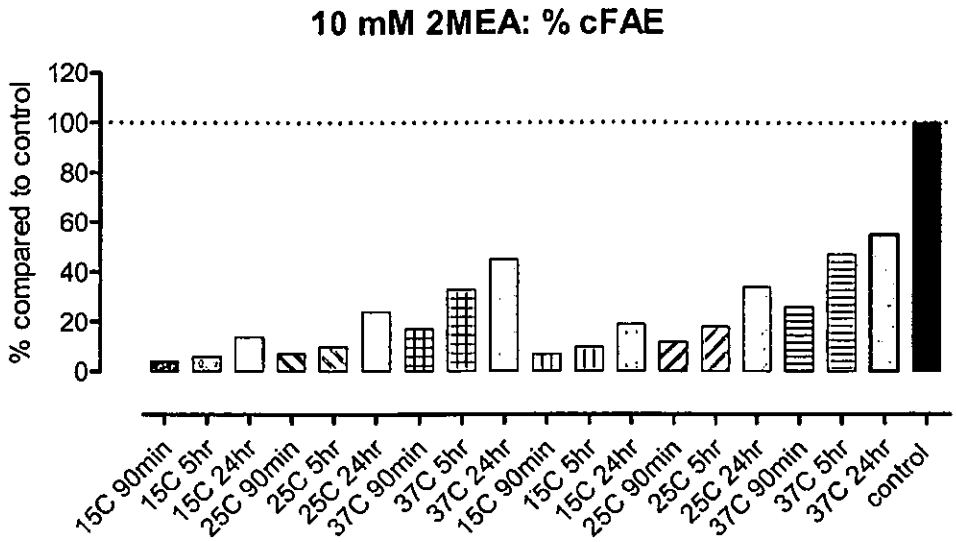


Figure 34

A


10 mM 2MEA: % cFAE
% compared to control
120
100
80
60
40
20
0
15C 90min
15C 5hr
15C 24hr
25C 90min
25C 5hr
25C 24hr
37C 90min
37C 5hr
37C 24hr
15C 90min
15C 5hr
15C 24hr
25C 90min
25C 5hr
25C 24hr
37C 90min
37C 5hr
37C 24hr
control

B

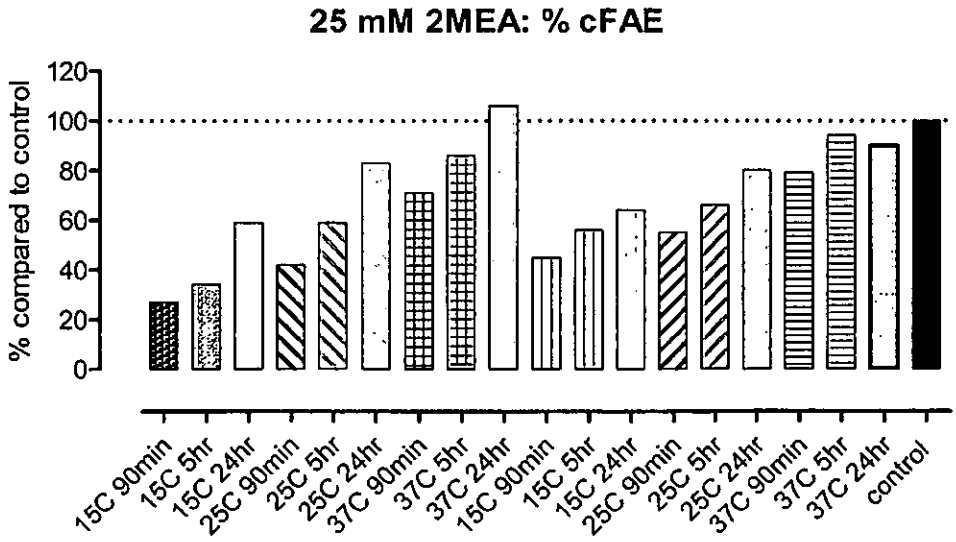
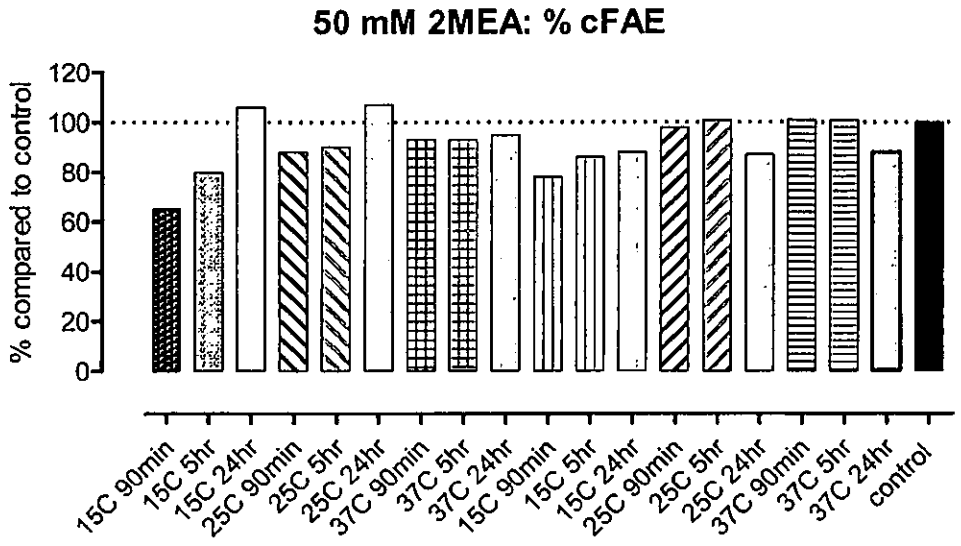

25 mM 2MEA: % cFAE
% compared to control
120
100
80
60
40
20
0
15C 90min
15C 5hr
15C 24hr
25C 90min
25C 5hr
25C 24hr
37C 90min
37C 5hr
37C 24hr
15C 90min
15C 5hr
15C 24hr
25C 90min
25C 5hr
25C 24hr
37C 90min
37C 5hr
37C 24hr
control

Figure 34 (continued)

C



D

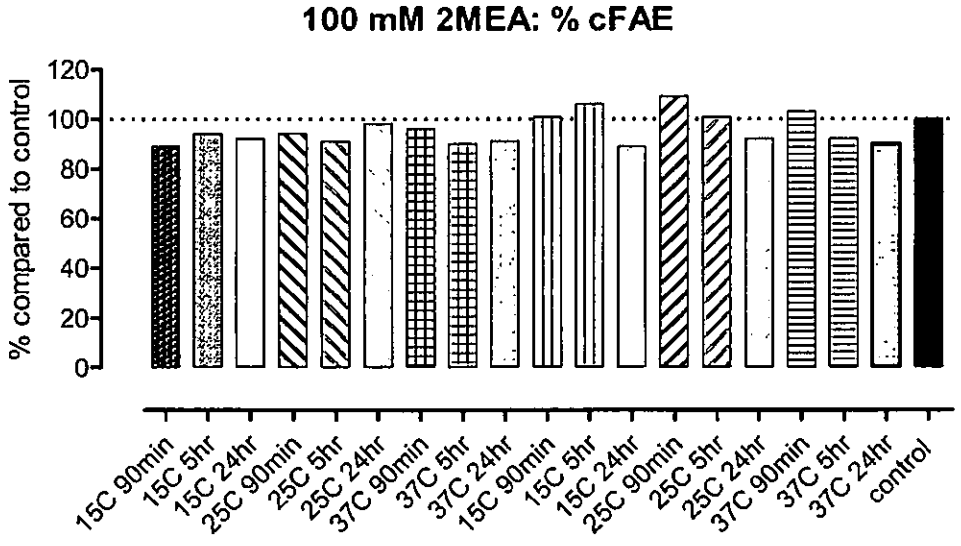
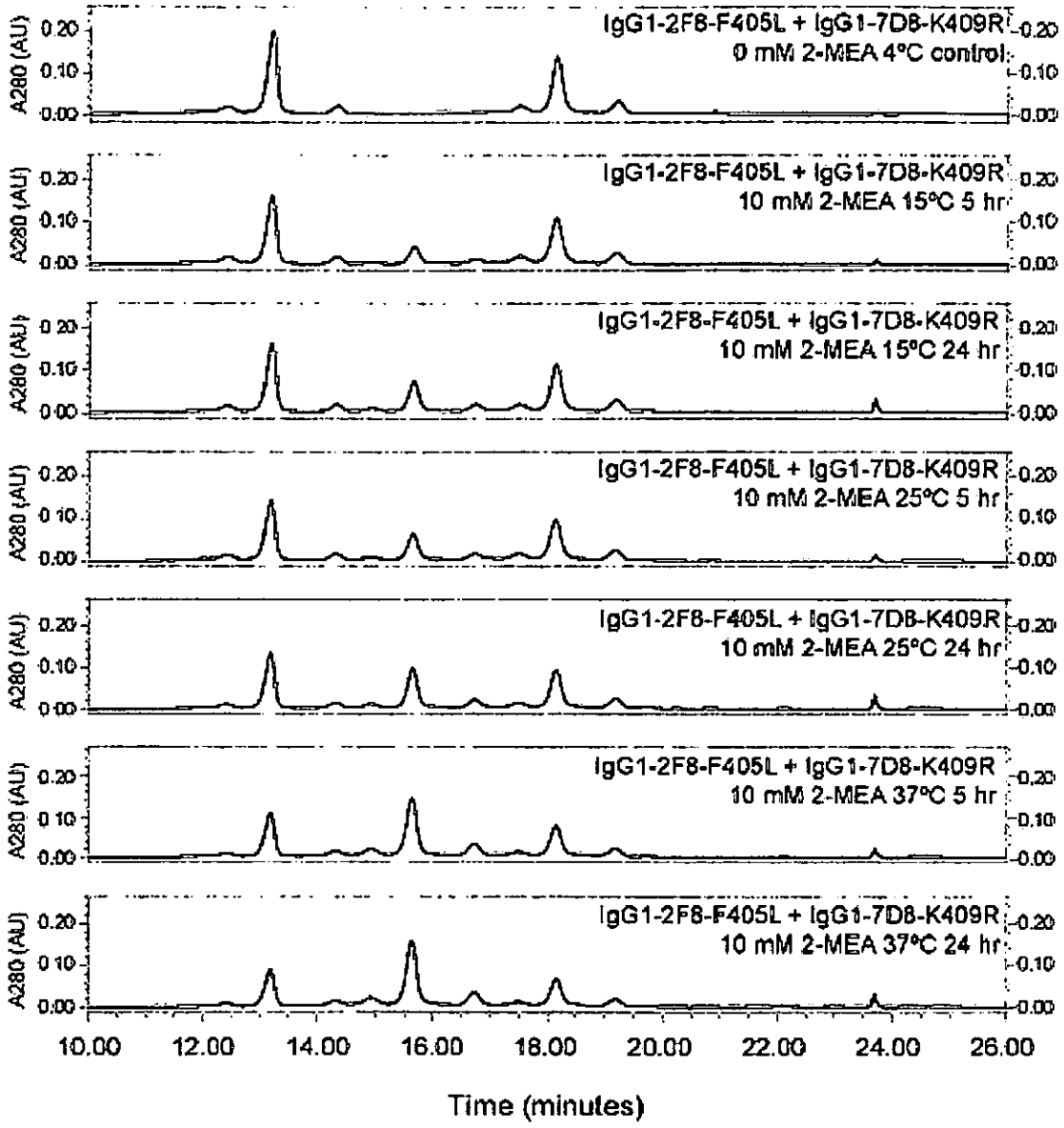
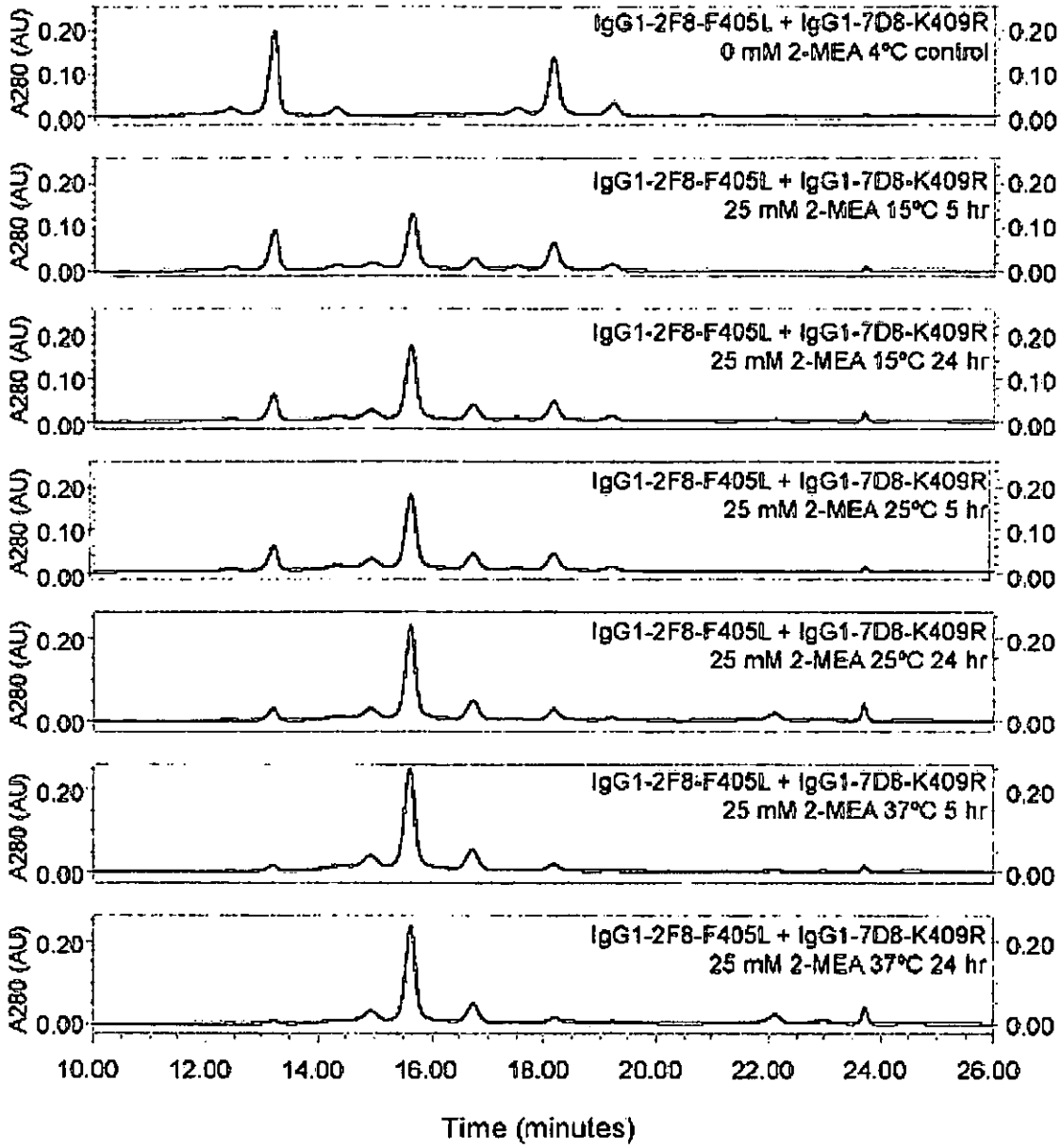


Figure 35

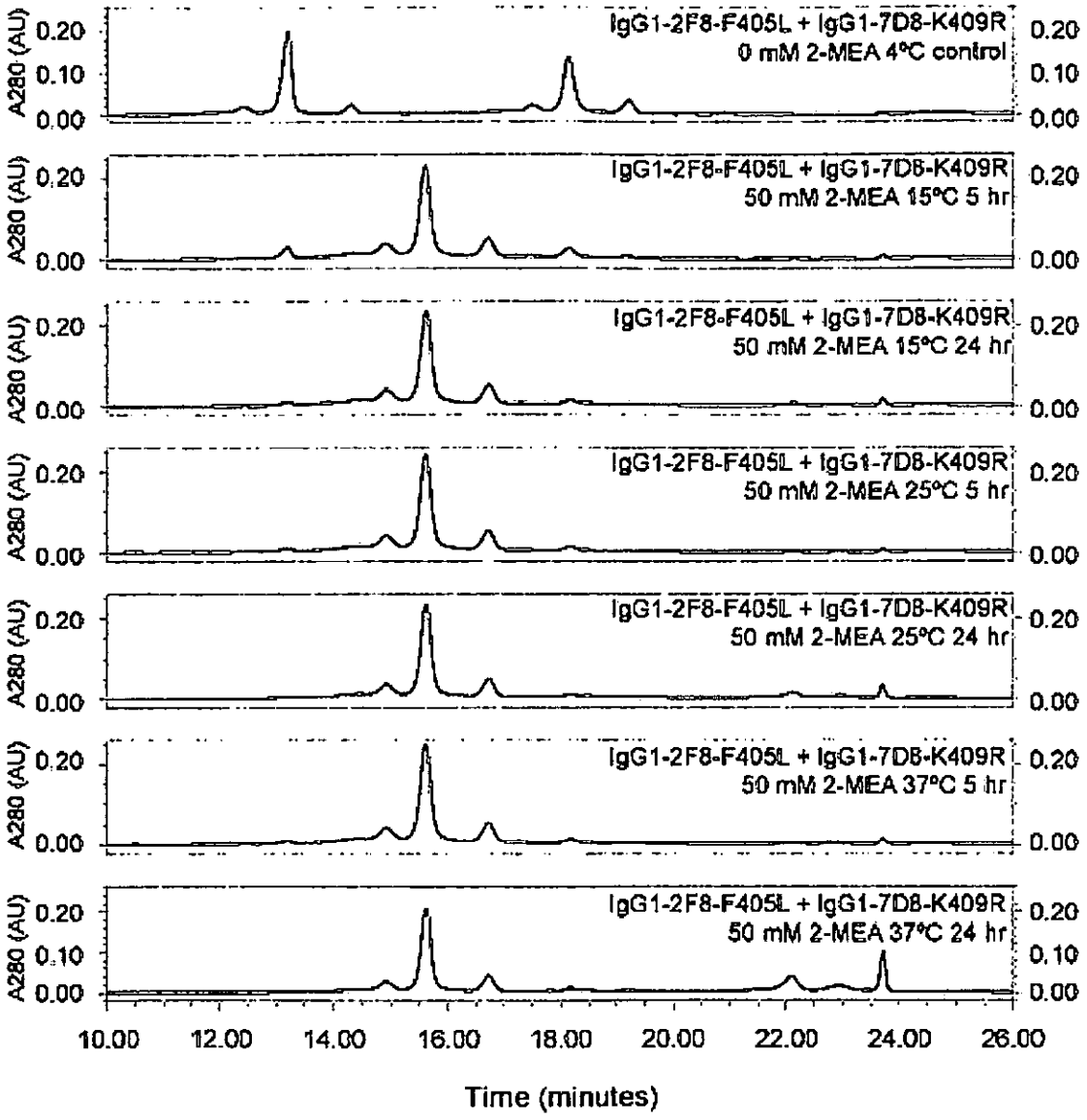
A


IgG1-2F8-F405L + IgG1-7D8-K409R
0 mM 2-MEA 4°C control
IgG1-2F8-F405L + IgG1-7D8-K409R
10 mM 2-MEA 15°C 5 hr
IgG1-2F8-F405L + IgG1-7D8-K409R
10 mM 2-MEA 15°C 24 hr
IgG1-2F8-F405L + IgG1-7D8-K409R
10 mM 2-MEA 25°C 5 hr
IgG1-2F8-F405L + IgG1-7D8-K409R
10 mM 2-MEA 25°C 24 hr
IgG1-2F8-F405L + IgG1-7D8-K409R
10 mM 2-MEA 37°C 5 hr
IgG1-2F8-F405L + IgG1-7D8-K409R
10 mM 2-MEA 37°C 24 hr
A280 (AU)
0.20
0.10
0.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
Time (minutes)

B


IgG1-2F8-F405L + IgG1-7D8-K409R
0 mM 2-MEA 4°C control
IgG1-2F8-F405L + IgG1-7D8-K409R
25 mM 2-MEA 15°C 5 hr
IgG1-2F8-F405L + IgG1-7D8-K409R
25 mM 2-MEA 15°C 24 hr
IgG1-2F8-F405L + IgG1-7D8-K409R
25 mM 2-MEA 25°C 5 hr
IgG1-2F8-F405L + IgG1-7D8-K409R
25 mM 2-MEA 25°C 24 hr
IgG1-2F8-F405L + IgG1-7D8-K409R
25 mM 2-MEA 37°C 5 hr
IgG1-2F8-F405L + IgG1-7D8-K409R
25 mM 2-MEA 37°C 24 hr
A280 (AU)
0.20
0.10
0.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
Time (minutes)

c


IgG1-2F8-F405L + IgG1-7D8-K409R
0 mM 2-MEA 4°C control
IgG1-2F8-F405L + IgG1-7D8-K409R
50 mM 2-MEA 15°C 5 hr
IgG1-2F8-F405L + IgG1-7D8-K409R
50 mM 2-MEA 15°C 24 hr
IgG1-2F8-F405L + IgG1-7D8-K409R
50 mM 2-MEA 25°C 5 hr
IgG1-2F8-F405L + IgG1-7D8-K409R
50 mM 2-MEA 25°C 24 hr
IgG1-2F8-F405L + IgG1-7D8-K409R
50 mM 2-MEA 37°C 5 hr
IgG1-2F8-F405L + IgG1-7D8-K409R
50 mM 2-MEA 37°C 24 hr
A280 (AU)
0.20
0.10
0.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
Time (minutes)

D

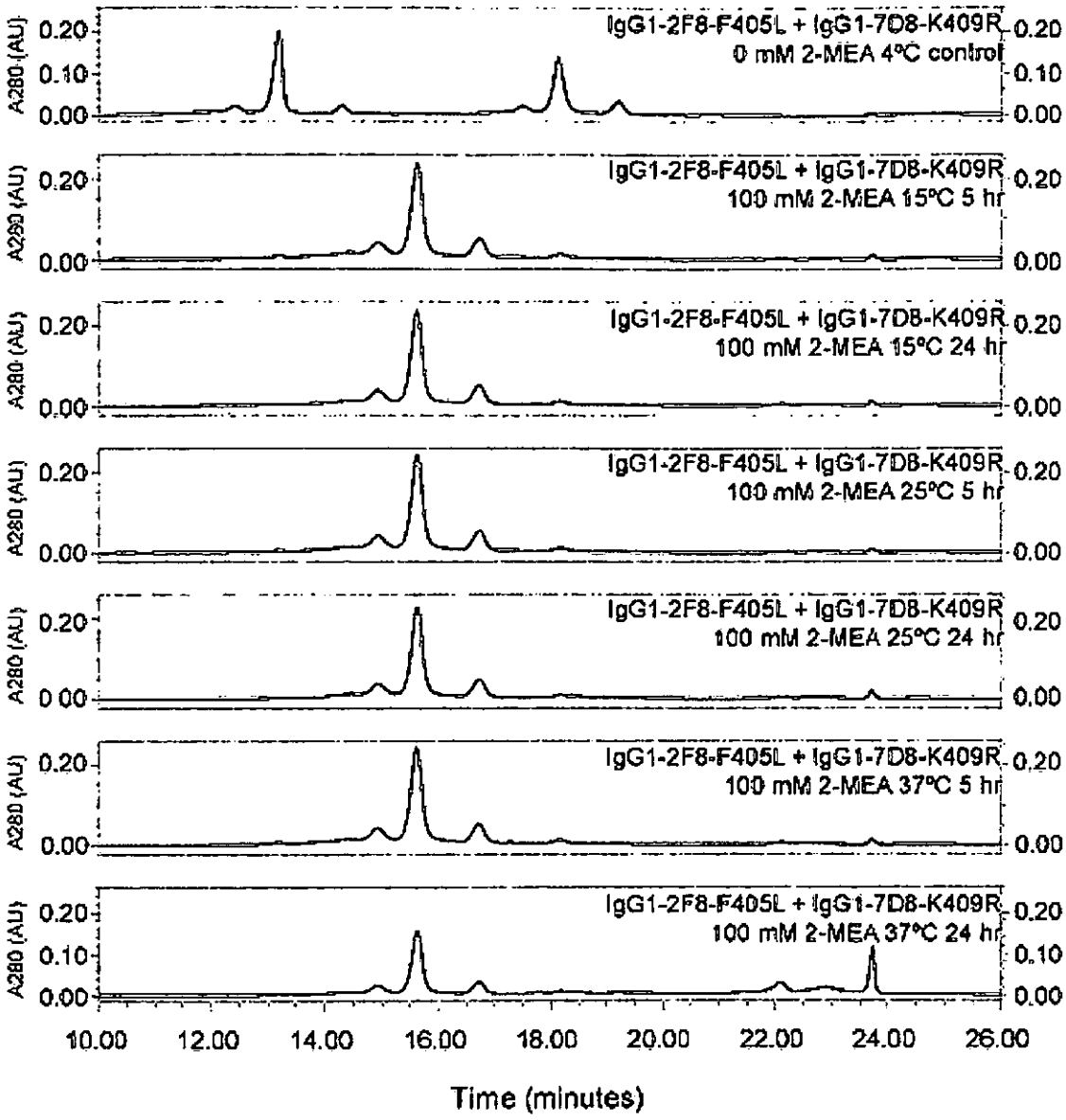
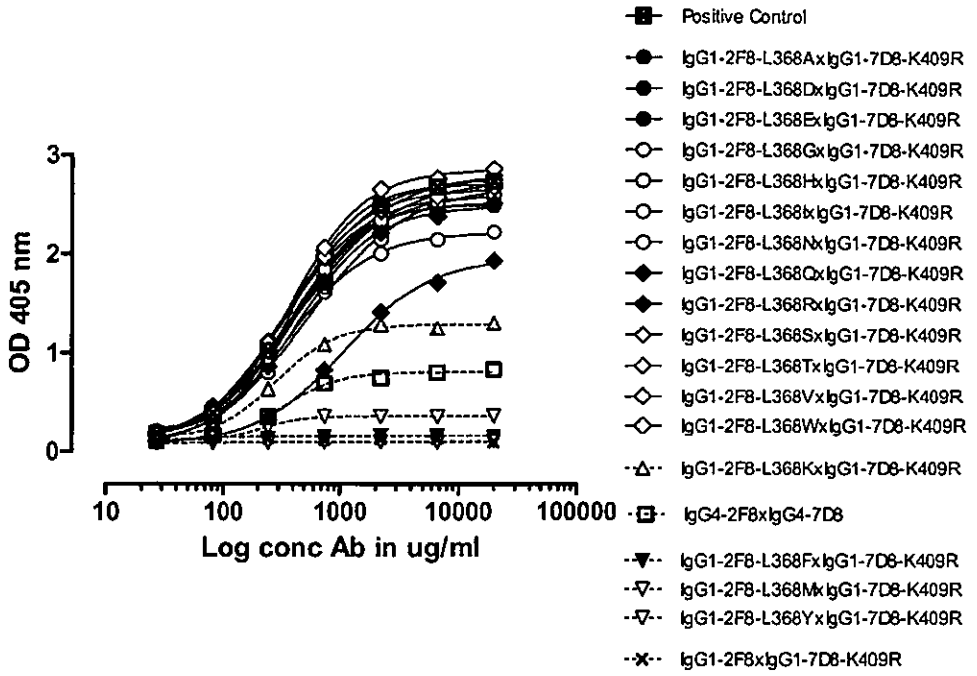

IgG1-2F8-F405L + IgG1-7D8-K409R
0 mM 2-MEA 4°C control
IgG1-2F8-F405L + IgG1-7D8-K409R
100 mM 2-MEA 15°C 5 hr
IgG1-2F8-F405L + IgG1-7D8-K409R
100 mM 2-MEA 15°C 24 hr
IgG1-2F8-F405L + IgG1-7D8-K409R
100 mM 2-MEA 25°C 5 hr
IgG1-2F8-F405L + IgG1-7D8-K409R
100 mM 2-MEA 25°C 24 hr
IgG1-2F8-F405L + IgG1-7D8-K409R
100 mM 2-MEA 37°C 5 hr
IgG1-2F8-F405L + IgG1-7D8-K409R
100 mM 2-MEA 37°C 24 hr
A280 (AU)
0.20
0.10
0.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
Time (minutes)

Figure 36

A


OD 405 nm
3
2
1
0
10
100
1000
10000
100000
Log conc Ab in ug/ml
Positive Control
IgG1-2F8-L368AxIgG1-7D8-K409R
IgG1-2F8-L368DxIgG1-7D8-K409R
IgG1-2F8-L368ExIgG1-7D8-K409R
IgG1-2F8-L368GxIgG1-7D8-K409R
IgG1-2F8-L368HxIgG1-7D8-K409R
IgG1-2F8-L368IxIgG1-7D8-K409R
IgG1-2F8-L368NxIgG1-7D8-K409R
IgG1-2F8-L368QxIgG1-7D8-K409R
IgG1-2F8-L368RxIgG1-7D8-K409R
IgG1-2F8-L368SxIgG1-7D8-K409R
IgG1-2F8-L368TxIgG1-7D8-K409R
IgG1-2F8-L368VxIgG1-7D8-K409R
IgG1-2F8-L368WxIgG1-7D8-K409R
IgG1-2F8-L368KxIgG1-7D8-K409R
IgG4-2F8xIgG4-7D8
IgG1-2F8-L368FxIgG1-7D8-K409R
IgG1-2F8-L368MxIgG1-7D8-K409R
IgG1-2F8-L368YxIgG1-7D8-K409R
IgG1-2F8xIgG1-7D8-K409R

B

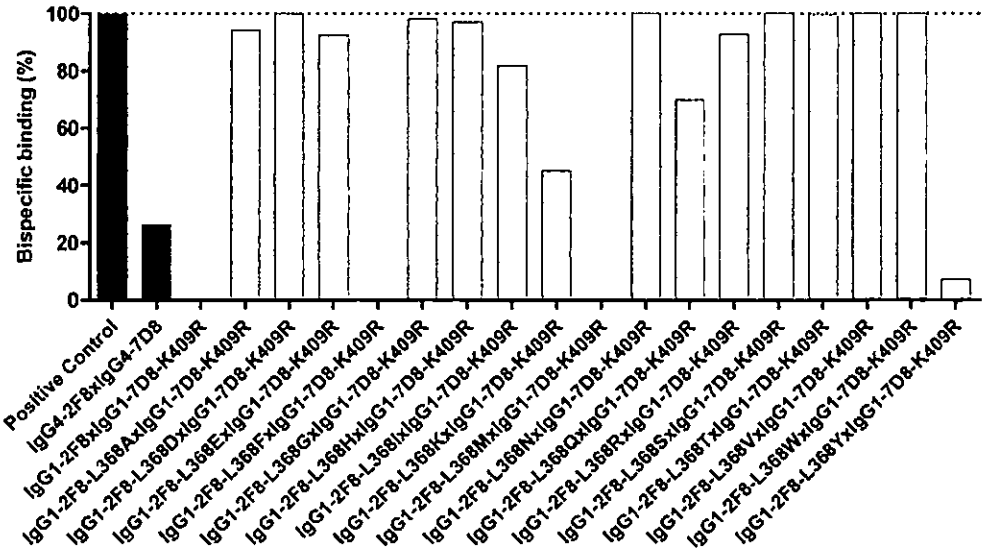
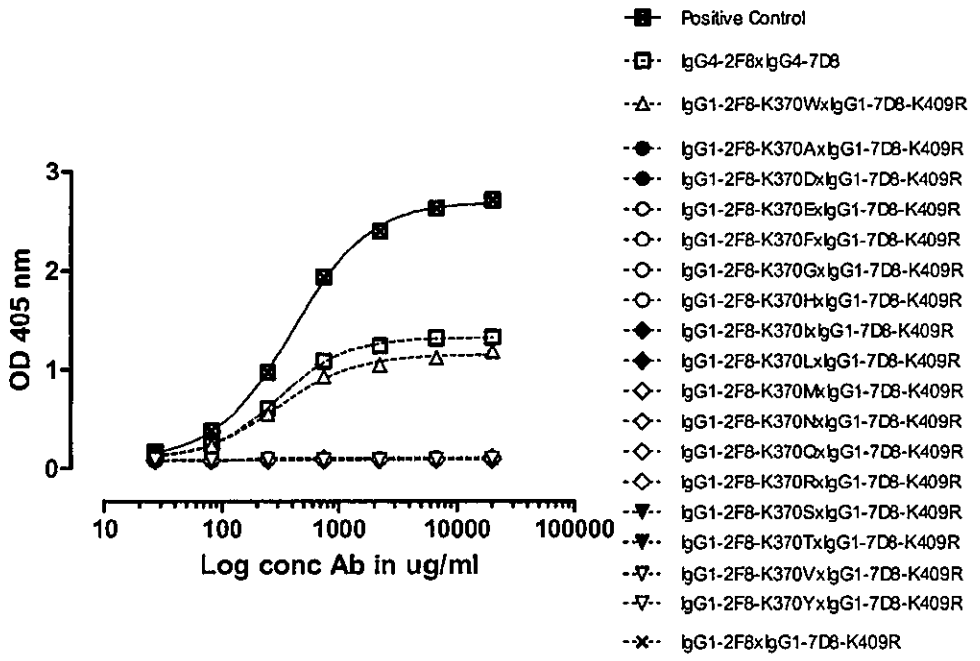

Bispecific binding (%)
100
80
60
40
20
0
Positive Control
IgG4-2F8xIgG4-7D8
IgG1-2F8xIgG1-7D8-K409R
IgG1-2F8-L368AxIgG1-7D8-K409R
IgG1-2F8-L368DxIgG1-7D8-K409R
IgG1-2F8-L368ExIgG1-7D8-K409R
IgG1-2F8-L368FxIgG1-7D8-K409R
IgG1-2F8-L368GxIgG1-7D8-K409R
IgG1-2F8-L368HxIgG1-7D8-K409R
IgG1-2F8-L368IxIgG1-7D8-K409R
IgG1-2F8-L368KxIgG1-7D8-K409R
IgG1-2F8-L368MxIgG1-7D8-K409R
IgG1-2F8-L368NxIgG1-7D8-K409R
IgG1-2F8-L368QxIgG1-7D8-K409R
IgG1-2F8-L368RxIgG1-7D8-K409R
IgG1-2F8-L368SxIgG1-7D8-K409R
IgG1-2F8-L368TxIgG1-7D8-K409R
IgG1-2F8-L368VxIgG1-7D8-K409R
IgG1-2F8-L368WxIgG1-7D8-K409R
IgG1-2F8-L368YxIgG1-7D8-K409R

Figure 37

A


OD 405 nm
3
2
1
0
10
100
1000
10000
100000
Log conc Ab in ug/ml
Positive Control
IgG4-2F8xIgG4-7D8
IgG1-2F8-K370WxIgG1-7D8-K409R
IgG1-2F8-K370AxIgG1-7D8-K409R
IgG1-2F8-K370DxIgG1-7D8-K409R
IgG1-2F8-K370ExIgG1-7D8-K409R
IgG1-2F8-K370FxIgG1-7D8-K409R
IgG1-2F8-K370GxIgG1-7D8-K409R
IgG1-2F8-K370HxIgG1-7D8-K409R
IgG1-2F8-K370IxIgG1-7D8-K409R
IgG1-2F8-K370LxIgG1-7D8-K409R
IgG1-2F8-K370MxIgG1-7D8-K409R
IgG1-2F8-K370NxIgG1-7D8-K409R
IgG1-2F8-K370QxIgG1-7D8-K409R
IgG1-2F8-K370RxIgG1-7D8-K409R
IgG1-2F8-K370SxIgG1-7D8-K409R
IgG1-2F8-K370TxIgG1-7D8-K409R
IgG1-2F8-K370VxIgG1-7D8-K409R
IgG1-2F8-K370YxIgG1-7D8-K409R
IgG1-2F8xIgG1-7D8-K409R

B

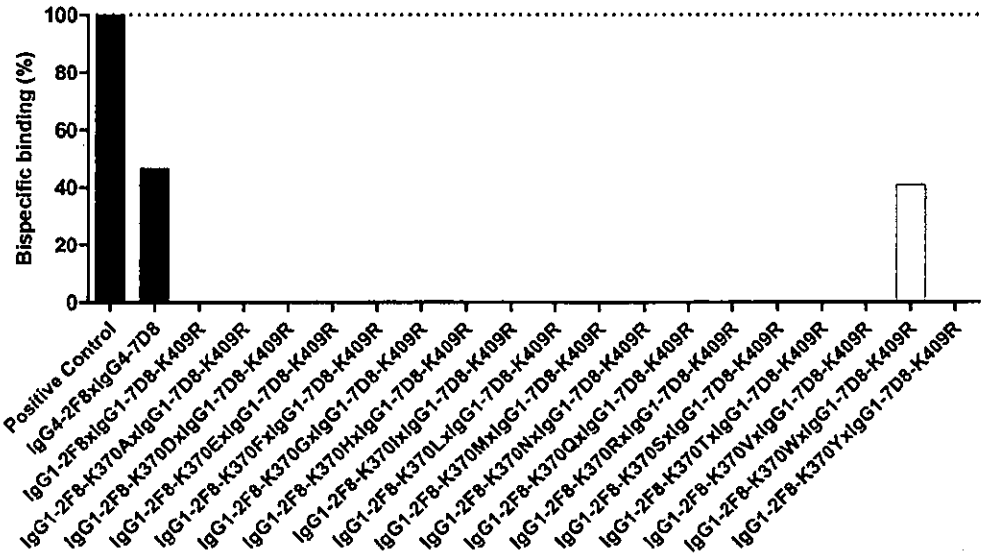
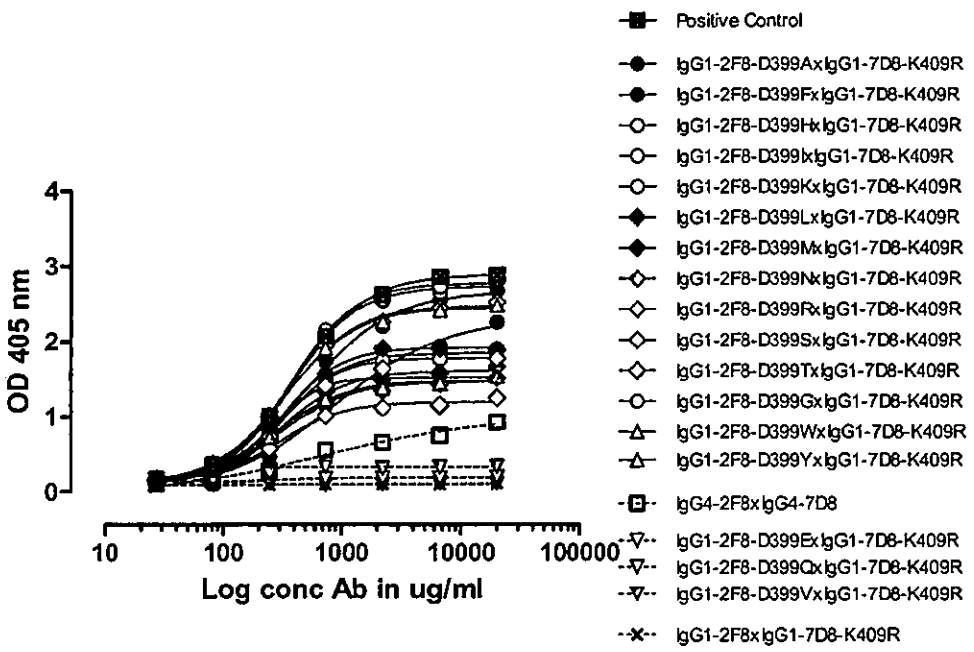

Bispecific binding (%)
100
80
60
40
20
0
Positive Control
IgG4-2F8xIgG4-7D8
IgG1-2F8xIgG1-7D8-K409R
IgG1-2F8-K370AxIgG1-7D8-K409R
IgG1-2F8-K370DxIgG1-7D8-K409R
IgG1-2F8-K370ExIgG1-7D8-K409R
IgG1-2F8-K370FxIgG1-7D8-K409R
IgG1-2F8-K370GxIgG1-7D8-K409R
IgG1-2F8-K370HxIgG1-7D8-K409R
IgG1-2F8-K370IxIgG1-7D8-K409R
IgG1-2F8-K370LxIgG1-7D8-K409R
IgG1-2F8-K370MxIgG1-7D8-K409R
IgG1-2F8-K370NxIgG1-7D8-K409R
IgG1-2F8-K370QxIgG1-7D8-K409R
IgG1-2F8-K370RxIgG1-7D8-K409R
IgG1-2F8-K370SxIgG1-7D8-K409R
IgG1-2F8-K370TxIgG1-7D8-K409R
IgG1-2F8-K370VxIgG1-7D8-K409R
IgG1-2F8-K370WxIgG1-7D8-K409R
IgG1-2F8-K370YxIgG1-7D8-K409R

Figure 38

A


OD 405 nm
0
1
2
3
4
10
100
1000
10000
100000
Log conc Ab in ug/ml
Positive Control
IgG1-2F8-D399AxIgG1-7D8-K409R
IgG1-2F8-D399FxIgG1-7D8-K409R
IgG1-2F8-D399HxIgG1-7D8-K409R
IgG1-2F8-D399IxIgG1-7D8-K409R
IgG1-2F8-D399KxIgG1-7D8-K409R
IgG1-2F8-D399LxIgG1-7D8-K409R
IgG1-2F8-D399MxIgG1-7D8-K409R
IgG1-2F8-D399NxIgG1-7D8-K409R
IgG1-2F8-D399RxIgG1-7D8-K409R
IgG1-2F8-D399SxIgG1-7D8-K409R
IgG1-2F8-D399TxIgG1-7D8-K409R
IgG1-2F8-D399GxIgG1-7D8-K409R
IgG1-2F8-D399WxIgG1-7D8-K409R
IgG1-2F8-D399YxIgG1-7D8-K409R
IgG4-2F8xIgG4-7D8
IgG1-2F8-D399ExIgG1-7D8-K409R
IgG1-2F8-D399QxIgG1-7D8-K409R
IgG1-2F8-D399VxIgG1-7D8-K409R
IgG1-2F8xIgG1-7D8-K409R

B

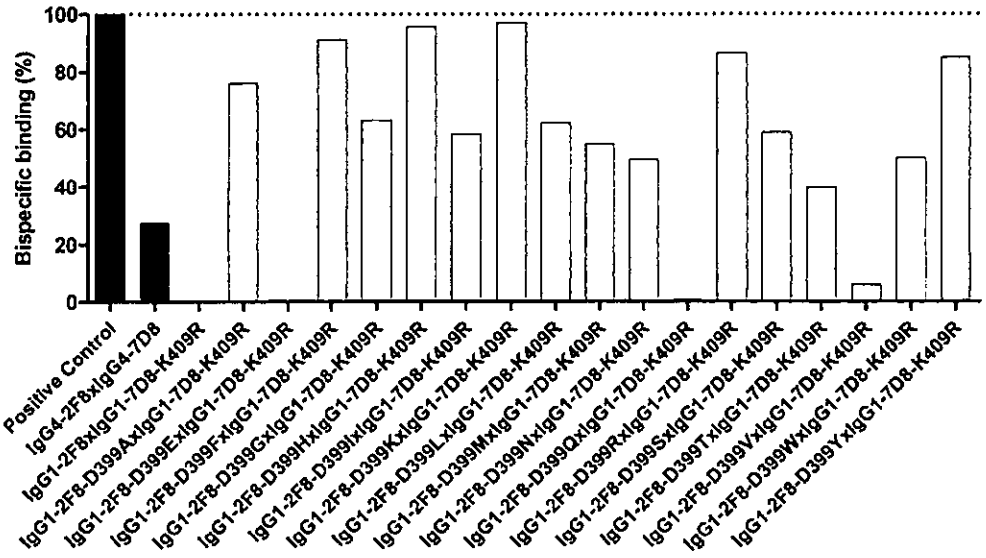

Bispecific binding (%)
0
20
40
60
80
100
Positive Control
IgG4-2F8xIgG4-7D8
IgG1-2F8xIgG1-7D8-K409R
IgG1-2F8-D399AxIgG1-7D8-K409R
IgG1-2F8-D399ExIgG1-7D8-K409R
IgG1-2F8-D399FxIgG1-7D8-K409R
IgG1-2F8-D399GxIgG1-7D8-K409R
IgG1-2F8-D399HxIgG1-7D8-K409R
IgG1-2F8-D399IxIgG1-7D8-K409R
IgG1-2F8-D399KxIgG1-7D8-K409R
IgG1-2F8-D399LxIgG1-7D8-K409R
IgG1-2F8-D399MxIgG1-7D8-K409R
IgG1-2F8-D399NxIgG1-7D8-K409R
IgG1-2F8-D399QxIgG1-7D8-K409R
IgG1-2F8-D399RxIgG1-7D8-K409R
IgG1-2F8-D399SxIgG1-7D8-K409R
IgG1-2F8-D399TxIgG1-7D8-K409R
IgG1-2F8-D399VxIgG1-7D8-K409R
IgG1-2F8-D399WxIgG1-7D8-K409R
IgG1-2F8-D399YxIgG1-7D8-K409R

Figure 39

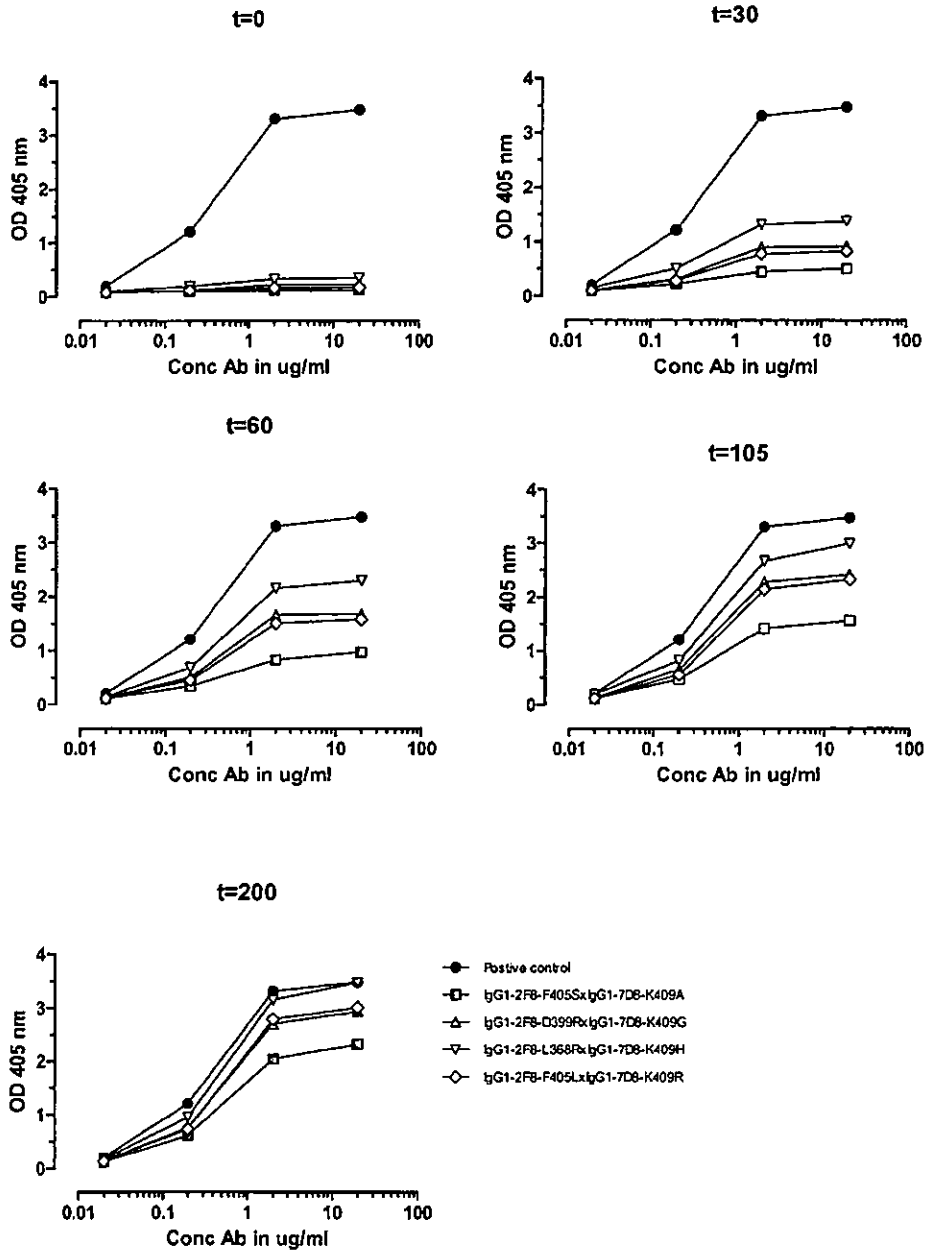

t=0
t=30
t=60
t=105
t=200
OD 405 nm
Conc Ab in ug/ml
0.01
0.1
1
10
100
Postive control
IgG1-2F8-F405S:IgG1-7D8-K409A
IgG1-2F8-D399R:IgG1-7D8-K409G
IgG1-2F8-L368R:IgG1-7D8-K409H
IgG1-2F8-F405L:IgG1-7D8-K409R

Figure 40

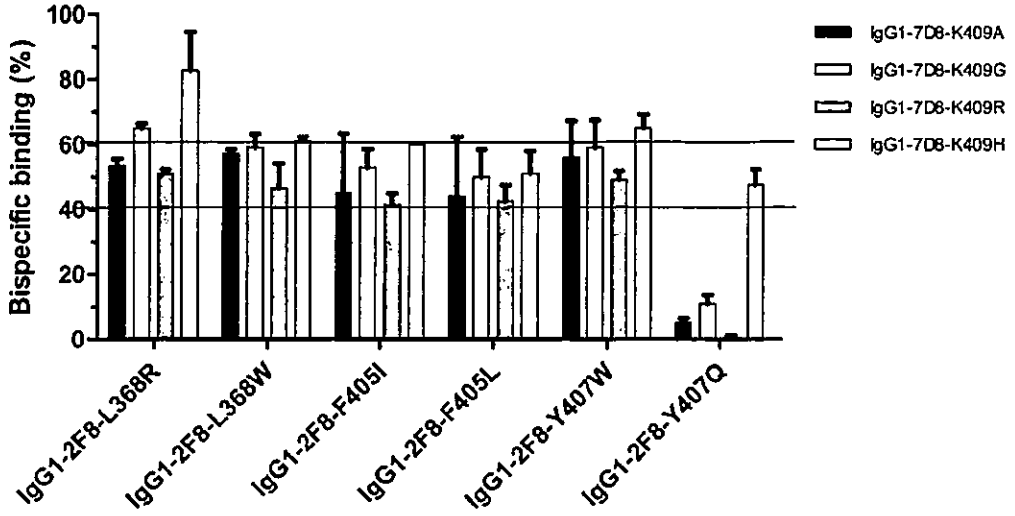

Bispecific binding (%)
100
80
60
40
20
0
IgG1-7D8-K409A
IgG1-7D8-K409G
IgG1-7D8-K409R
IgG1-7D8-K409H
IgG1-2F8-L368R
IgG1-2F8-L368W
IgG1-2F8-F405I
IgG1-2F8-F405L
IgG1-2F8-Y407W
IgG1-2F8-Y407Q

Figure 41

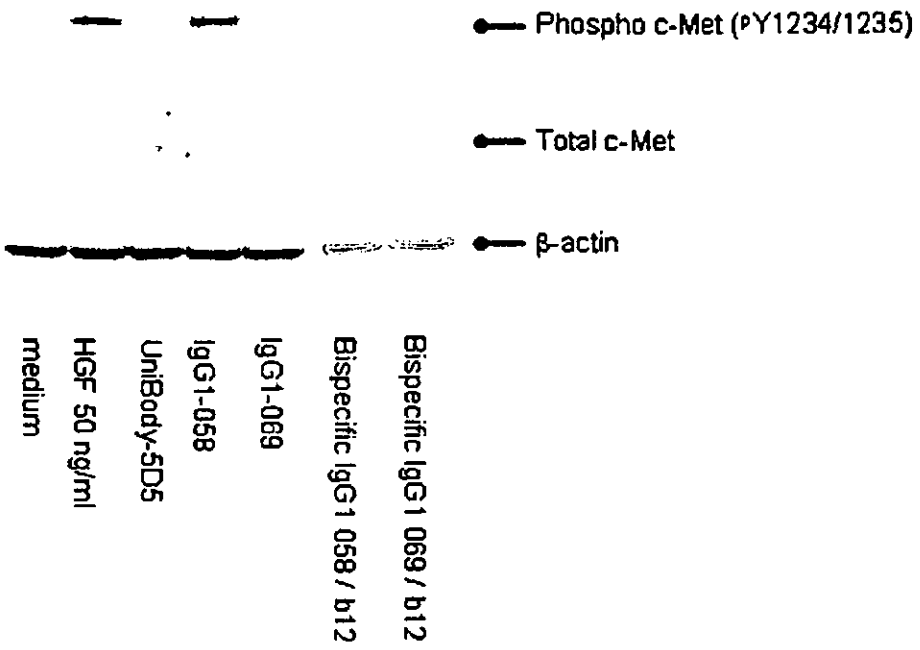

Phospho c-Met (pY1234/1235)
Total c-Met
β-actin
medium
HGF 50 ng/ml
UniBody-5D5
IgG1-058
IgG1-069
Bispecific IgG1 058 / b12
Bispecific IgG1 069 / b12

Figure 42

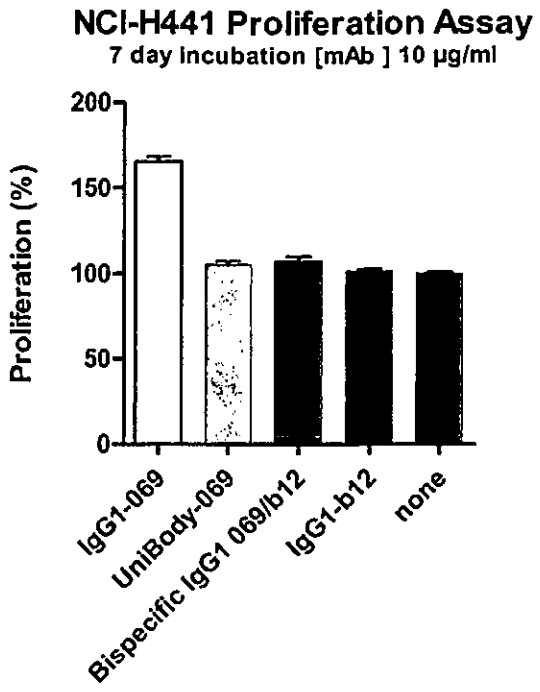
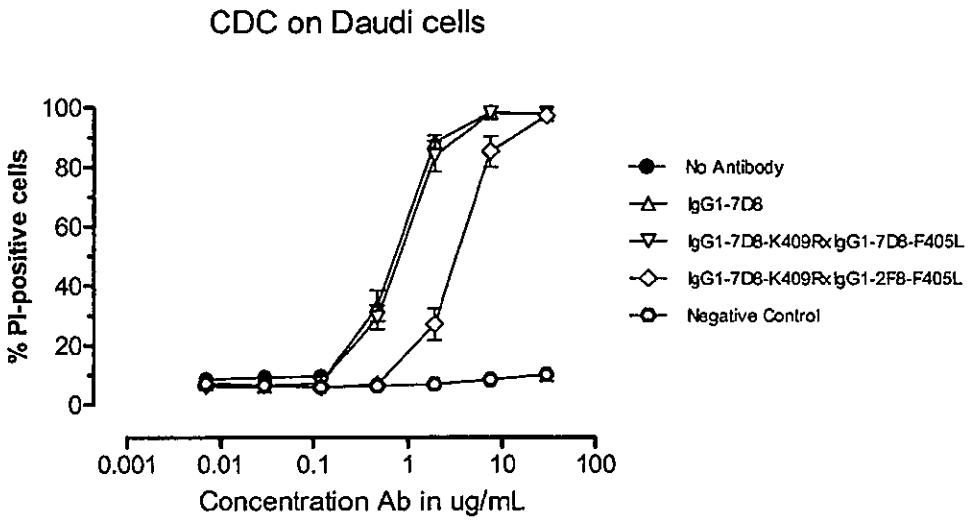

NCI-H441 Proliferation Assay
7 day incubation [mAb] 10 µg/ml
Proliferation (%)
200
150
100
50
0
IgG1-069
UniBody-069
Bispecific IgG1 069/b12
IgG1-b12
none

Figure 43

A


CDC on Daudi cells
% PI-positive cells
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
Concentration Ab in ug/mL
No Antibody
IgG1-7D8
IgG1-7D8-K409RxIgG1-7D8-F405L
IgG1-7D8-K409RxIgG1-2F8-F405L
Negative Control

B

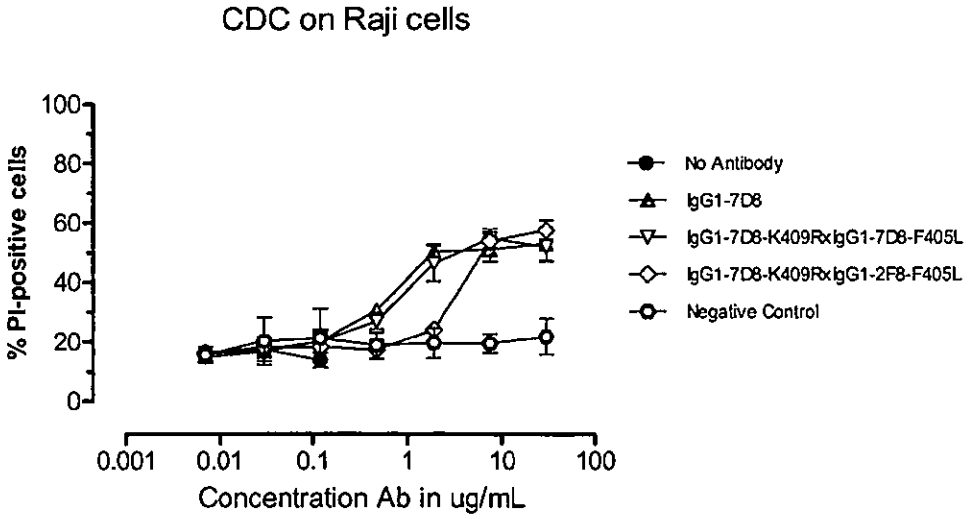

CDC on Raji cells
% PI-positive cells
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
Concentration Ab in ug/mL
No Antibody
IgG1-7D8
IgG1-7D8-K409RxIgG1-7D8-F405L
IgG1-7D8-K409RxIgG1-2F8-F405L
Negative Control

Figure 44

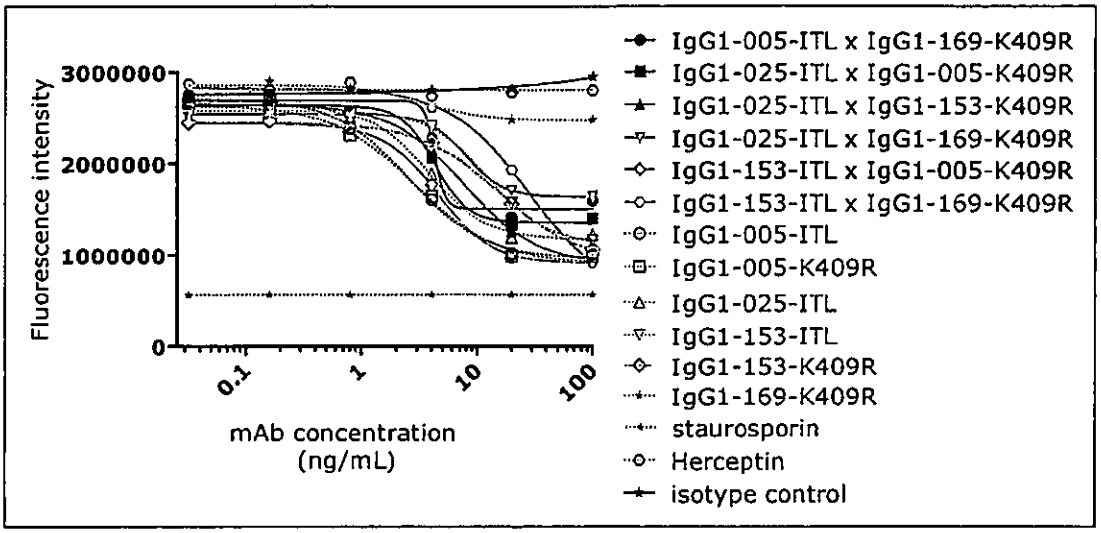

Fluorescence intensity
3000000
2000000
1000000
0
0.1
1
10
100
mAb concentration
(ng/mL)
IgG1-005-ITL x IgG1-169-K409R
IgG1-025-ITL x IgG1-005-K409R
IgG1-025-ITL x IgG1-153-K409R
IgG1-025-ITL x IgG1-169-K409R
IgG1-153-ITL x IgG1-005-K409R
IgG1-153-ITL x IgG1-169-K409R
IgG1-005-ITL
IgG1-005-K409R
IgG1-025-ITL
IgG1-153-ITL
IgG1-153-K409R
IgG1-169-K409R
staurosporin
Herceptin
isotype control

Figure 45

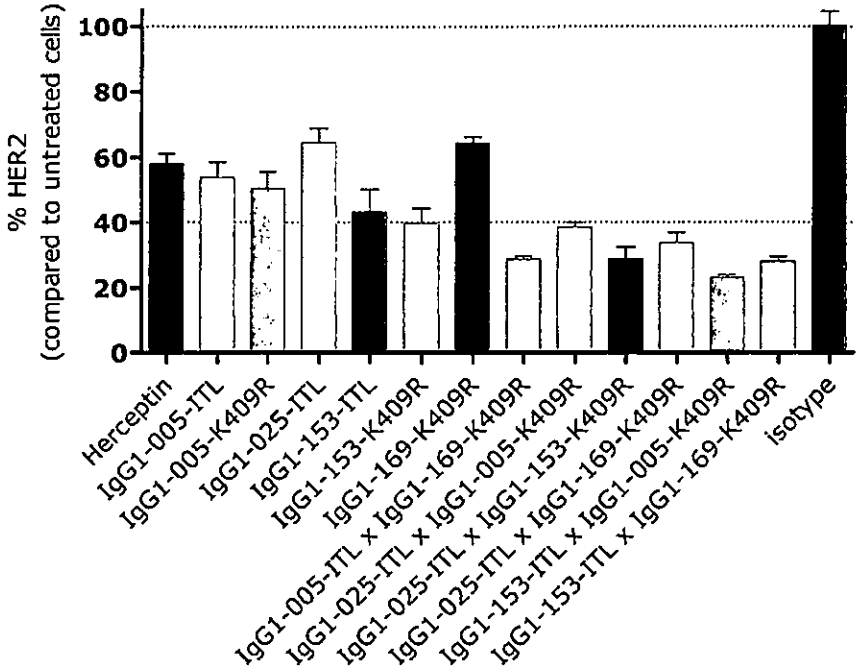
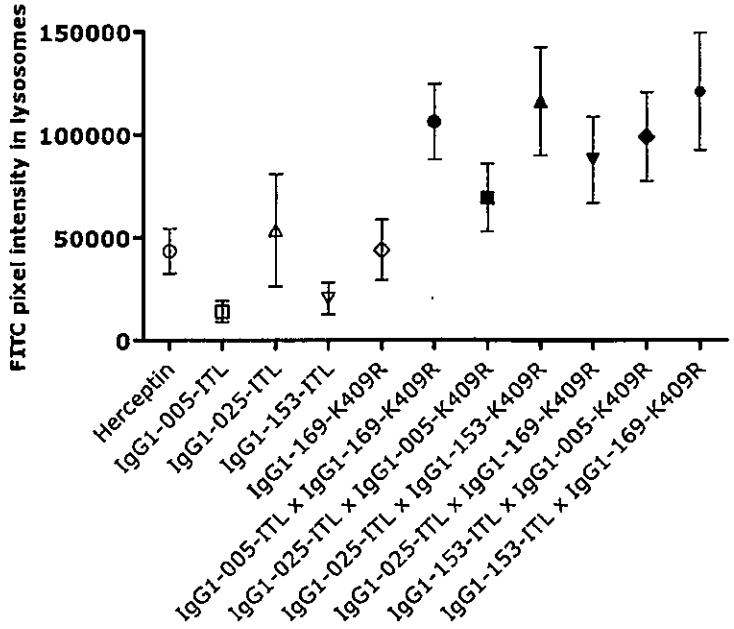

% HER2
(compared to untreated cells)
100
80
60
40
20
0
Herceptin
IgG1-005-ITL
IgG1-005-K409R
IgG1-025-ITL
IgG1-153-ITL
IgG1-153-K409R
IgG1-169-K409R
IgG1-005-ITL x IgG1-169-K409R
IgG1-025-ITL x IgG1-005-K409R
IgG1-025-ITL x IgG1-153-K409R
IgG1-025-ITL x IgG1-169-K409R
IgG1-153-ITL x IgG1-005-K409R
IgG1-153-ITL x IgG1-169-K409R
isotype

Figure 46

A


FITC pixel intensity in lysosomes
150000
100000
50000
0
Herceptin
IgG1-005-ITL
IgG1-025-ITL
IgG1-153-ITL
IgG1-169-K409R
IgG1-005-ITL x IgG1-169-K409R
IgG1-025-ITL x IgG1-153-K409R
IgG1-025-ITL x IgG1-169-K409R
IgG1-025-ITL x IgG1-005-K409R
IgG1-153-ITL x IgG1-169-K409R
IgG1-153-ITL x IgG1-005-K409R

B

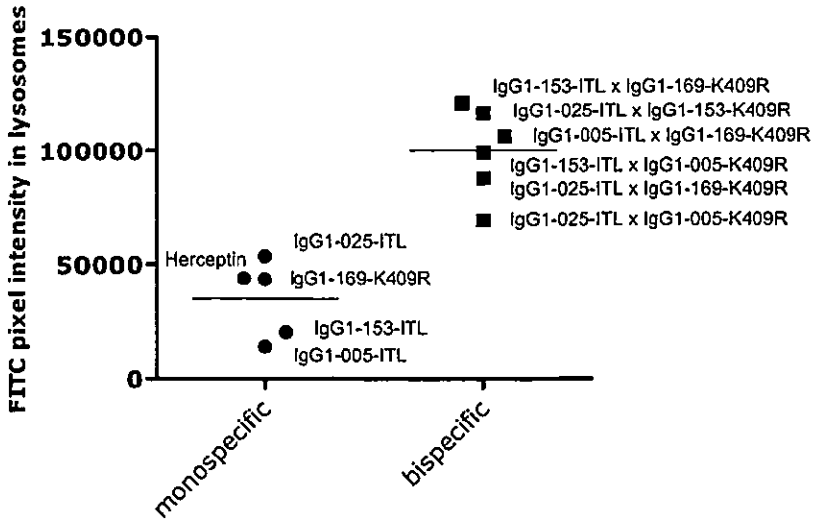

FITC pixel intensity in lysosomes
150000
100000
50000
0
IgG1-153-ITL x IgG1-169-K409R
IgG1-025-ITL x IgG1-153-K409R
IgG1-005-ITL x IgG1-169-K409R
IgG1-153-ITL x IgG1-005-K409R
IgG1-025-ITL x IgG1-169-K409R
IgG1-025-ITL x IgG1-005-K409R
Herceptin
IgG1-025-ITL
IgG1-169-K409R
IgG1-153-ITL
IgG1-005-ITL
monospecific
bispecific

Figure 47

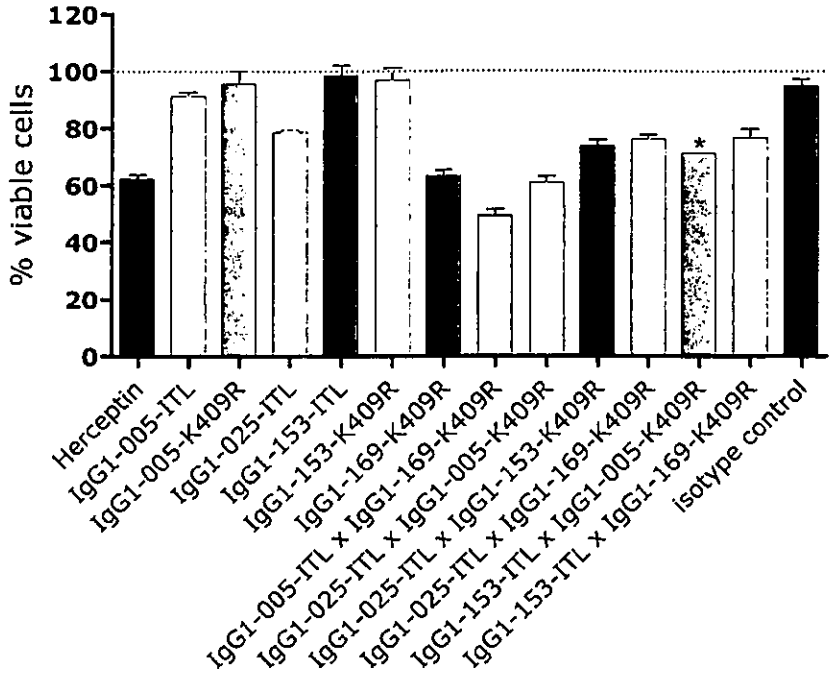

120
100
80
60
40
20
0
% viable cells
*
Herceptin
IgG1-005-ITL
IgG1-005-K409R
IgG1-025-ITL
IgG1-153-ITL
IgG1-153-K409R
IgG1-169-K409R
IgG1-005-ITL x IgG1-169-K409R
IgG1-025-ITL x IgG1-005-K409R
IgG1-025-ITL x IgG1-153-K409R
IgG1-025-ITL x IgG1-169-K409R
IgG1-025-ITL x IgG1-005-K409R
IgG1-153-ITL x IgG1-169-K409R
isotype control

Figure 48

A

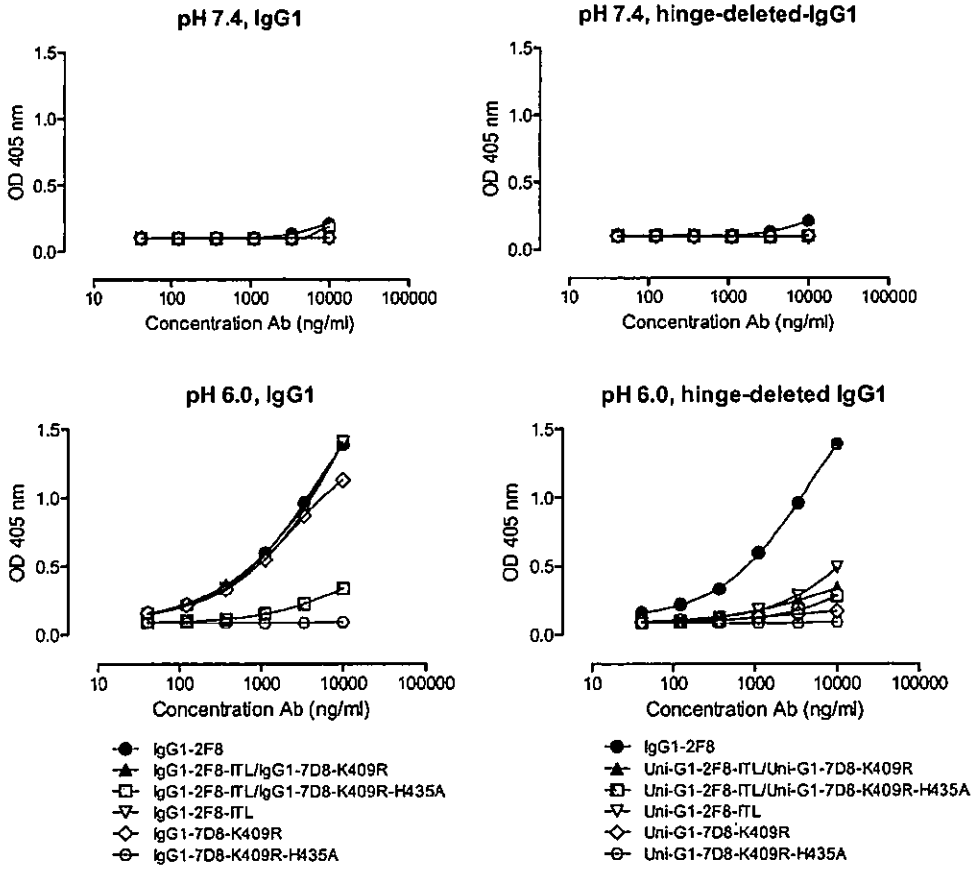

pH 7.4, IgG1
pH 7.4, hinge-deleted-IgG1
pH 6.0, IgG1
pH 6.0, hinge-deleted IgG1
OD 405 nm
Concentration Ab (ng/ml)
IgG1-2F8
IgG1-2F8-ITL/IgG1-7D8-K409R
IgG1-2F8-ITL/IgG1-7D8-K409R-H435A
IgG1-2F8-ITL
IgG1-7D8-K409R
IgG1-7D8-K409R-H435A
Uni-G1-2F8-ITL/Uni-G1-7D8-K409R
Uni-G1-2F8-ITL/Uni-G1-7D8-K409R-H435A
Uni-G1-2F8-ITL
Uni-G1-7D8-K409R
Uni-G1-7D8-K409R-H435A

Figure 48 (continued)

B

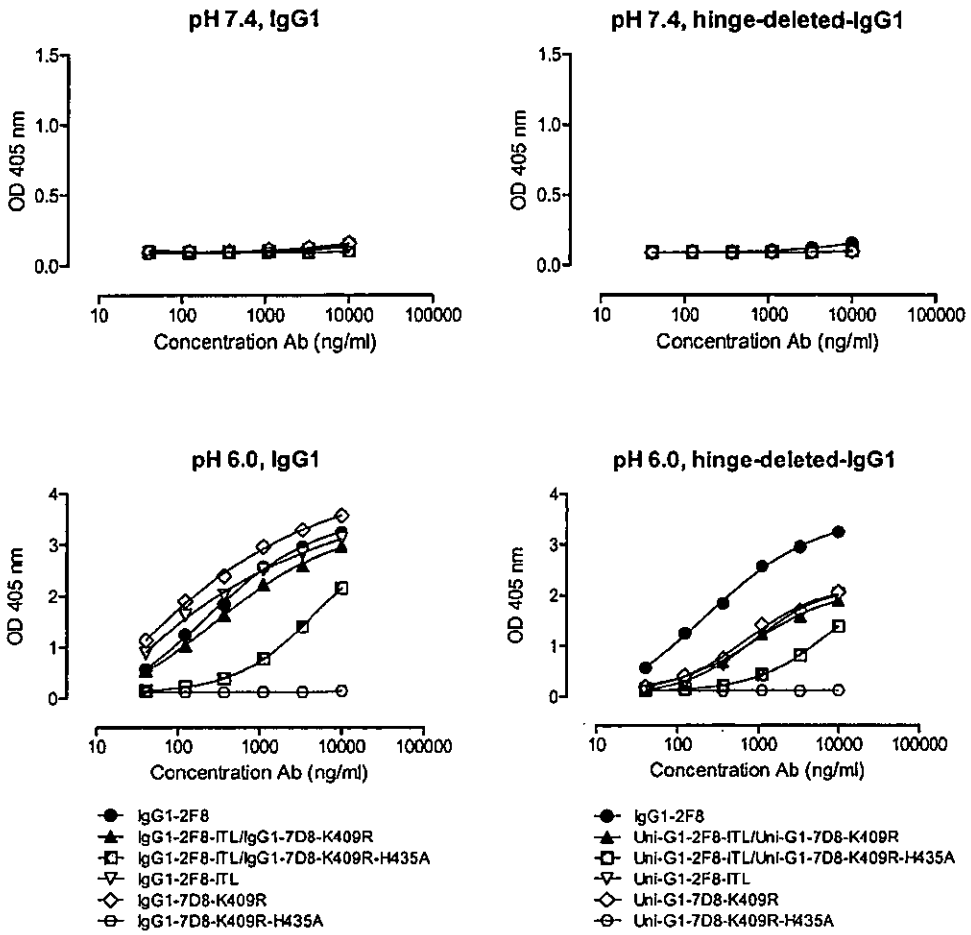


Figure 49

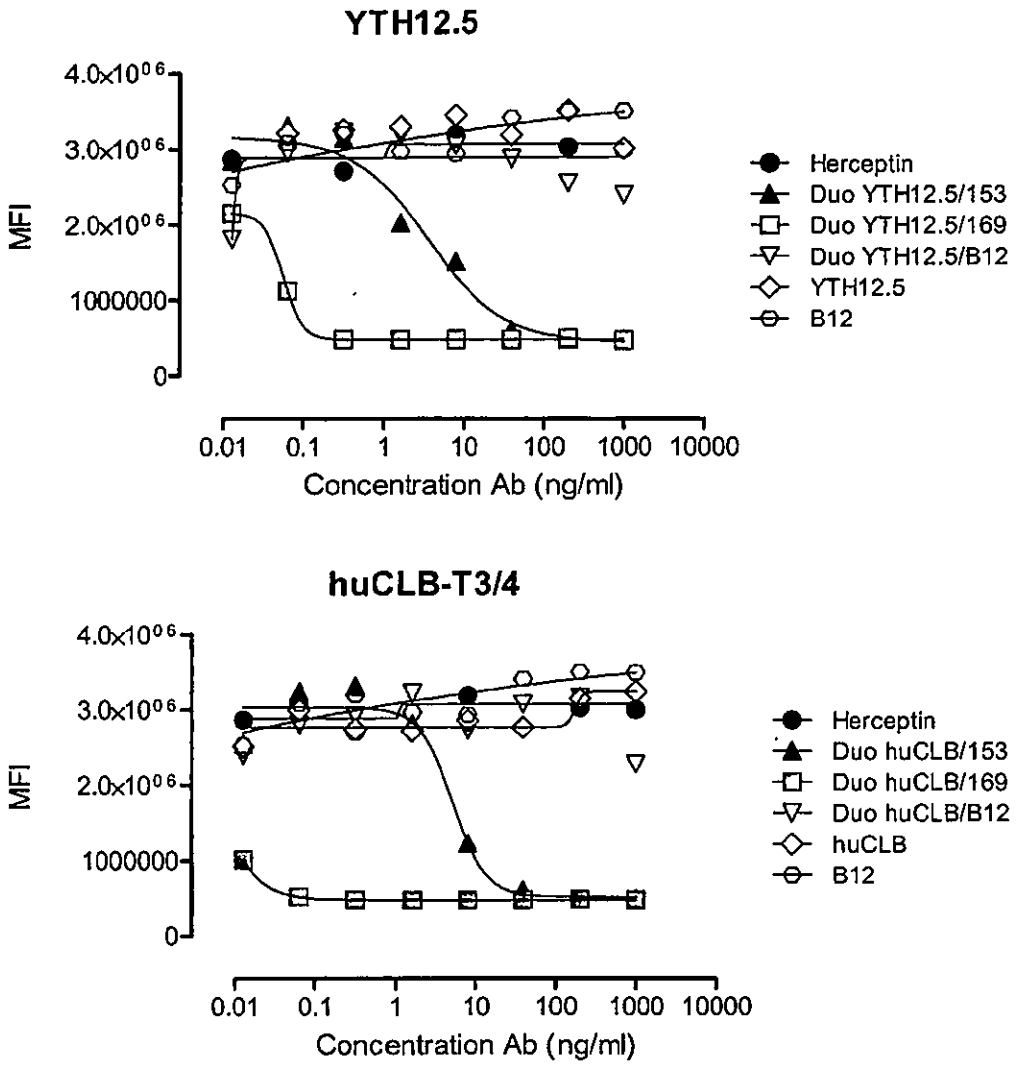

YTH12.5
4.0x10^06
3.0x10^06
2.0x10^06
1000000
0
MFI
0.01
0.1
1
10
100
1000
10000
Concentration Ab (ng/ml)
Herceptin
Duo YTH12.5/153
Duo YTH12.5/169
Duo YTH12.5/B12
YTH12.5
B12
huCLB-T3/4
4.0x10^06
3.0x10^06
2.0x10^06
1000000
0
MFI
0.01
0.1
1
10
100
1000
10000
Concentration Ab (ng/ml)
Herceptin
Duo huCLB/153
Duo huCLB/169
Duo huCLB/B12
huCLB
B12

Figure 49 (continued)

