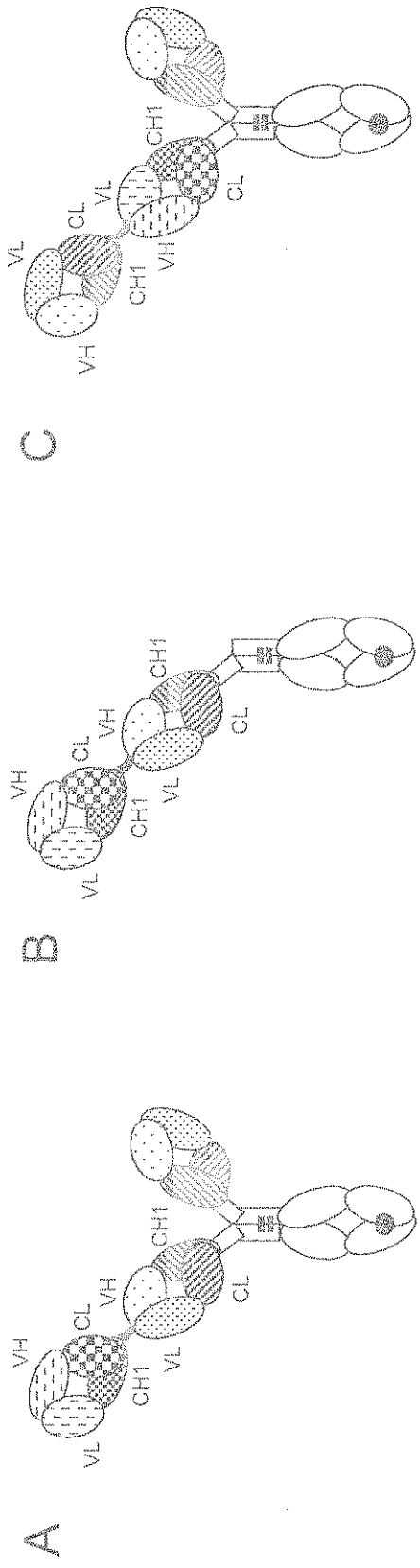

A
VL
CL
VH
CH1
VH
CL
VL
CH1
B
VH
CL
VL
CH1
VL
CH1
VH
CL
C
VL
CL
VH
CH1
VH
CL
VL
CH1
D
VH
CH1
VL
CL
VL
VH
CL
CH1

Figure 1

Figure 2

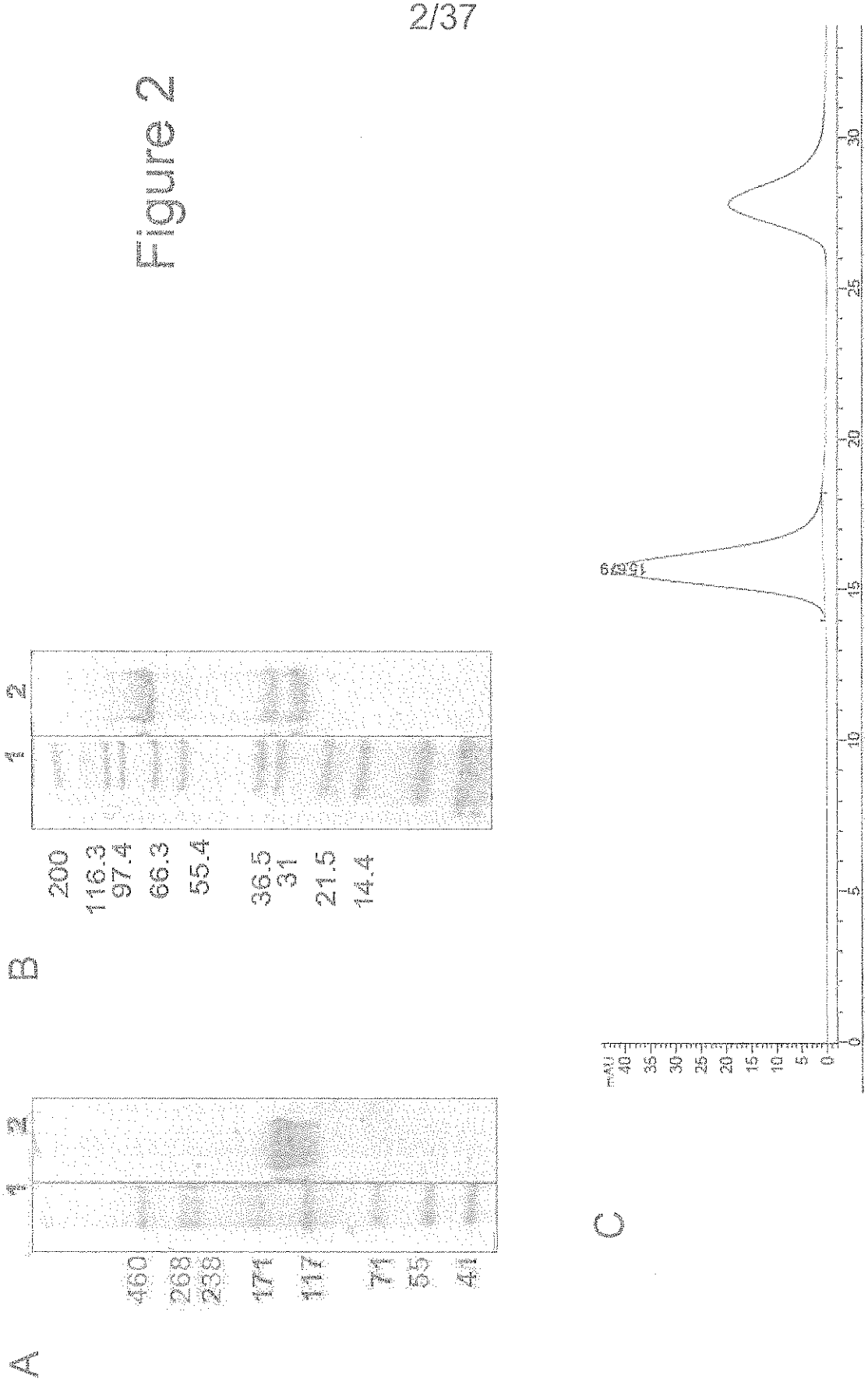

A
B
C
1
2
3
460
268
238
171
117
71
55
41
200
116.3
97.4
66.3
55.4
36.5
31
21.5
14.4
6.51
0
5
10
15
20
25
30
35
40

Figure 3

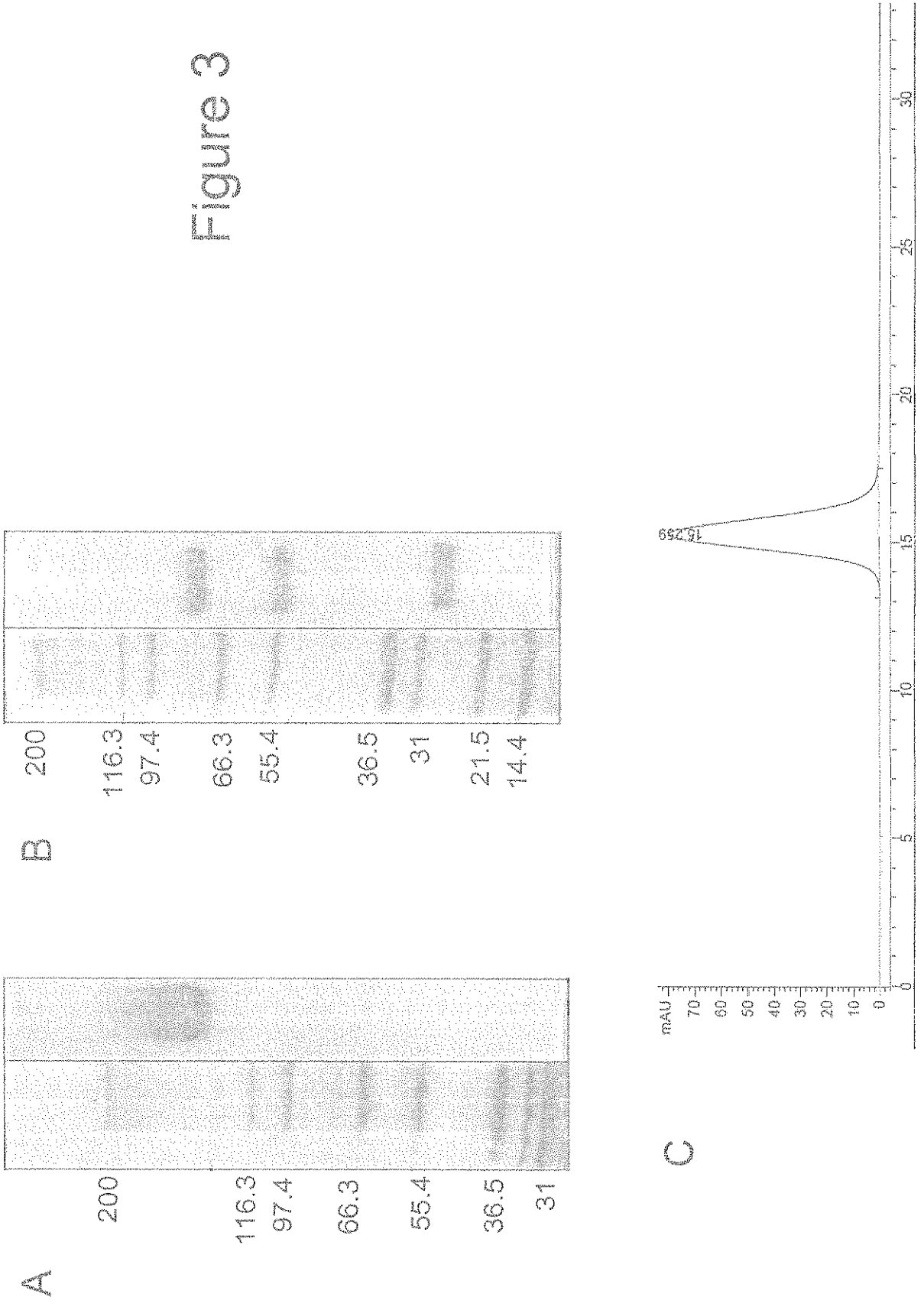

A
200
116.3
97.4
66.3
55.4
36.5
31
B
200
116.3
97.4
66.3
55.4
36.5
31
21.5
14.4
C
15.289
mAU
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30

Figure 4

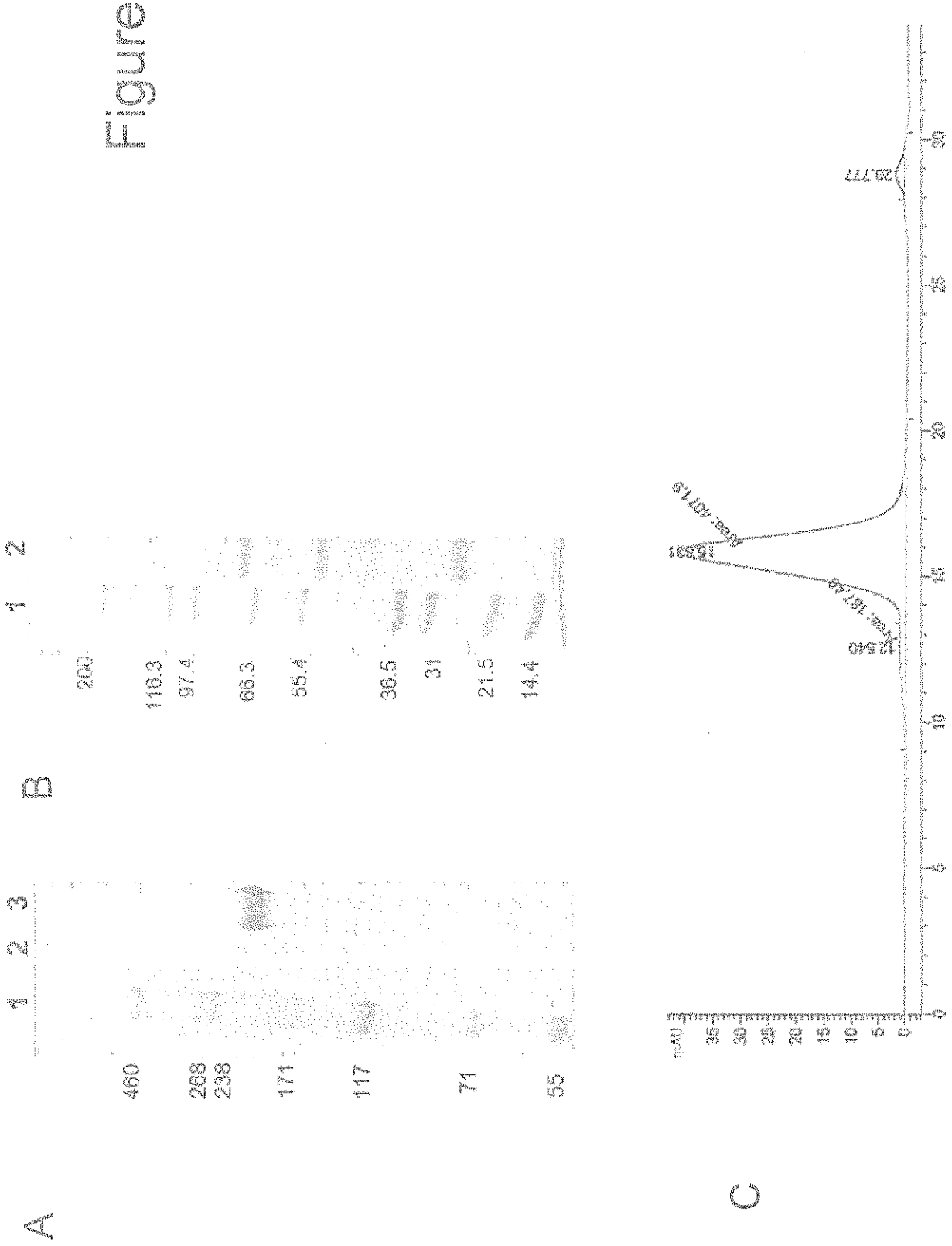

A
1 2 3
460
268
238
171
117
71
55
B
1 2
200
116.3
97.4
66.3
55.4
36.5
31
21.5
14.4
C
mAU
35
30
25
20
15
10
5
0
0
5
10
15
20
25
30
25.540
Area: 167.98
15.881
Area: 107.19
28.777

Figure 5

A
B
[FU]
[kDa]
[s]
4,5
9,0
15,6
49,2
95
150
240
298,8
240,0
193,5
159,8
147,7
125,6
Correct Ab
Crossed and non-crossed LCs
Unused HC
Fused HC
4,5
8,0
15,3
26,6
49,2
94,1
194,9
240,0

Figure 6

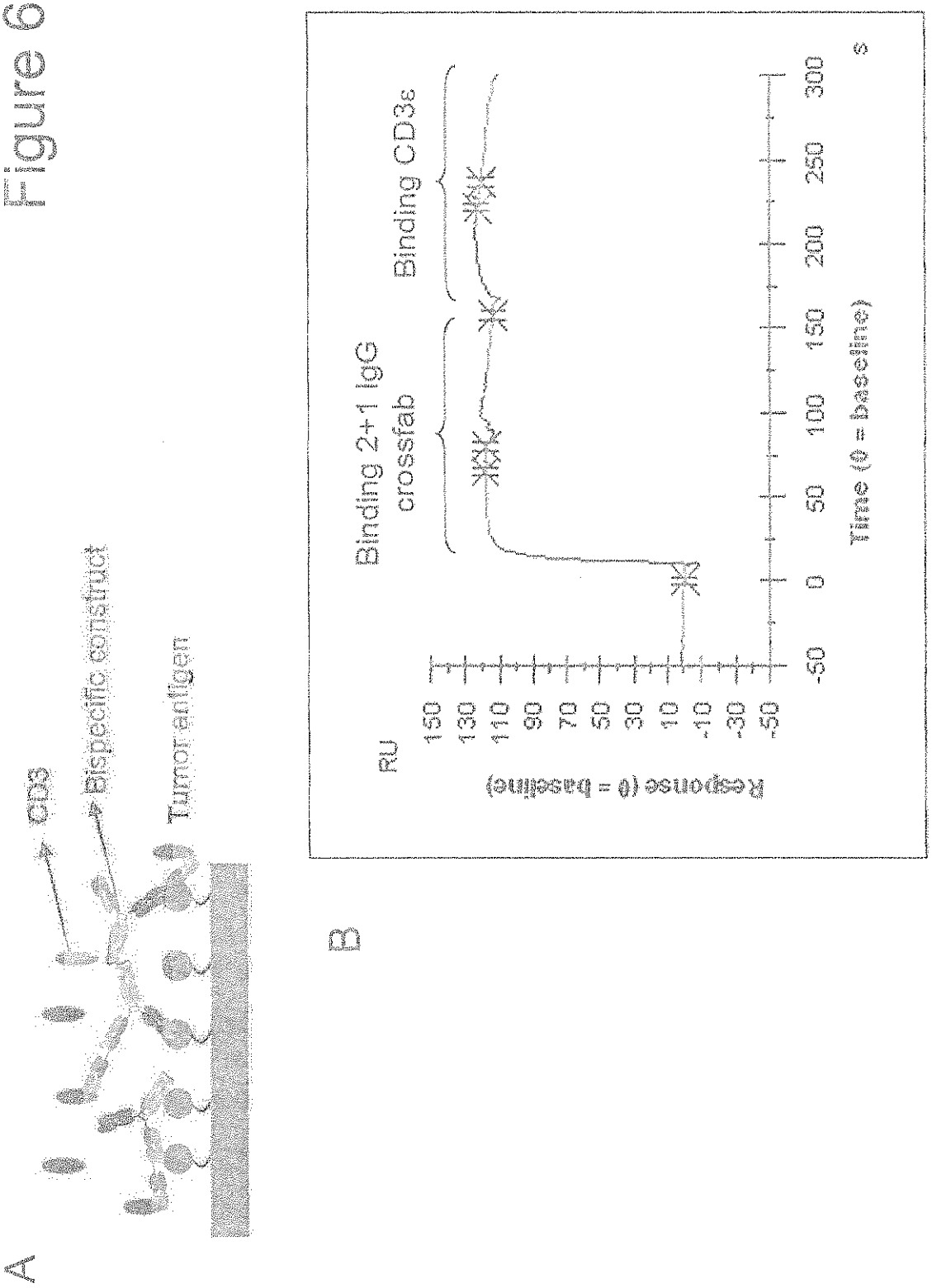

A
CD3
Bispecific construct
Tumor antigen
B
Binding 2+1 IgG crossfab
Binding CD3ε
RU
150
130
110
90
70
50
30
10
-10
-30
-50
Response (0 = baseline)
-50
0
50
100
150
200
250
300
Time (0 = baseline)
s

Figure 7

A

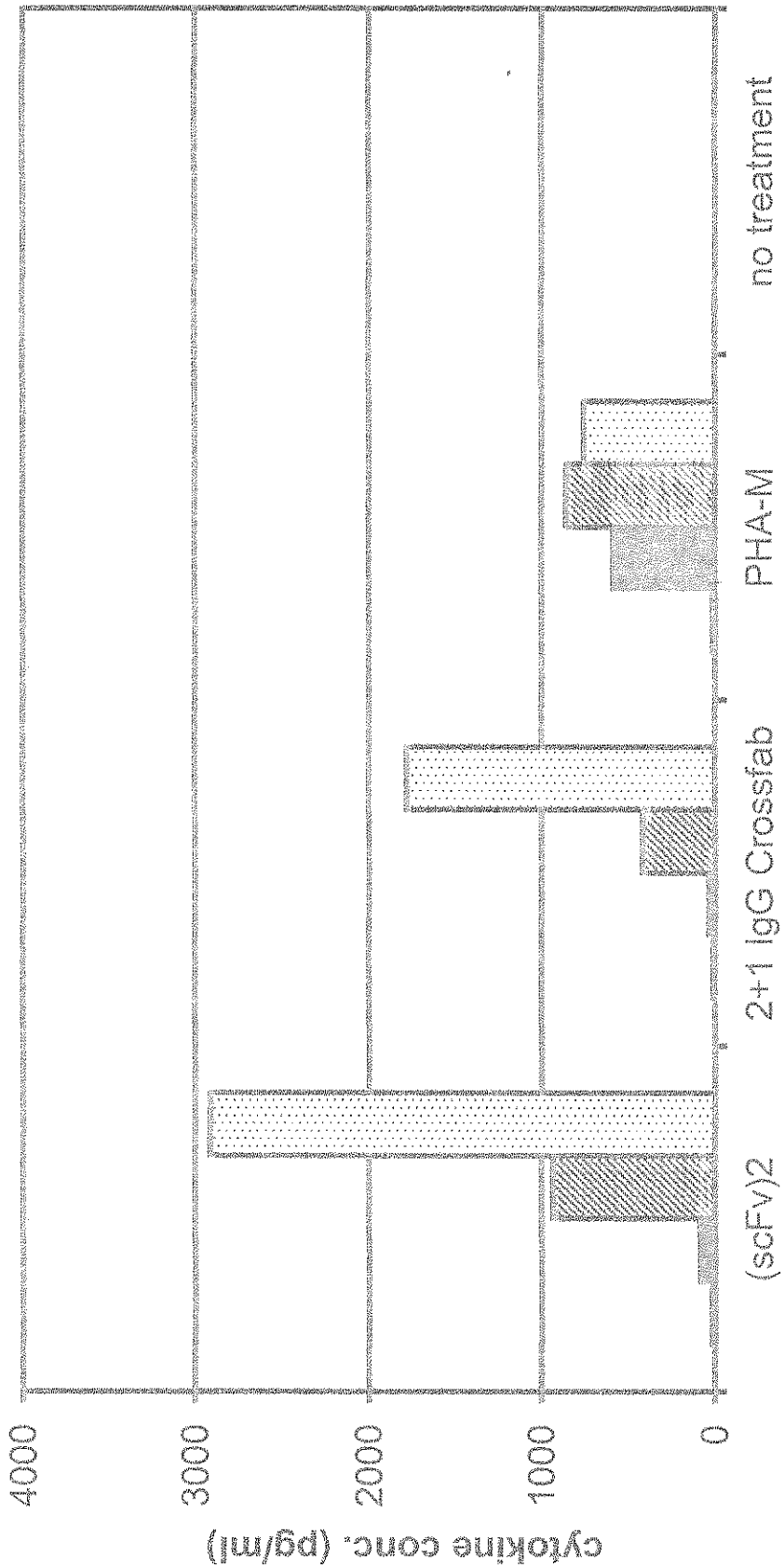

hu IL-4
hu IL-10
hu TNF
hu IFNg
cytokine conc. (pg/ml)
4000
3000
2000
1000
0
(scFv)2
2+1 IgG Crossfab
PHA-M
no treatment

Figure 7

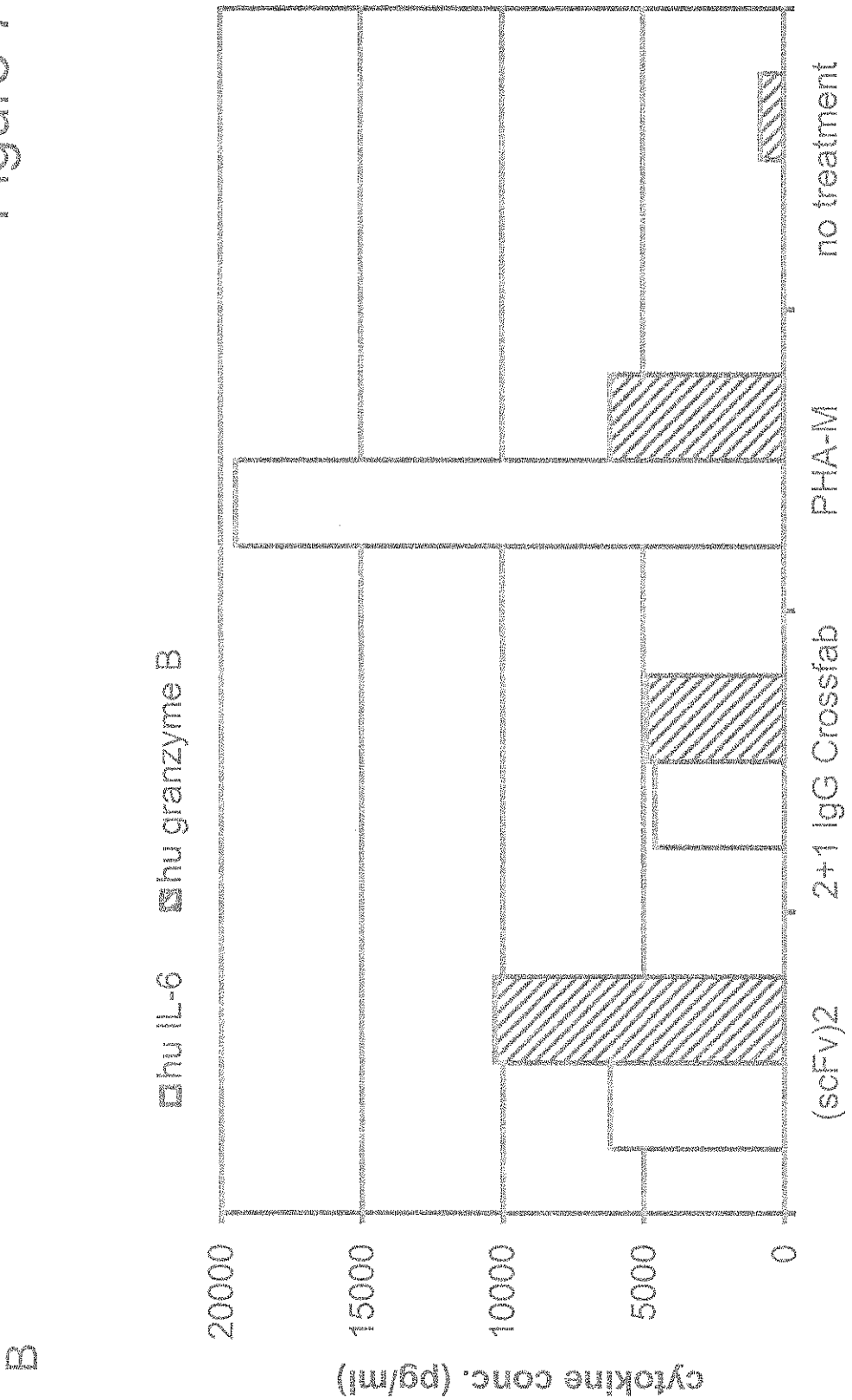

B
cytokine conc. (pg/ml)
20000
15000
10000
5000
0
hu IL-6
hu granzyme B
(scFv)2
2+1 IgG Crossfab
PHA-M
no treatment

Figure 7

C

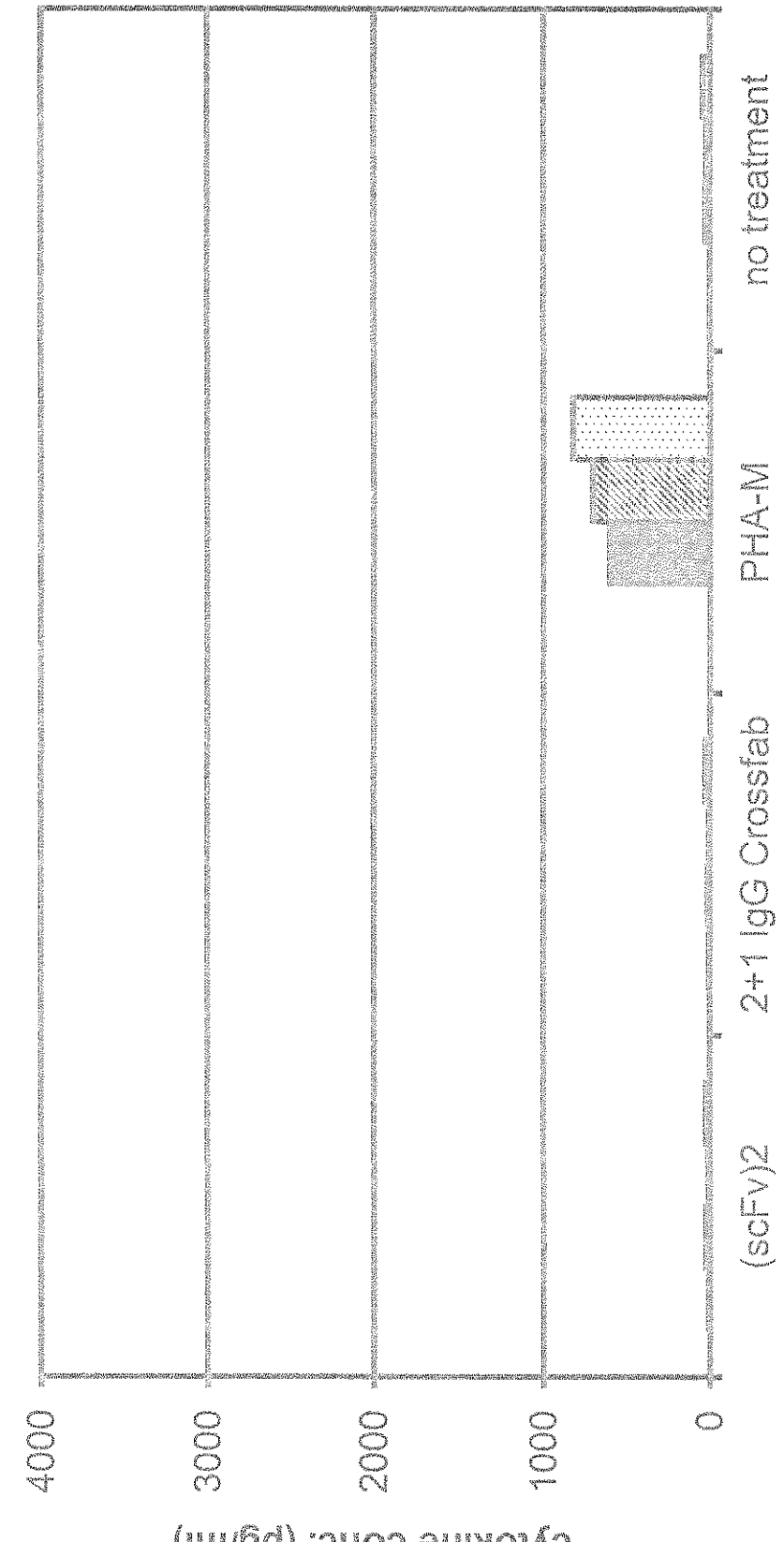

cytokine conc. (pg/ml)
4000
3000
2000
1000
0
hu IL-4
hu IL-10
hu TNF
hu IFNg
(scFv)2
2+1 IgG Crossfab
PHA-M
no treament

Figure 7

D

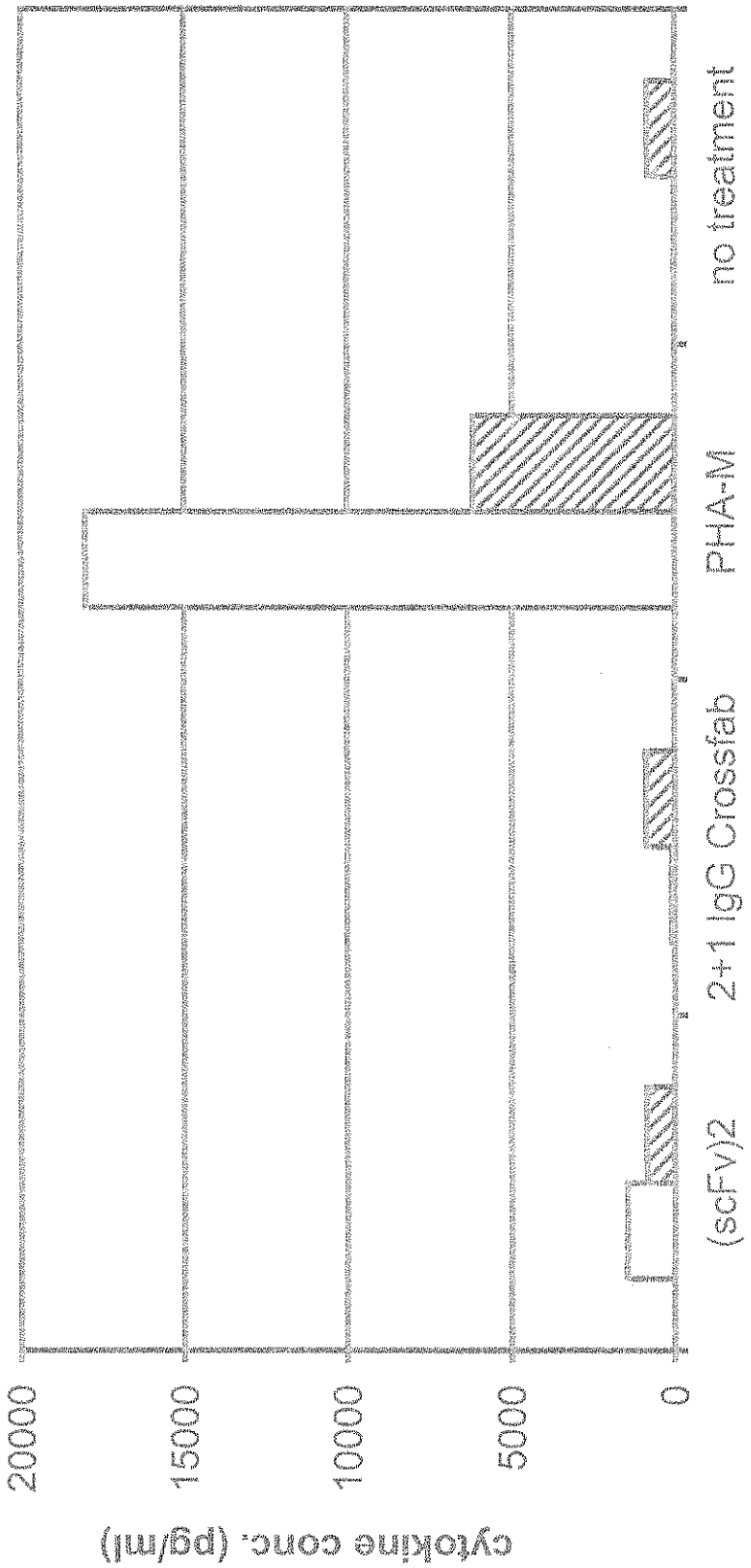

hu IL-6
hu granzyme B
cytokine conc. (pg/ml)
20000
15000
10000
5000
0
(scFv)2
2+1 IgG Crossfab
PHA-M
no treatment

Figure 8

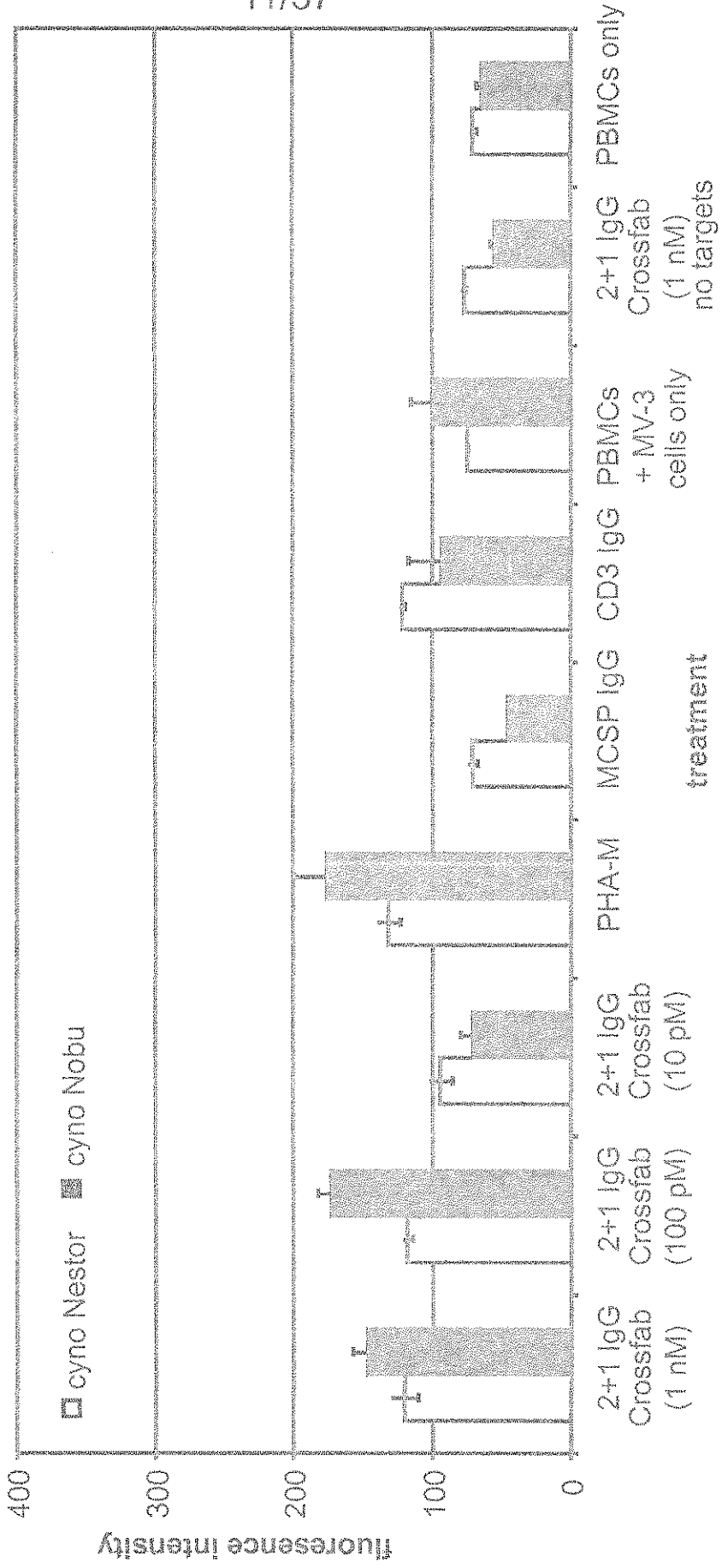

fluorescence intensity
400
300
200
100
0
cyno Nestor
cyno Nobu
2+1 IgG Crossfab (1 nM)
2+1 IgG Crossfab (100 pM)
2+1 IgG Crossfab (10 pM)
PHA-M
MCSP IgG
CD3 IgG
PBMCs + MV-3 cells only
2+1 IgG Crossfab (1 nM) no targets
PBMCs only
treatment

Figure 9

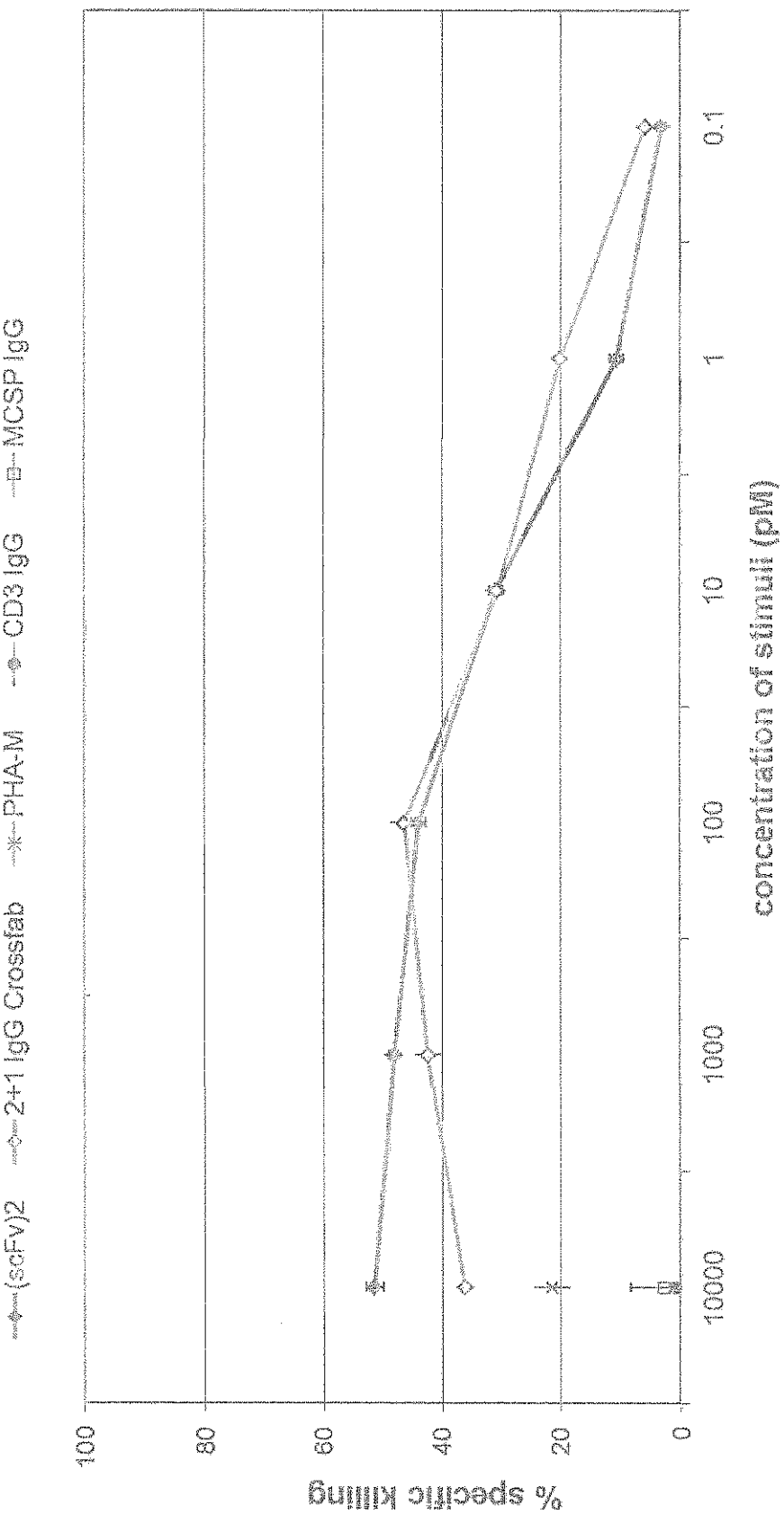

(scFv)2
2+1 IgG Crossfab
PHA-M
CD3 IgG
MCSP IgG
% specific killing
100
80
60
40
20
0
10000
1000
100
10
1
0.1
concentration of stimuli (pM)

Figure 10

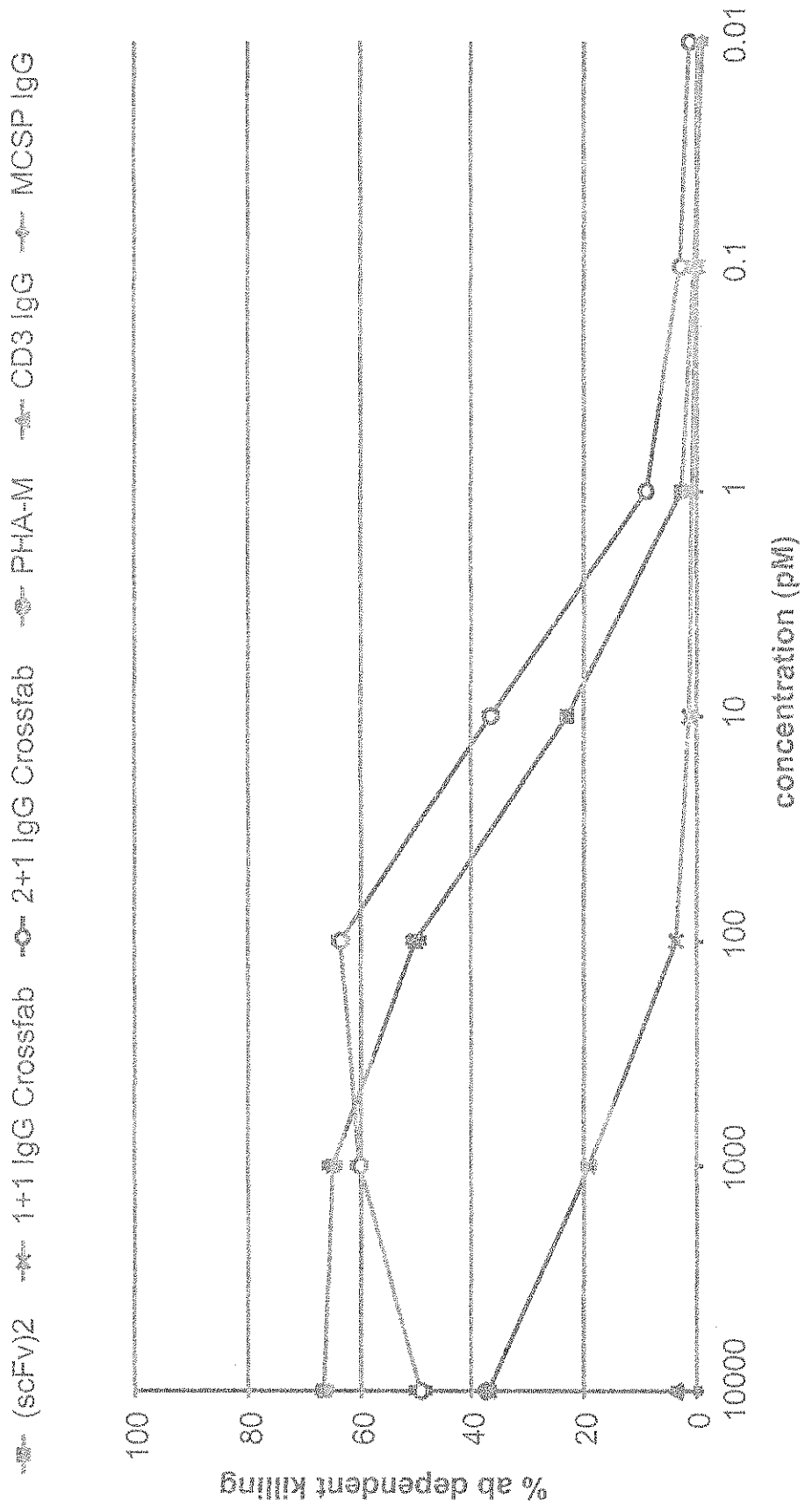

(scFv)2
1+1 IgG Crossfab
2+1 IgG Crossfab
PHA-M
CD3 IgG
MCSP IgG
% ab dependent killing
100
80
60
40
20
0
10000
1000
100
10
1
0.1
0.01
concentration (pM)

Figure 14

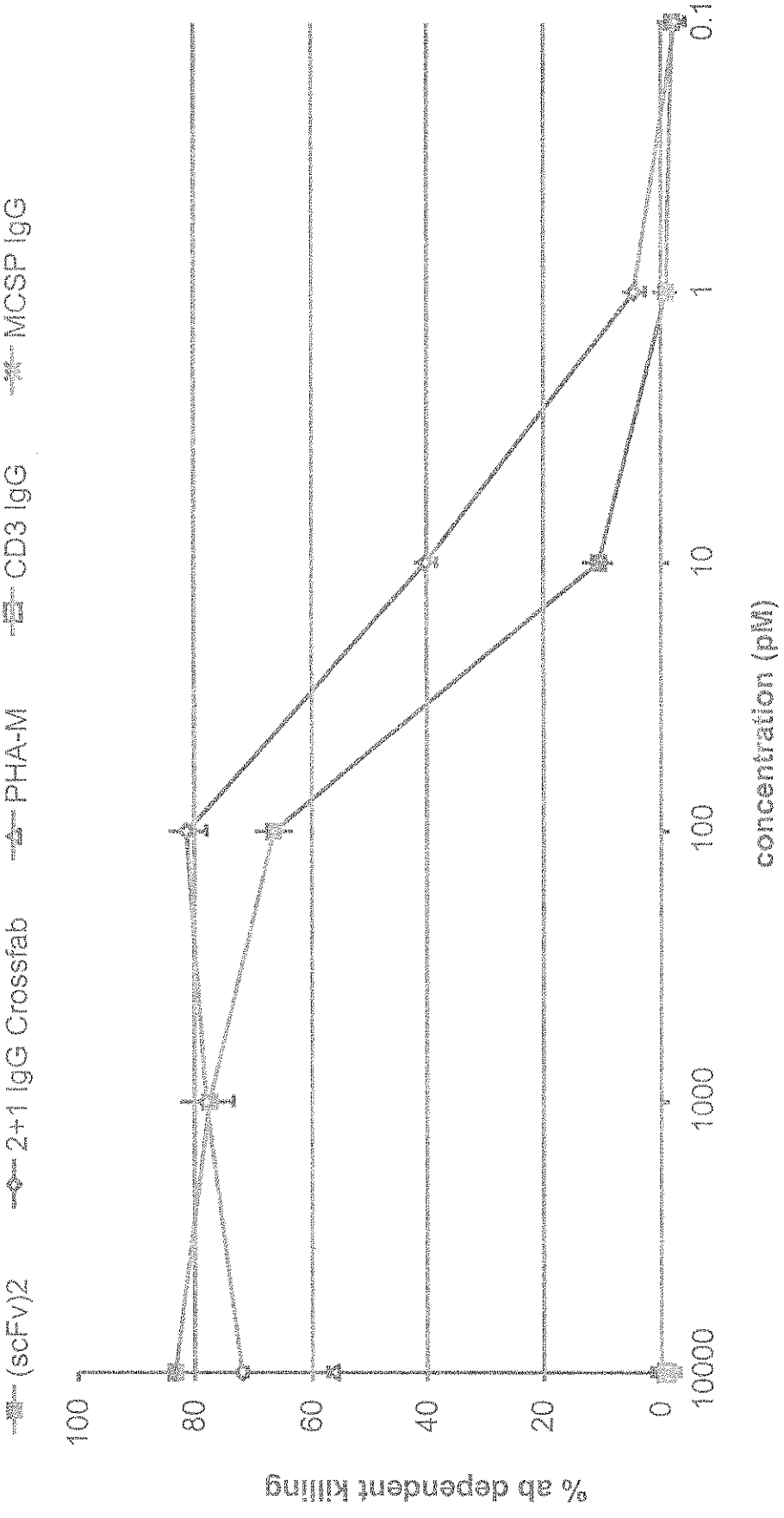

(scFv)2
2+1 IgG Crossfab
PHA-M
CD3 IgG
MCSP IgG
% ab dependent killing
100
80
60
40
20
0
10000
1000
100
10
1
0.1
concentration (pM)

Figure 12

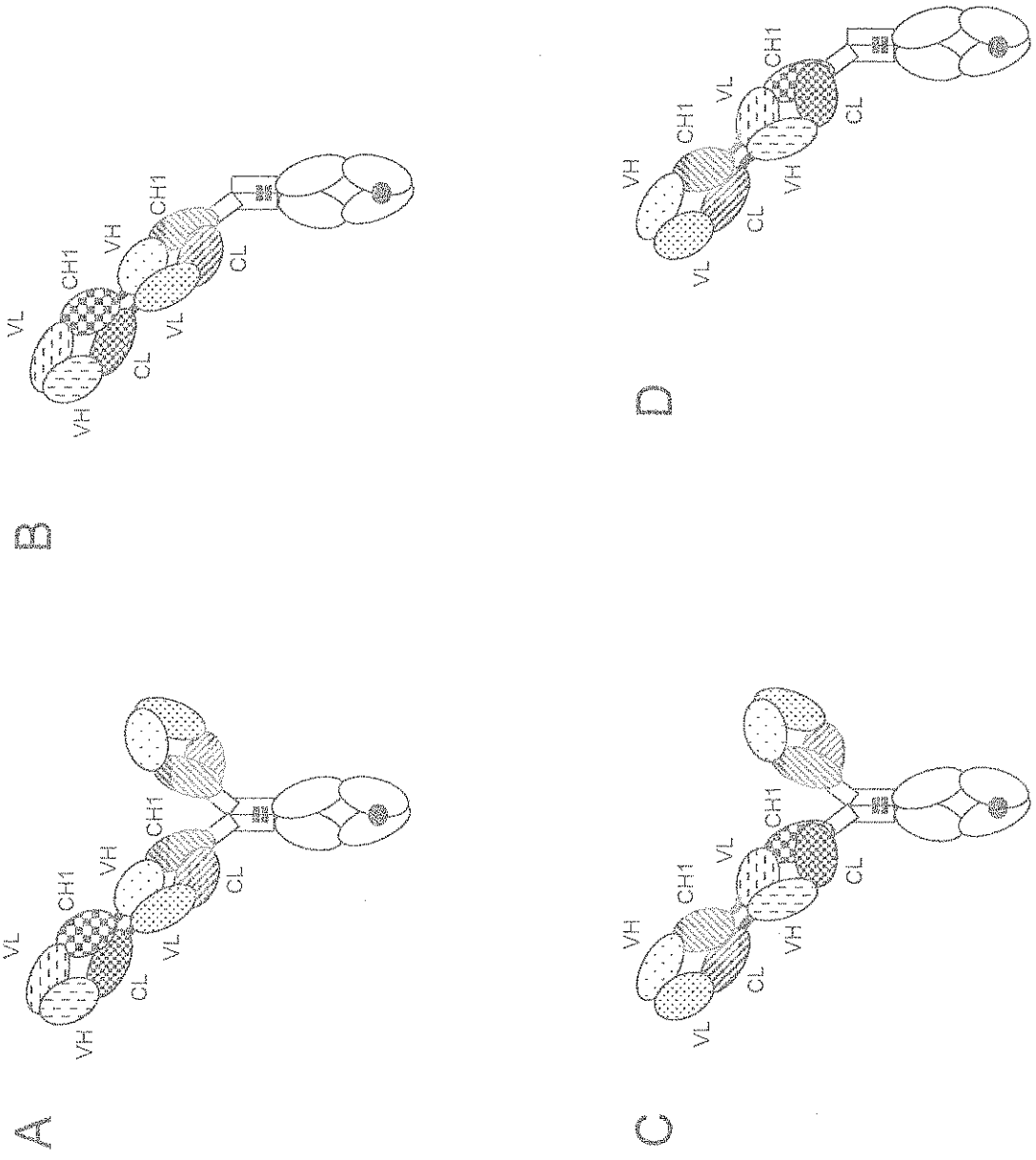

A
VL
CH1
VH
CH1
CL
VL
CL
VH
B
VL
CH1
VH
CH1
CL
VL
CL
VH
C
VH
CH1
VL
CH1
CL
VH
CL
VL
D
VH
CH1
VL
CH1
CL
VH
CL
VL

Figure 13

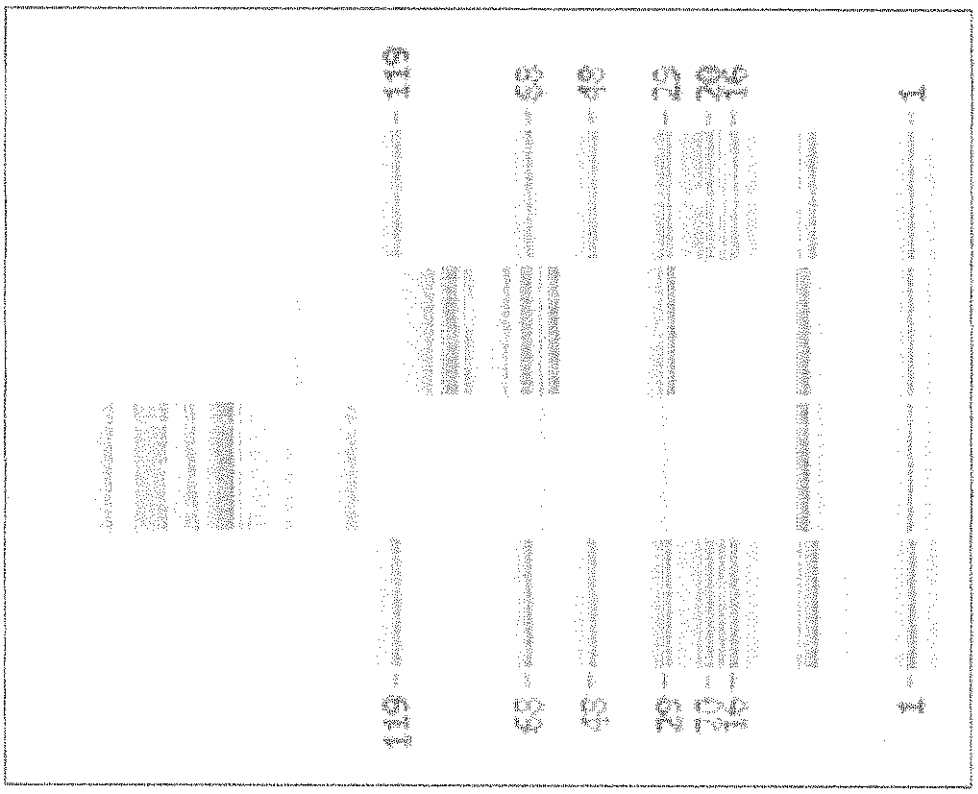
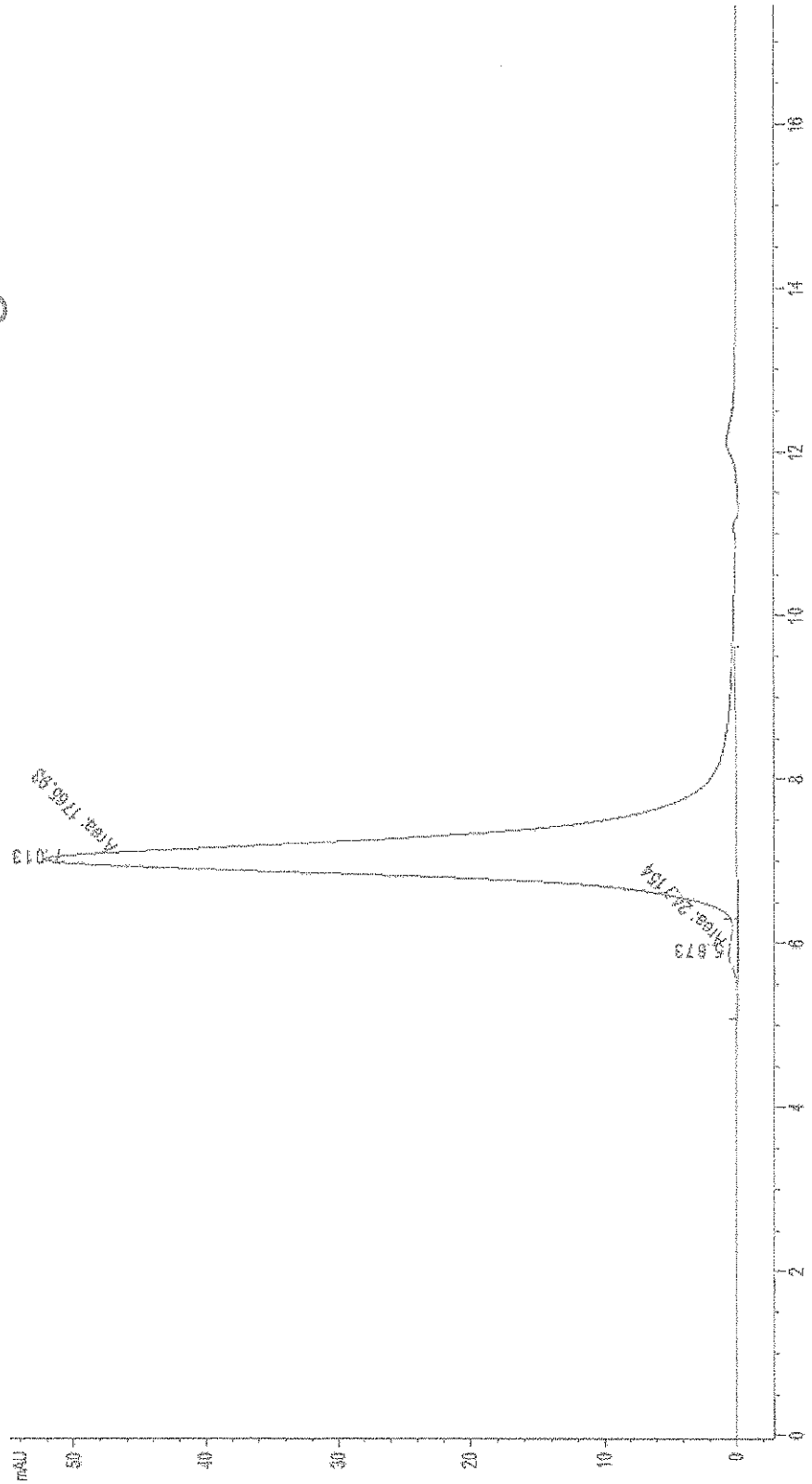


FIG 13A


mAU
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
5.013
Area: 1766.92
5.673
Area: 2237.154

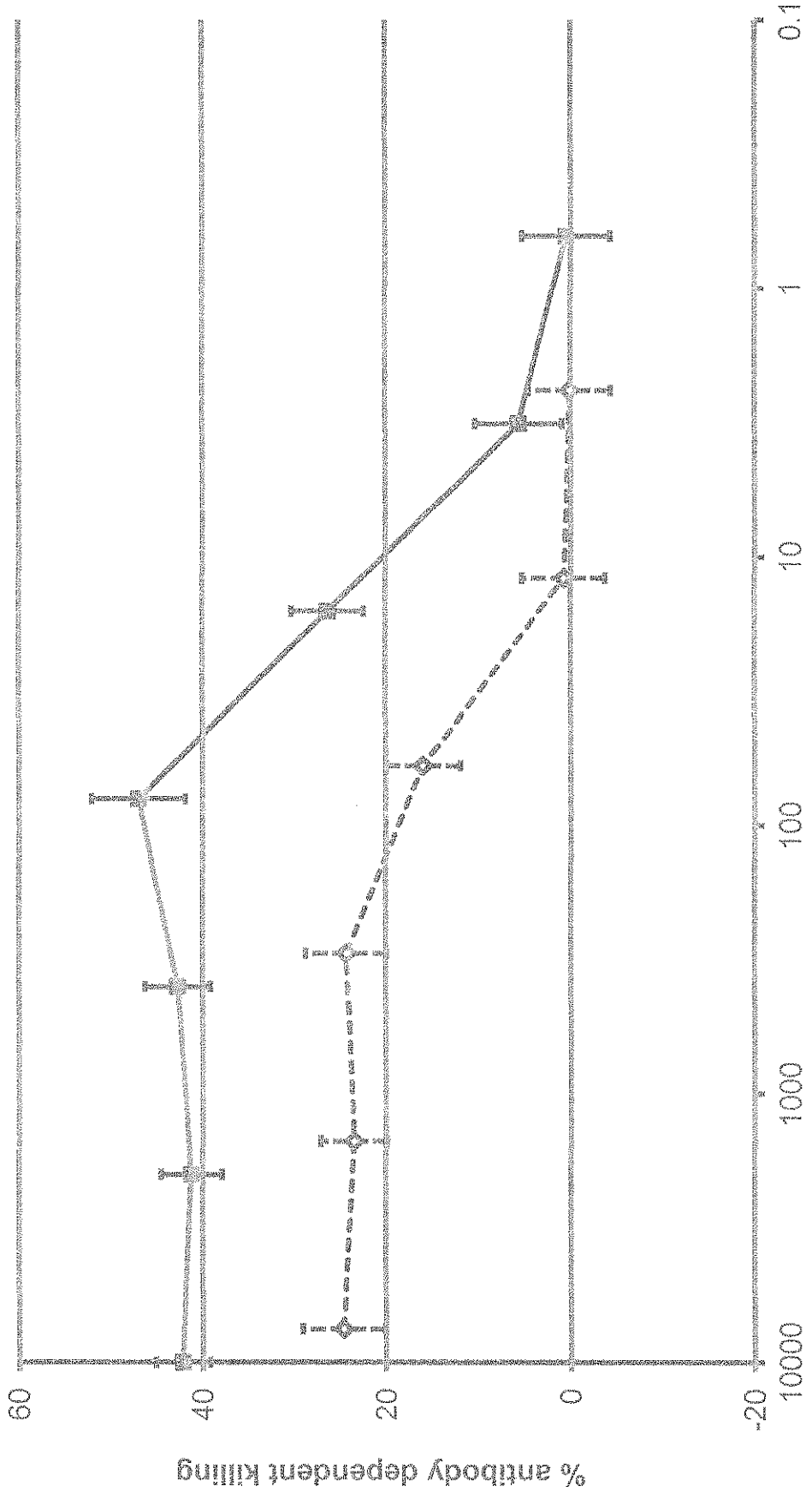

2+1 IgG Crossfab
2+1 IgG Crossfab, linked LC
% antibody dependent killing
60
40
20
0
-20
10000
1000
100
10
1
0.1
concentration (pM)

Figure 15

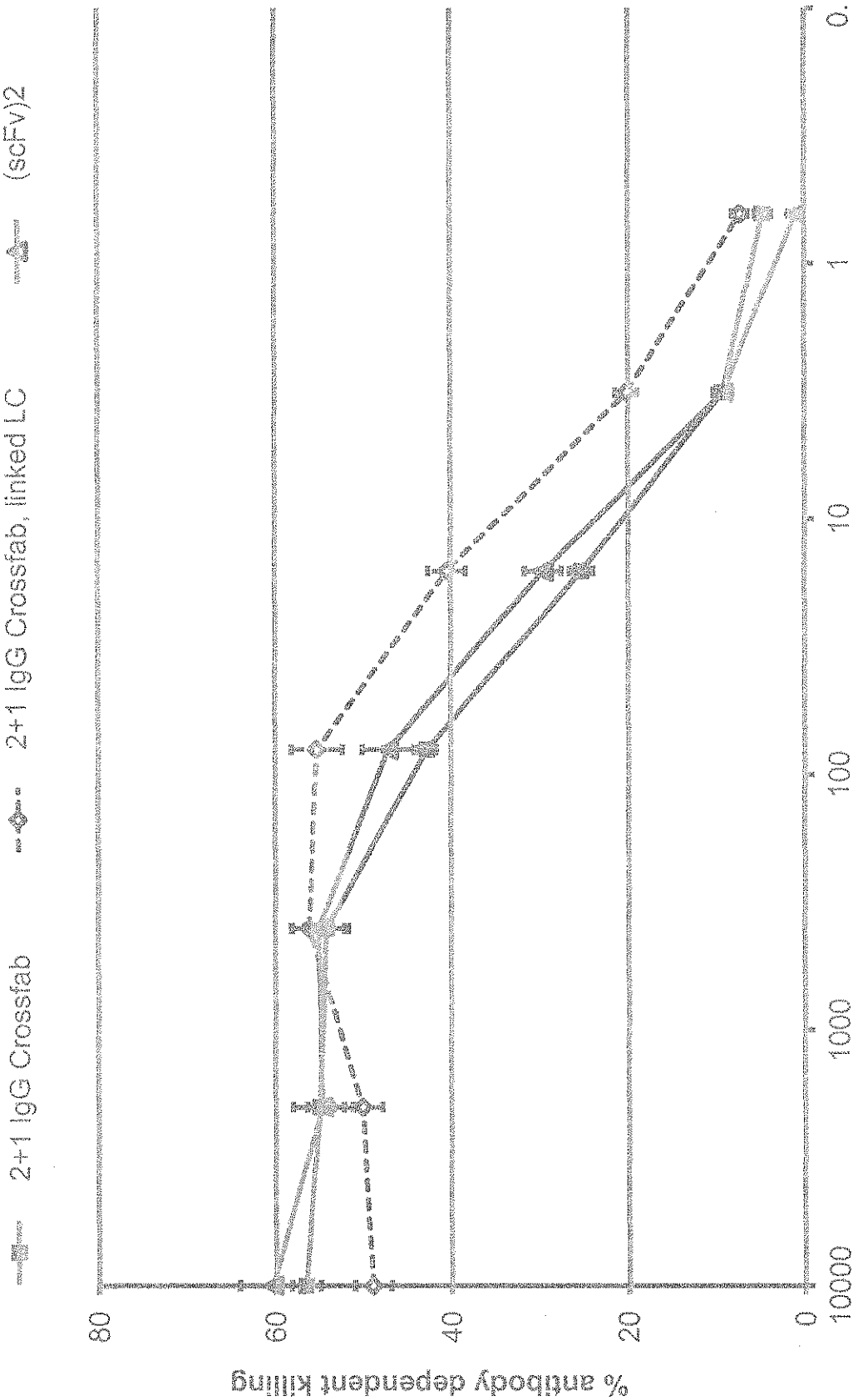

2+1 IgG Crossfab
2+1 IgG Crossfab, linked LC
(scFv)2
% antibody dependent killing
80
60
40
20
0
10000
1000
100
10
1
0.1
concentration (pM)

Figure 16

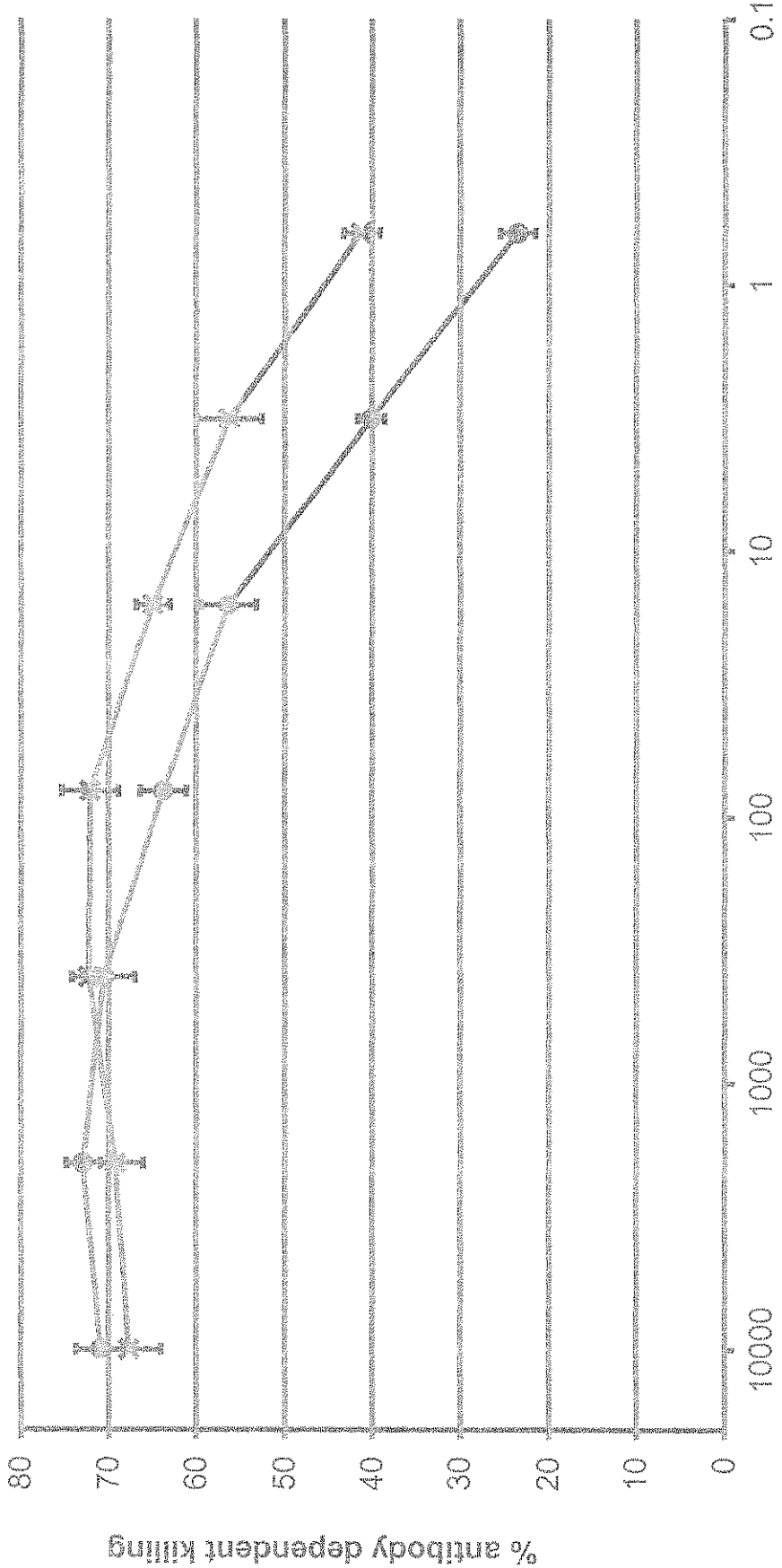

2+1 IgG Crossfab, linked LC
2+1 IgG Crossfab
% antibody dependent killing
concentration (pM)
80
70
60
50
40
30
20
10
0
10000
1000
100
10
1
0.1

Figure 17

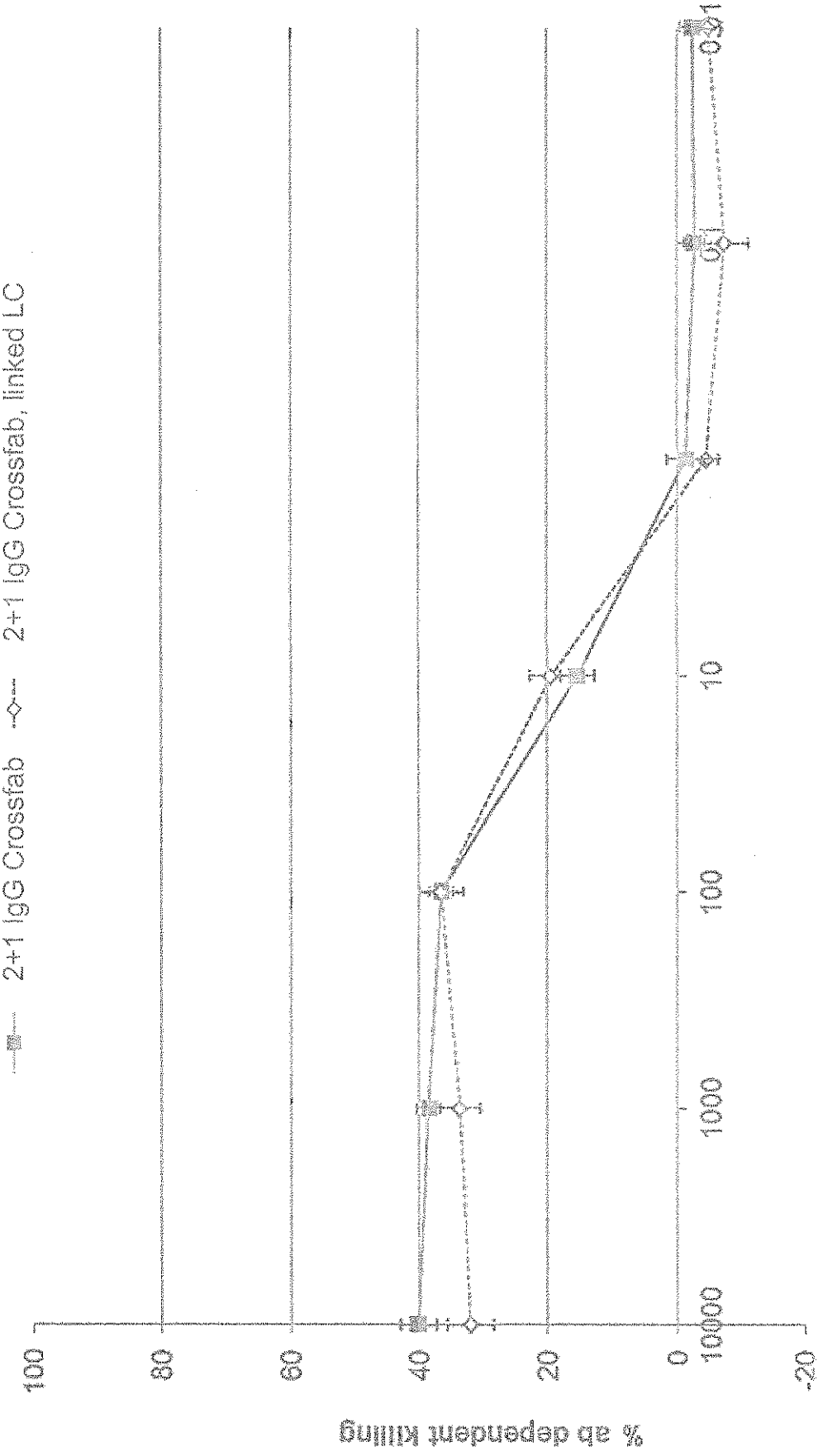

% ab dependent killing
100
80
60
40
20
0
-20
0.1
1
10
100
1000
10000
final antibody concentration (pM)
2+1 IgG Crossfab
2+1 IgG Crossfab, linked LC

Figure 18

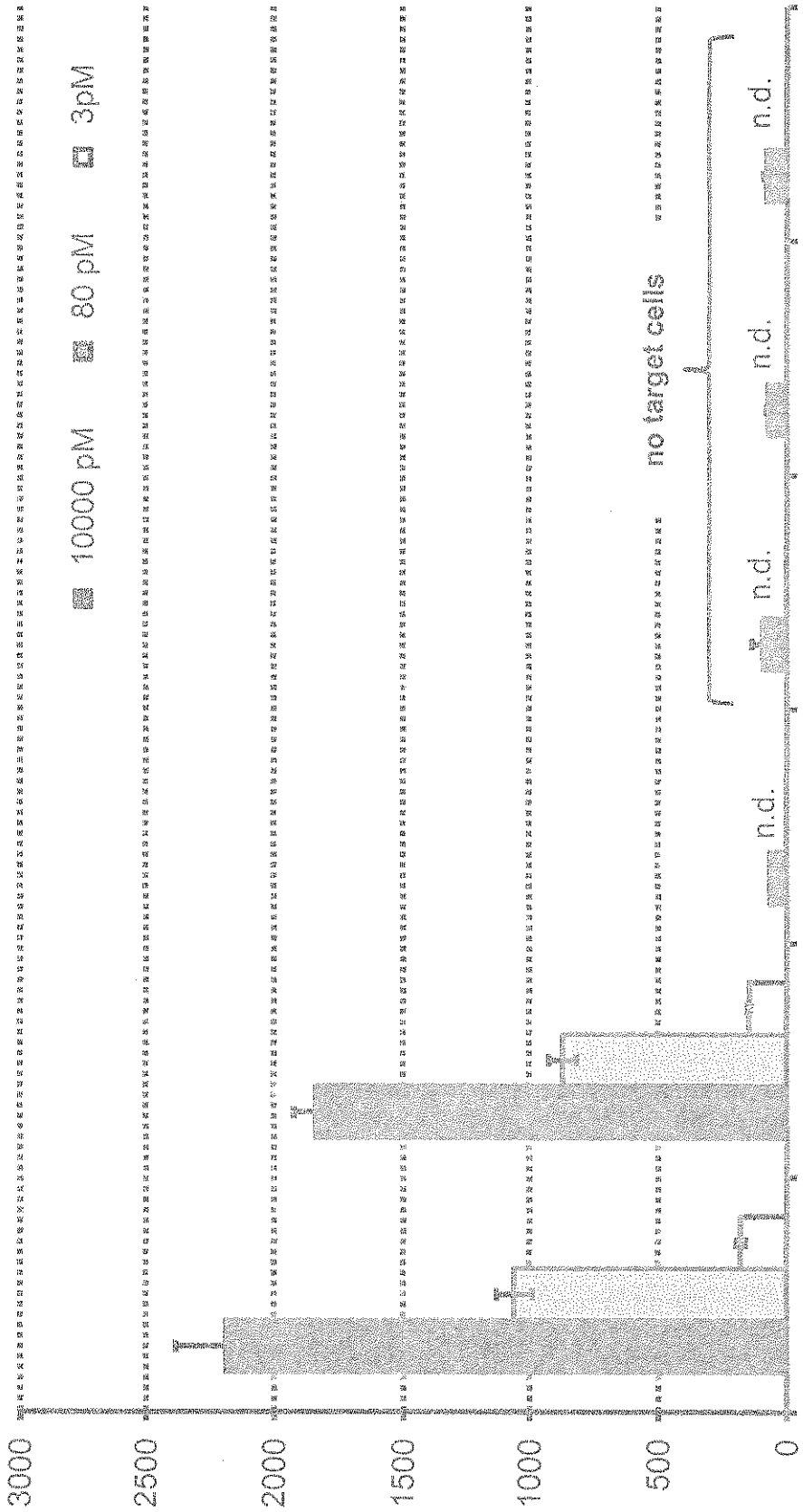

median fluorescence intensity [RU]
3000
2500
2000
1500
1000
500
0
10000 pM
80 pM
3pM
n.d.
n.d.
n.d.
n.d.
no target cells
2+1 CrossFab-IgG, linked LC
2+1 CrossFab-IgG
PBMC + targets
2+1 CrossFab-IgG, linked LC
2+1 CrossFab-IgG
PBMC

Figure 19A

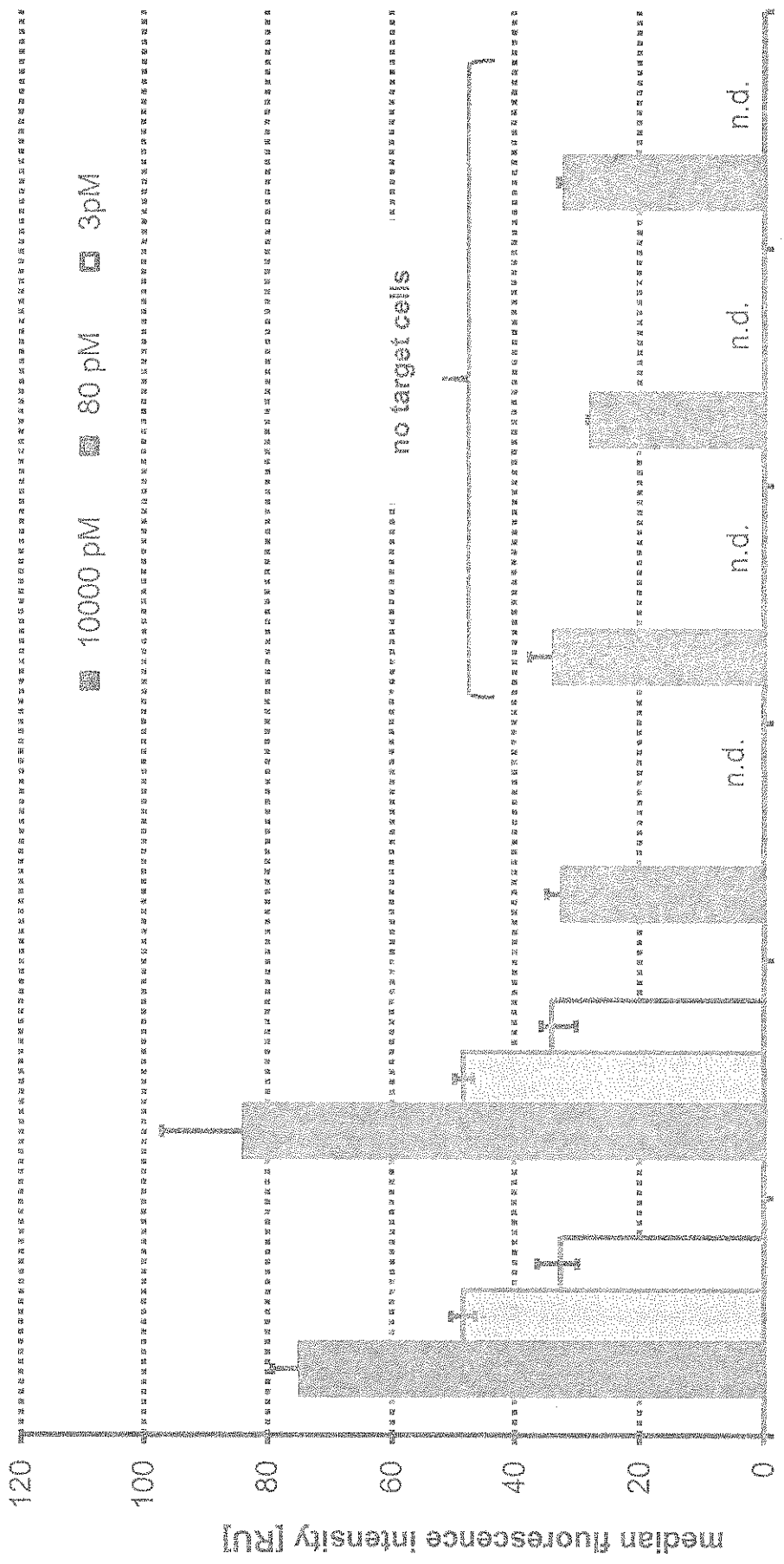

median fluorescence intensity [RU]
120
100
80
60
40
20
0
10000 pM
80 pM
3pM
no target cells
n.d.
2+1 CrossFab-IgG, linked LC
2+1 CrossFab-IgG
PBMC + targets
2+1 CrossFab-IgG, linked LC
2+1 CrossFab-IgG
PBMC

Figure 19B

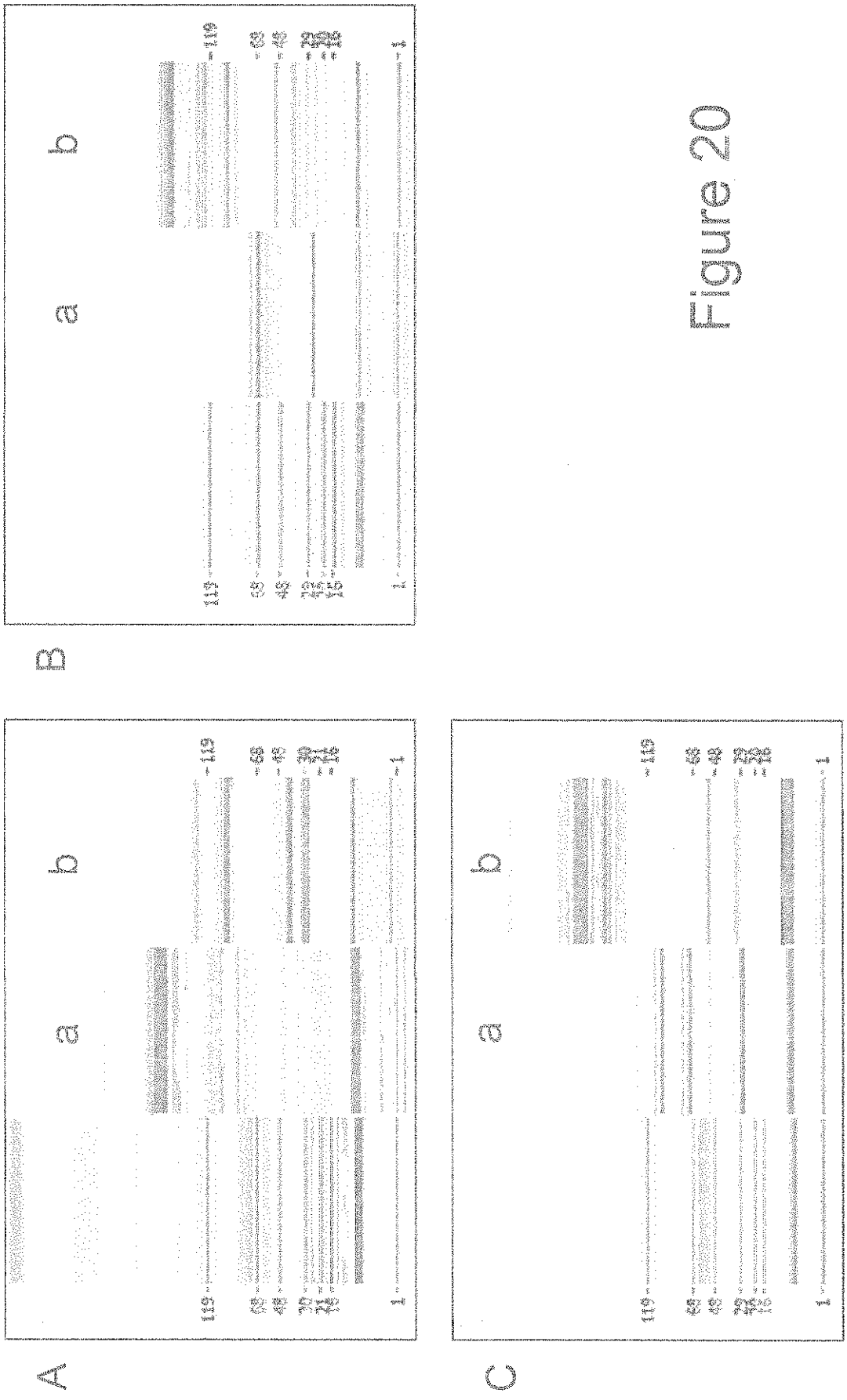


Figure 20

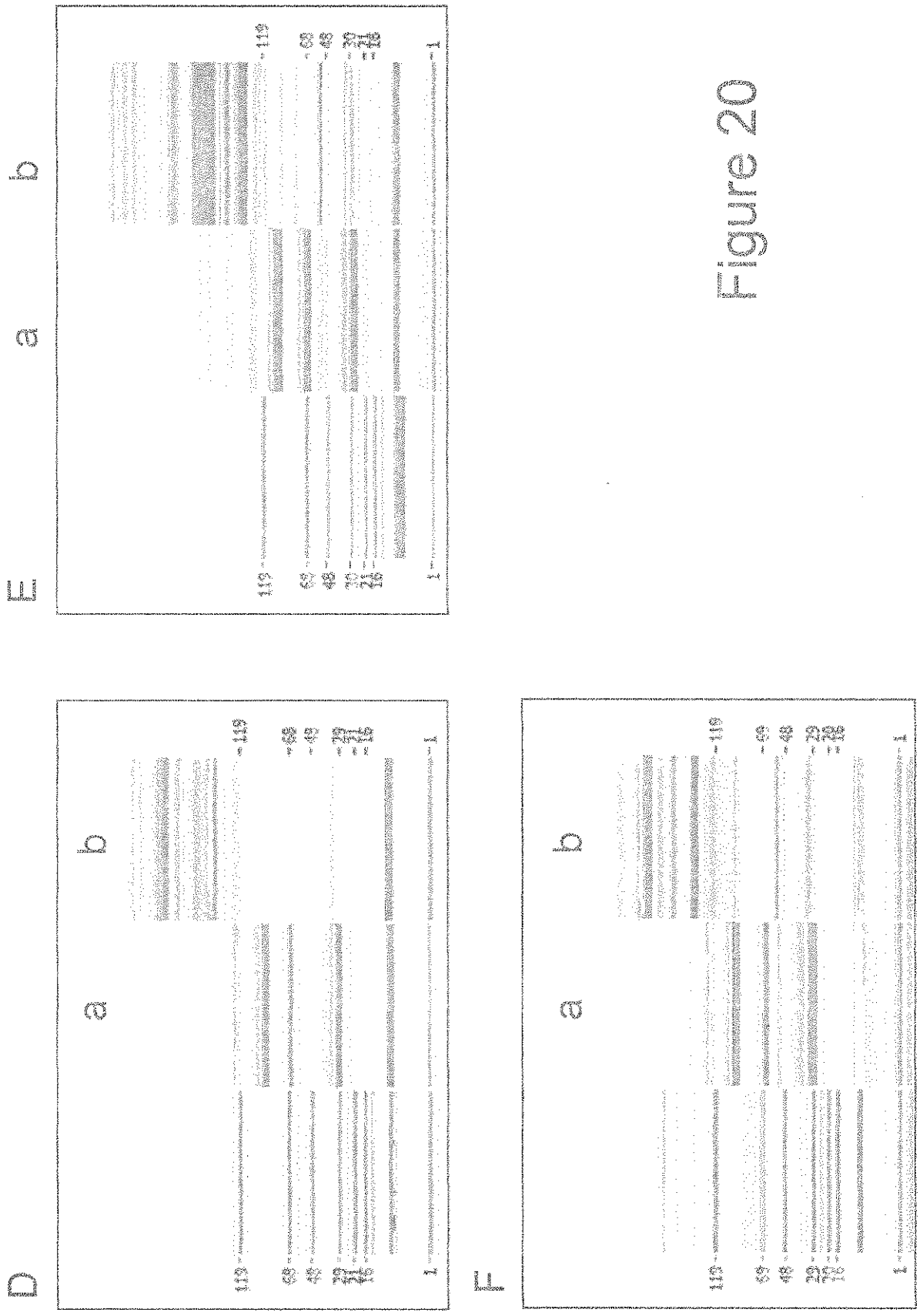


Figure 20

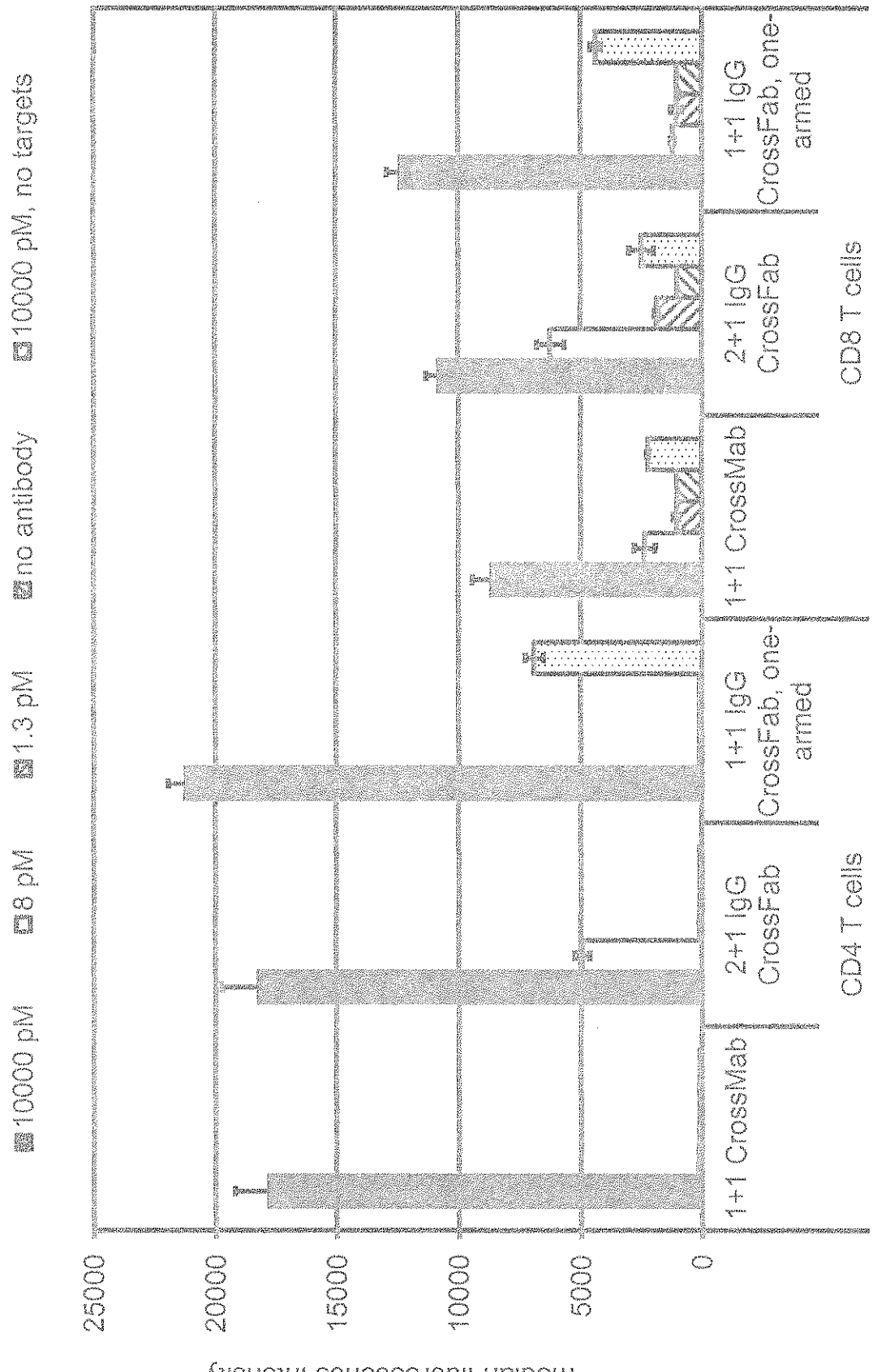

median fluorescence intensity
25000
20000
15000
10000
5000
0
10000 pM
8 pM
1.3 pM
no antibody
10000 pM, no targets
1+1 CrossMab
2+1 IgG CrossFab
1+1 IgG CrossFab, one-armed
1+1 CrossMab
2+1 IgG CrossFab
1+1 IgG CrossFab, one-armed
CD4 T cells
CD8 T cells

Figure 21A

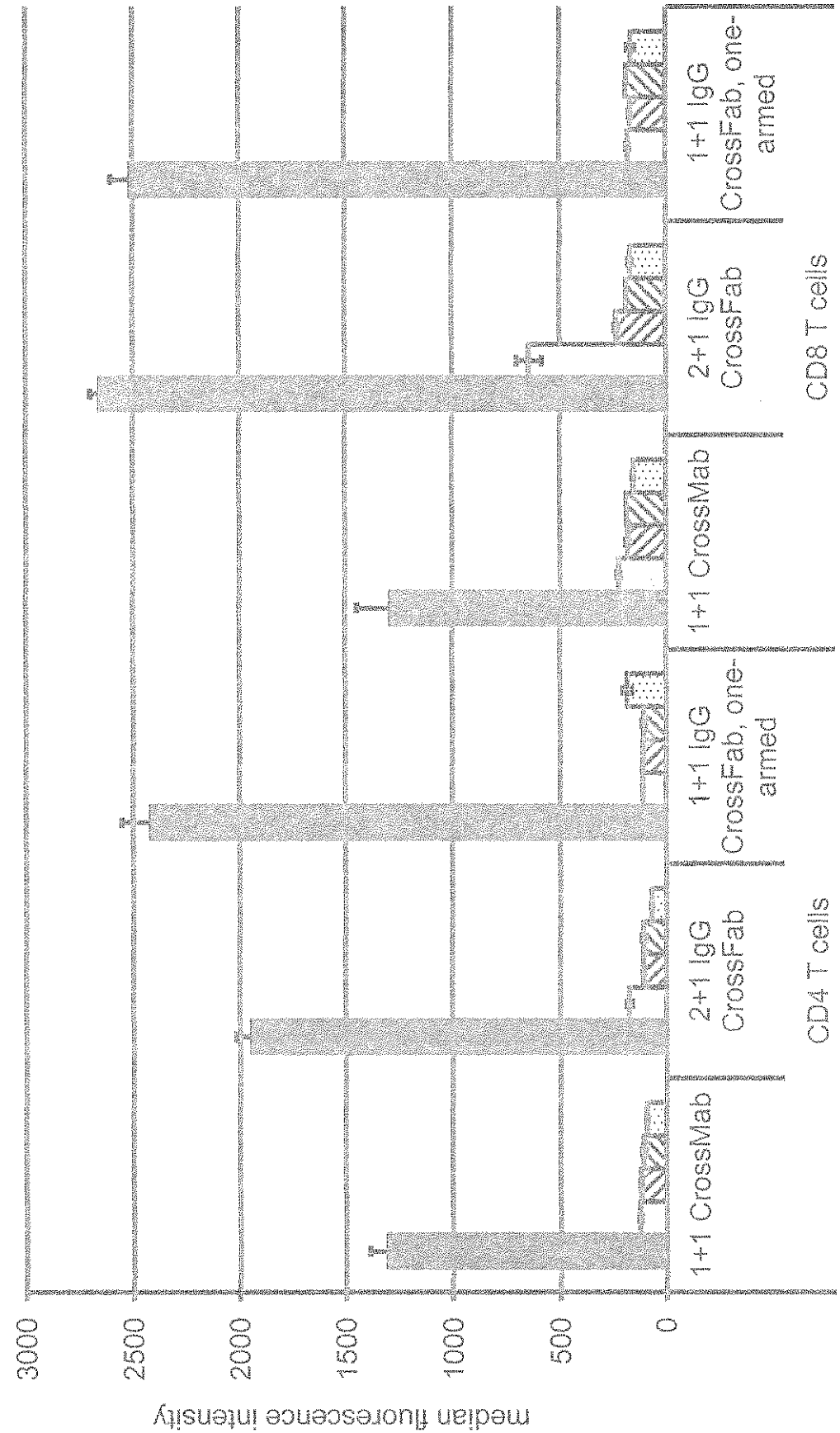

median fluorescence intensity
3000
2500
2000
1500
1000
500
0
10000 pM
8 pM
1.3 pM
no antibody
10000 pM, no targets
1+1 CrossMab
2+1 IgG CrossFab
1+1 IgG CrossFab, one-armed
1+1 CrossMab
2+1 IgG CrossFab
1+1 IgG CrossFab, one-armed
CD4 T cells
CD8 T cells

Figure 21B

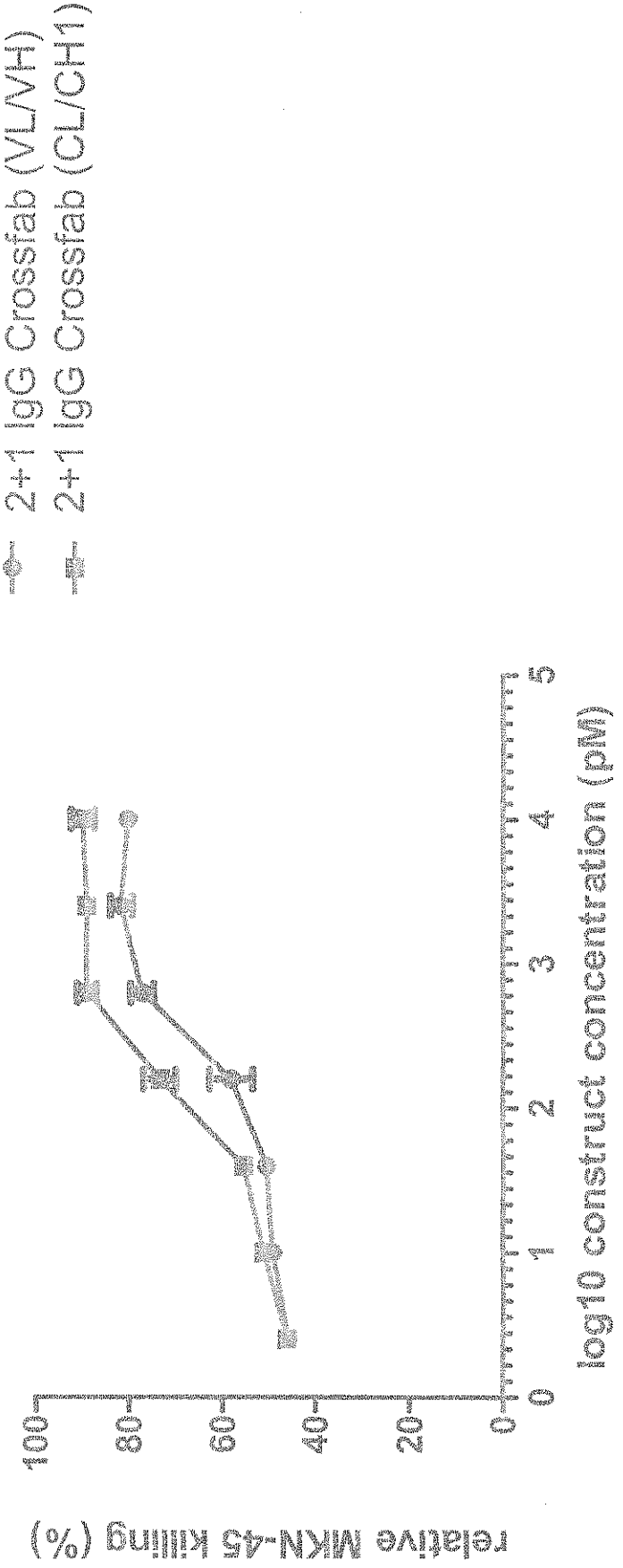

2+1 IgG Crossfab (VL/VH)
2+1 IgG Crossfab (CL/CH1)
relative MKN-45 killing (%)
100
80
60
40
20
0
log10 construct concentration (nM)
0
1
2
3
4
5

Figure 22A

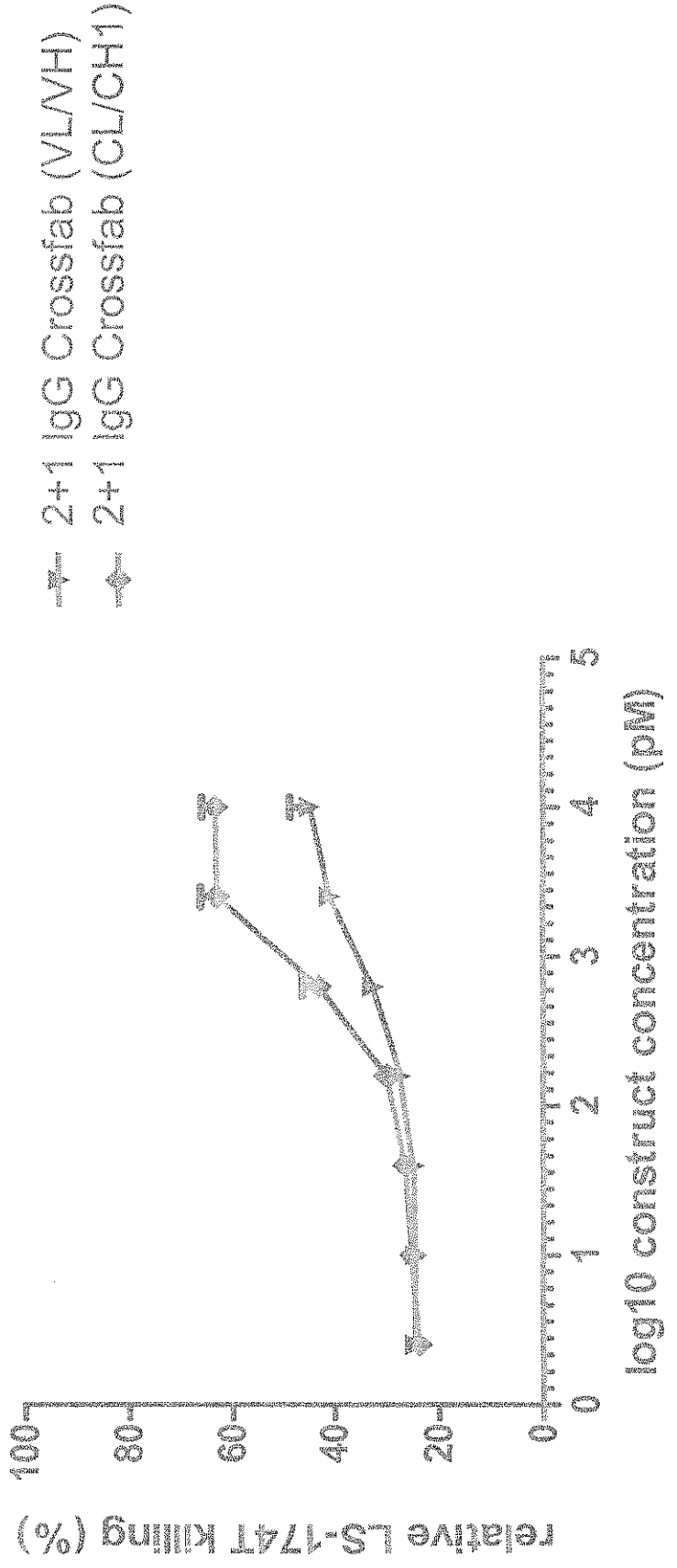

2+1 IgG Crossfab (VL/VH)
2+1 IgG Crossfab (CL/CH1)
relative LS-174T killing (%)
100
80
60
40
20
0
log10 construct concentration (pM)
5
4
3
2
1
0

Figure 22B

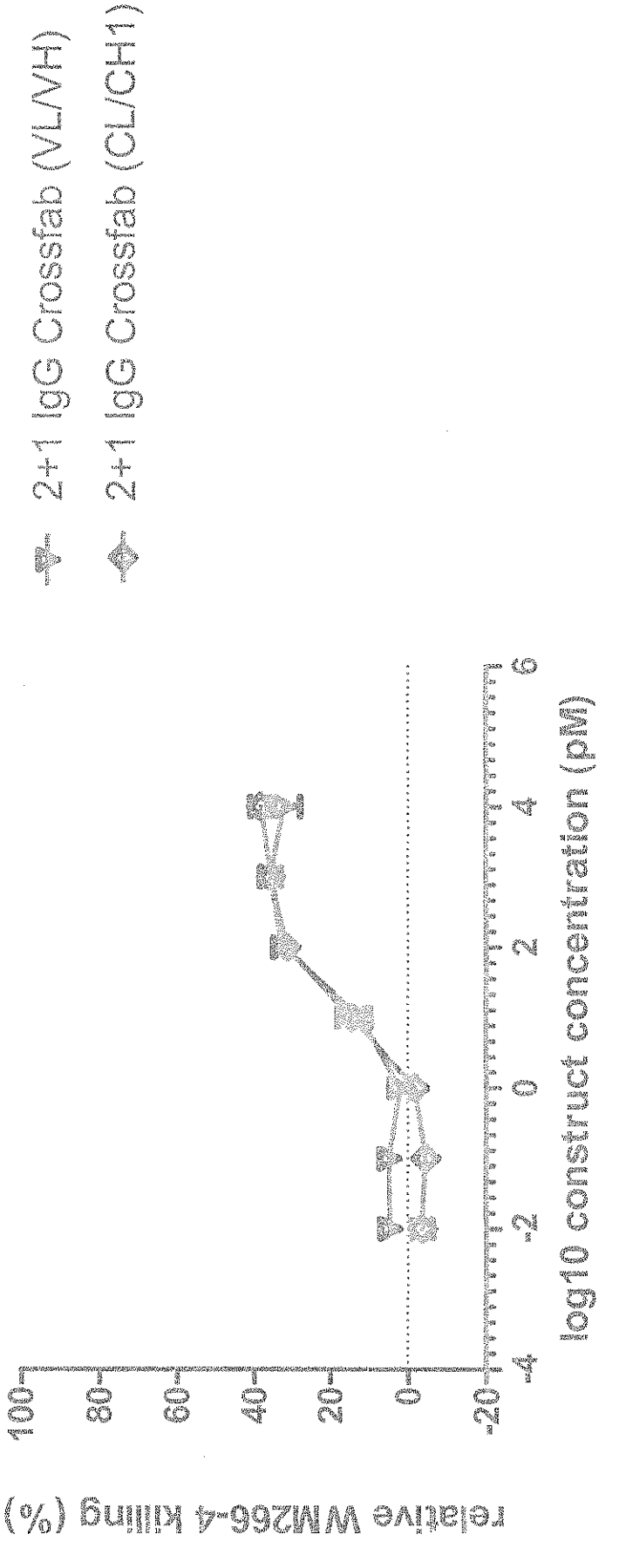

2+1 IgG Crossfab (VL/VH)
2+1 IgG Crossfab (CL/CH1)
relative WM266-4 killing (%)
100
80
60
40
20
0
-20
log10 construct concentration (pM)
-4
-2
0
2
4
6

Figure 23

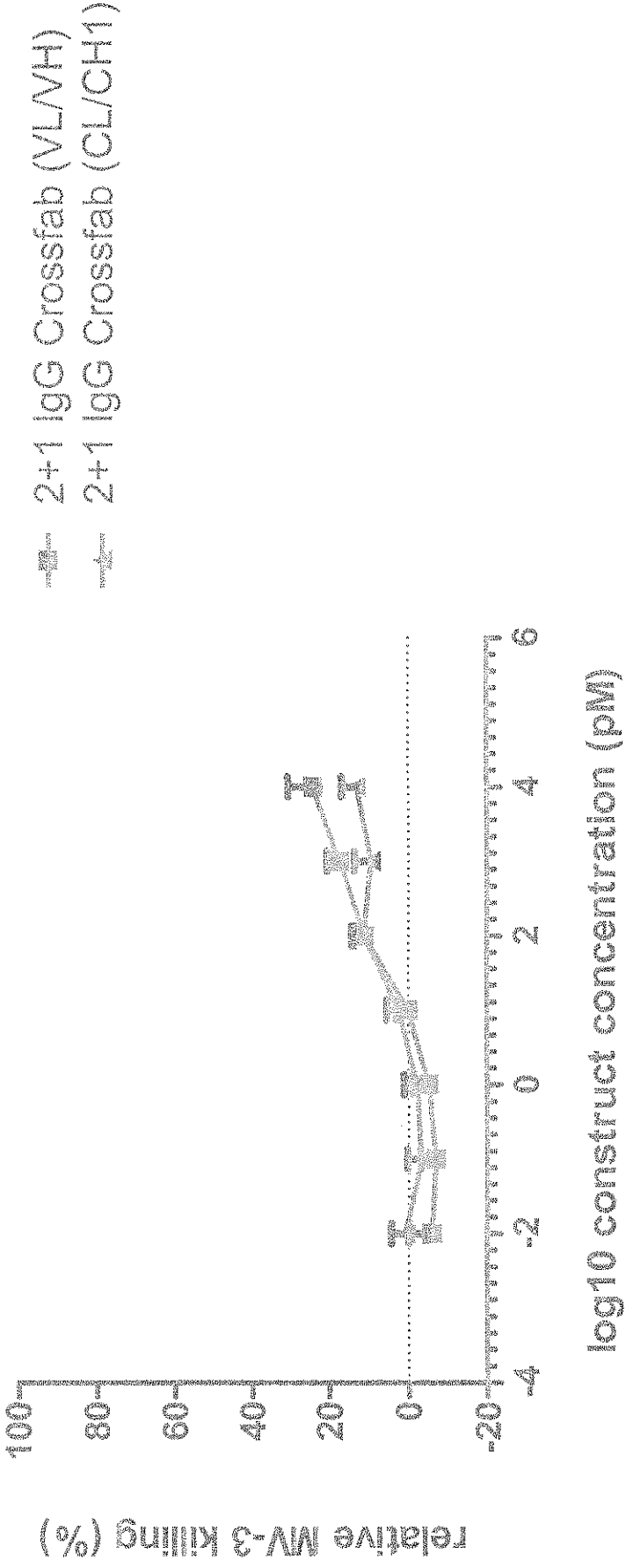

2+1 bg crossfab (VL/VH)
2+1 bg crossfab (CL/CH1)
relative MV-3 killing (%)
100
80
60
40
20
0
-20
-4
-2
0
2
4
6
log10 construct concentration (nM)

Fig. 24

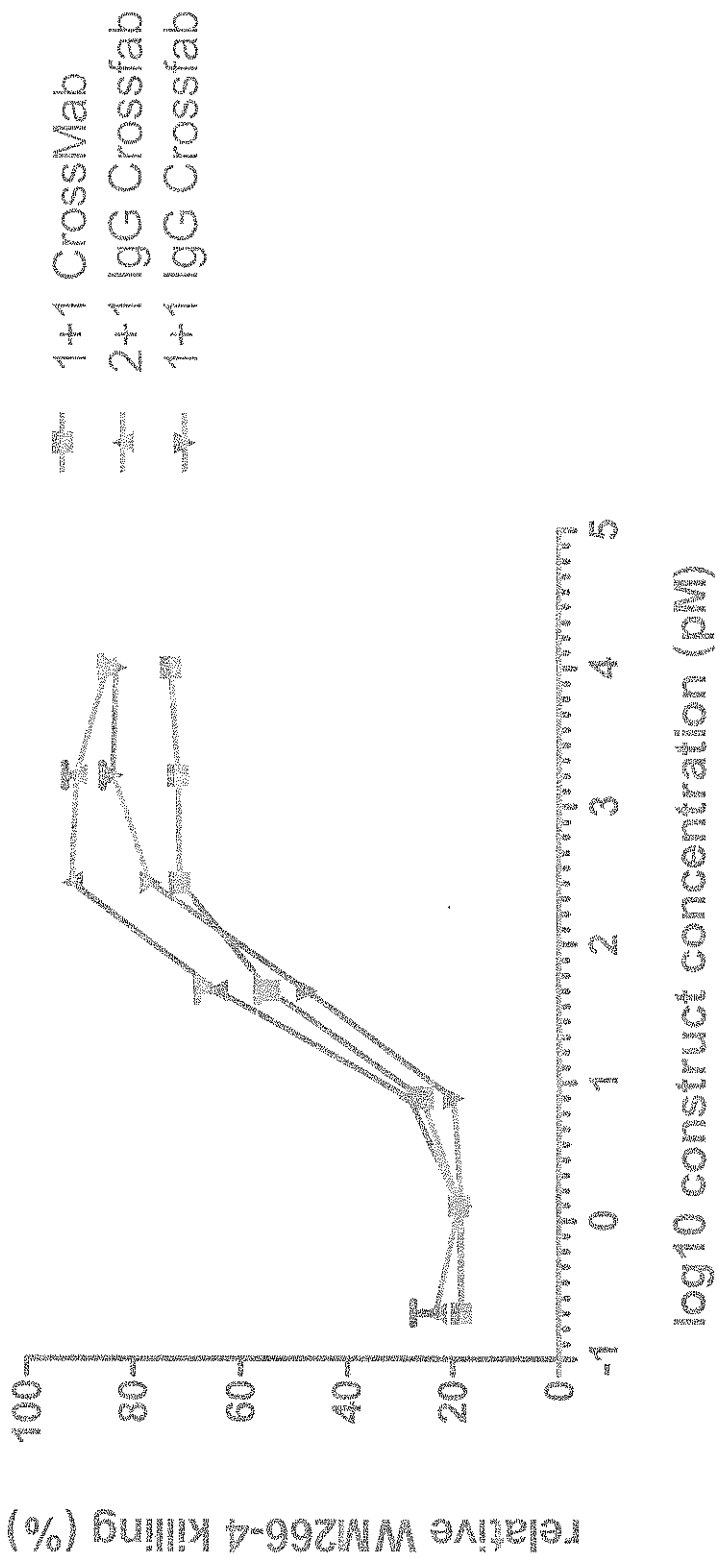

1+1 CrossMab
2+1 IgG Crossfab
1+1 IgG Crossfab
relative WM266-4 killing (%)
100
80
60
40
20
0
-1
0
1
2
3
4
5
log10 construct concentration (pM)

Figure 25A

1+1 CrossMab
2+1 IgG Crossfab
1+1 IgG Crossfab
relative MV-3 killing (%)
100
80
60
40
20
0
-4
-3
-2
-1
0
1
2
3
4
5
log10 construct concentration (pM)

Figure 25B

anti-CD3 IgG
2ndary antibody only
cells only
2+1 IgG Crossfab (CL/CH1)
2+1 IgG Crossfab (VL/VH)
median fluorescence intensity
1000
800
600
400
200
0
-1
0
1
2
3
4
5
Antibody concentration [nM]

Figure 29A

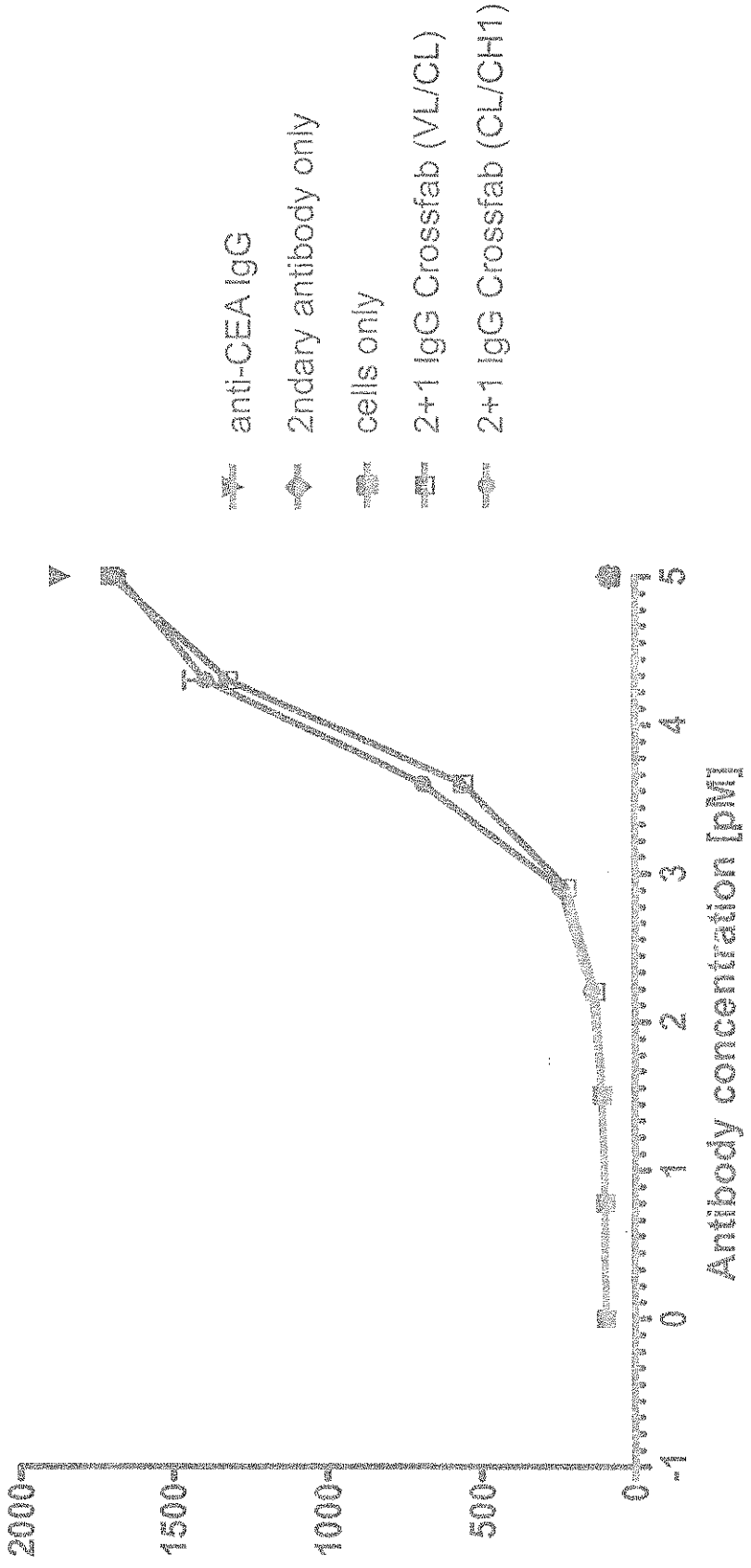

anti-CEA IgG
2ndary antibody only
cells only
2+1 IgG Crossfab (VL/CL)
2+1 IgG Crossfab (CL/CH1)
median fluorescence intensity
2500
1500
1000
500
0
-1
0
1
2
3
4
5
Antibody concentration [pM]

Figure 26B

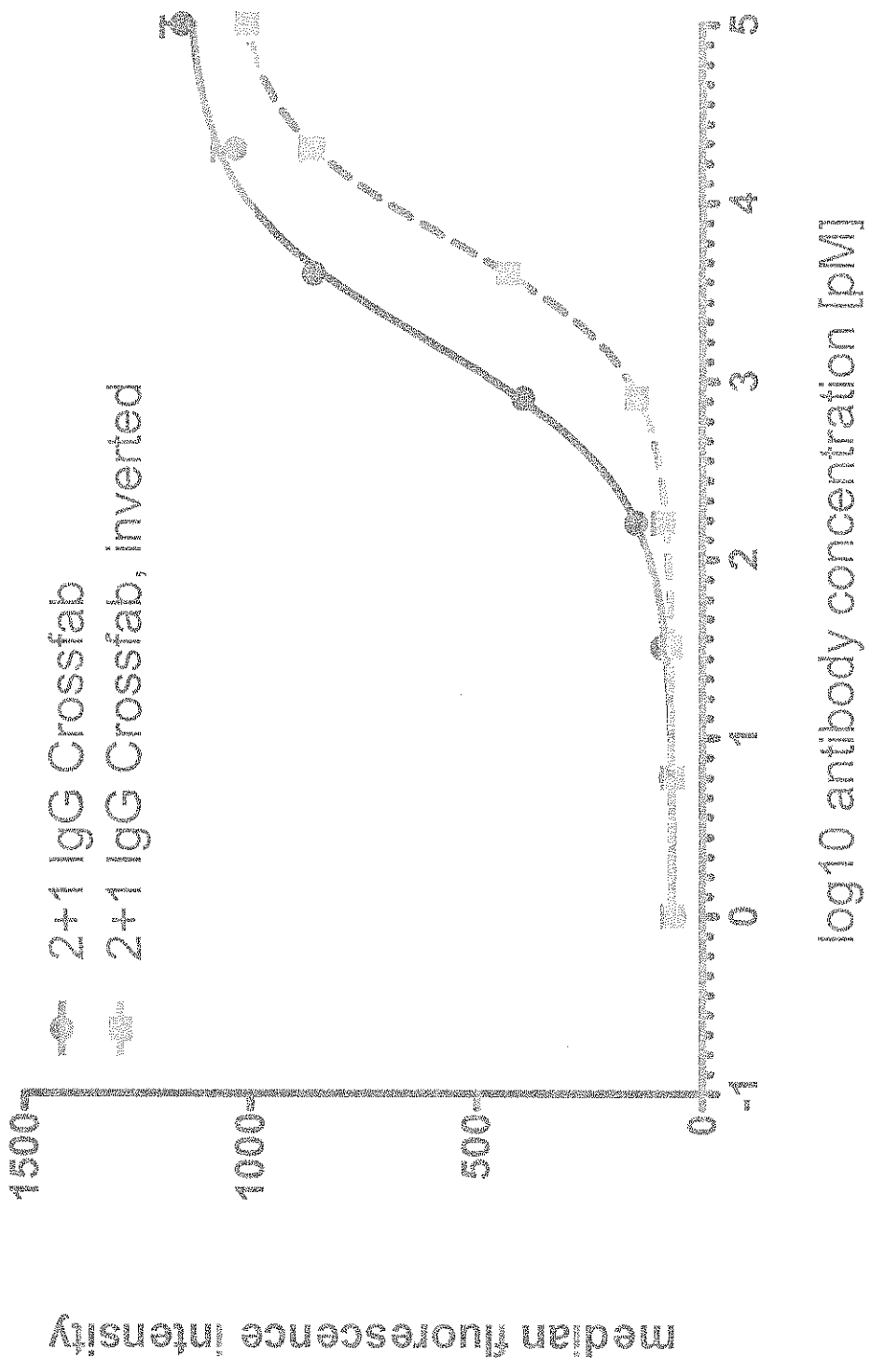

median fluorescence intensity
1500
1000
500
0
-1
0
1
2
3
4
5
log10 antibody concentration [pM]
2+1 IgG Crosstab
2+1 IgG Crosstab, inverted

Figure 27A

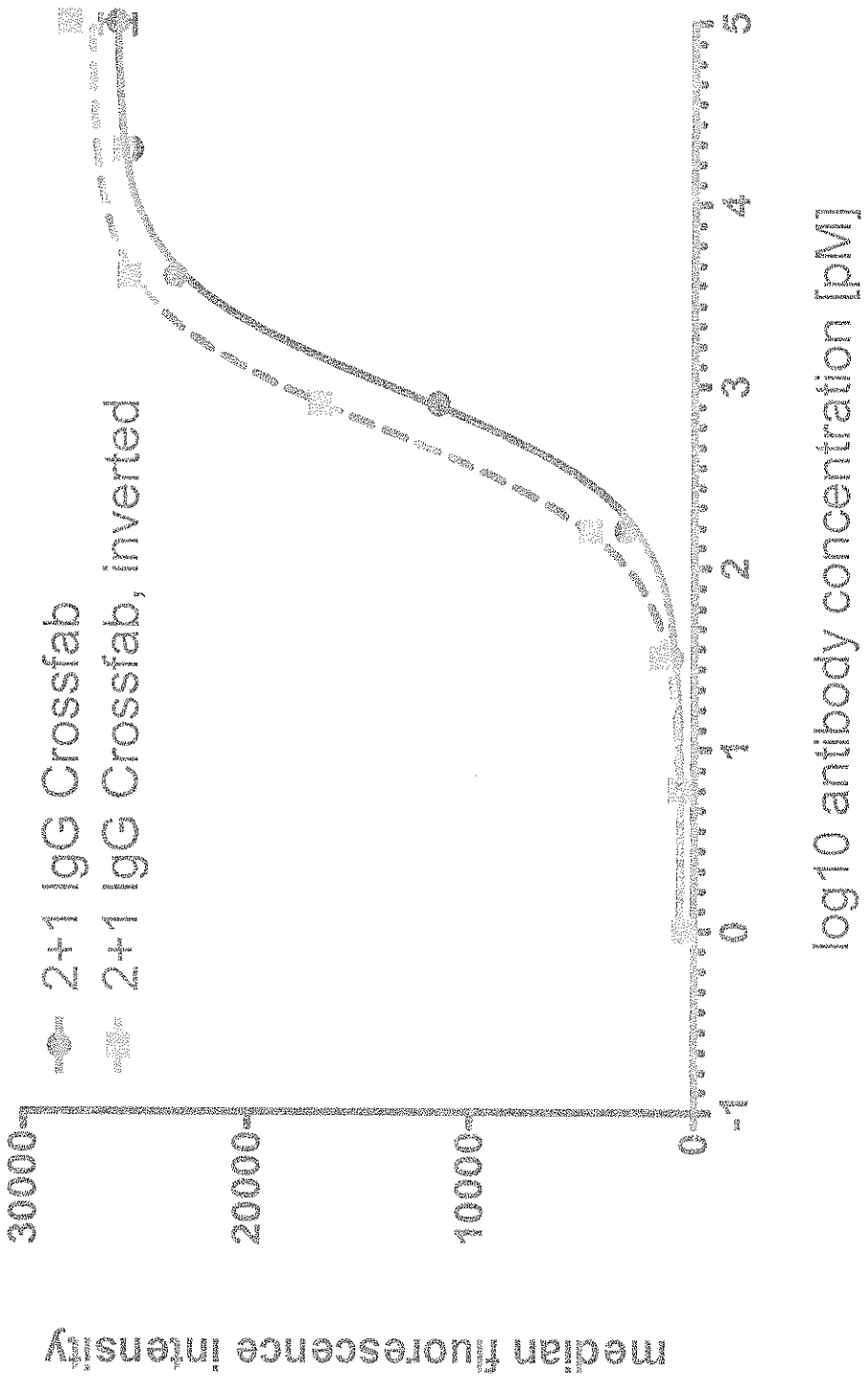

median fluorescence intensity
30000
20000
10000
0
-1
0
1
2
3
4
5
log10 antibody concentration [nM]
2+1 IgG Crossfab
2+1 IgG Crossfab, inverted

Figure 27B