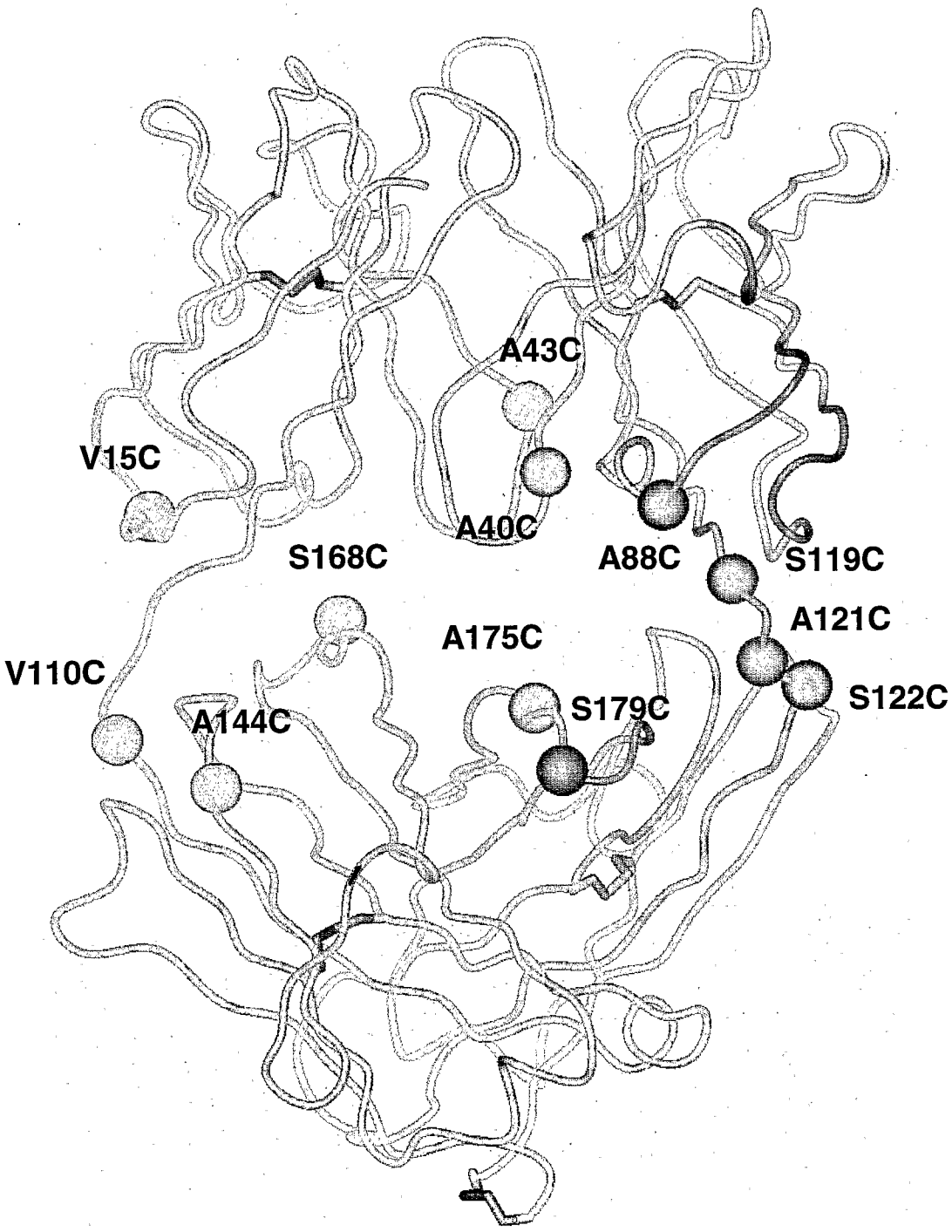

A43C
V15C
A40C
S168C
A88C
S119C
A121C
A175C
V110C
S179C
S122C
A144C

Figure 1A

Sequential Numbering

```
                    10         20         30
4d5v7fabH   EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHW
            **********************************
4d5v7fabH   EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHW
                    10         20         30
```

Kabat Numbering

```
              40         50         60         70         80
4d5v7fabH   VRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSL
            ************************************************
4d5v7fabH   VRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSL
              40         50  a      60         70         80  abc

              90        100        110        120        130
4d5v7fabH   RAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSK
            ************************************************
4d5v7fabH   RAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSK
                   90        100abc        110        120

             140        150        160        170        180
4d5v7fabH   STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS
            ************************************************
4d5v7fabH   STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS
          130        140        150        160        170

             190        200        210        220
4d5v7fabH   SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
            **********************************
4d5v7fabH   SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
          180        190        200        210
```

Figure 1B

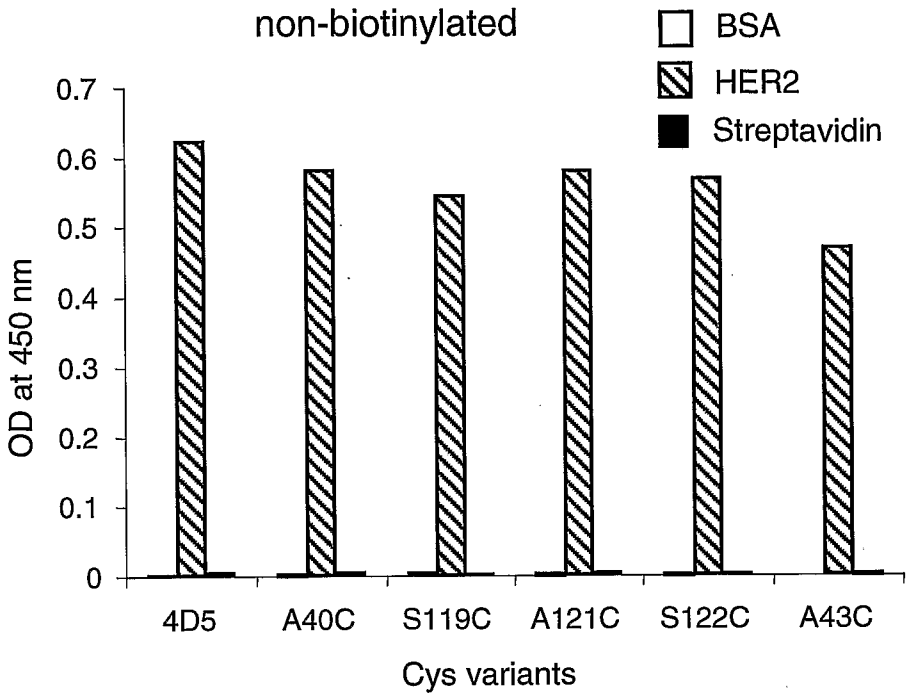


Figure 2A

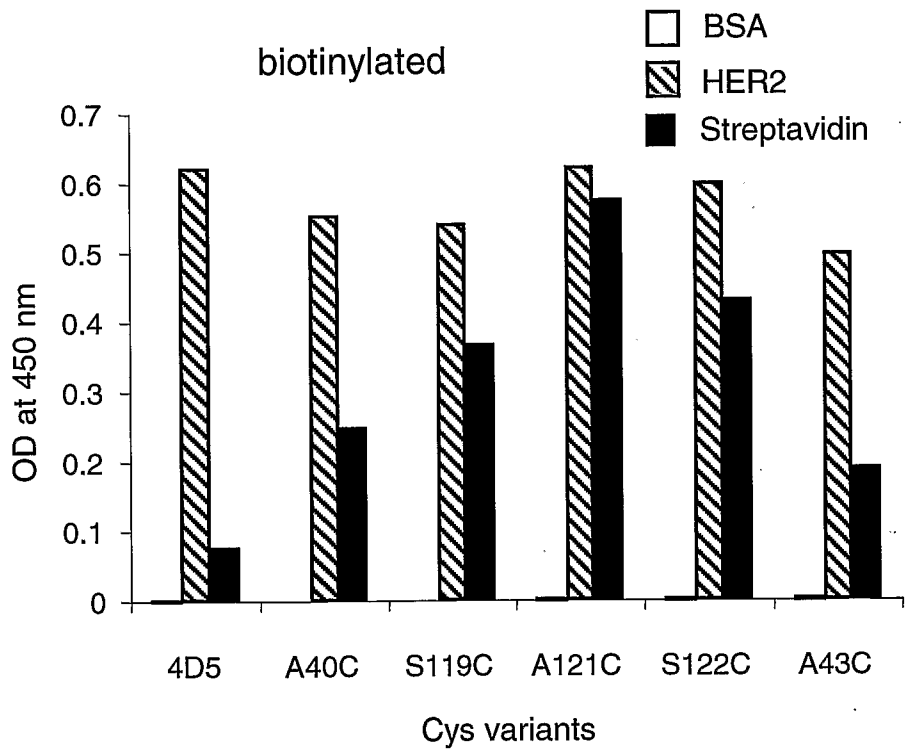


Figure 2B

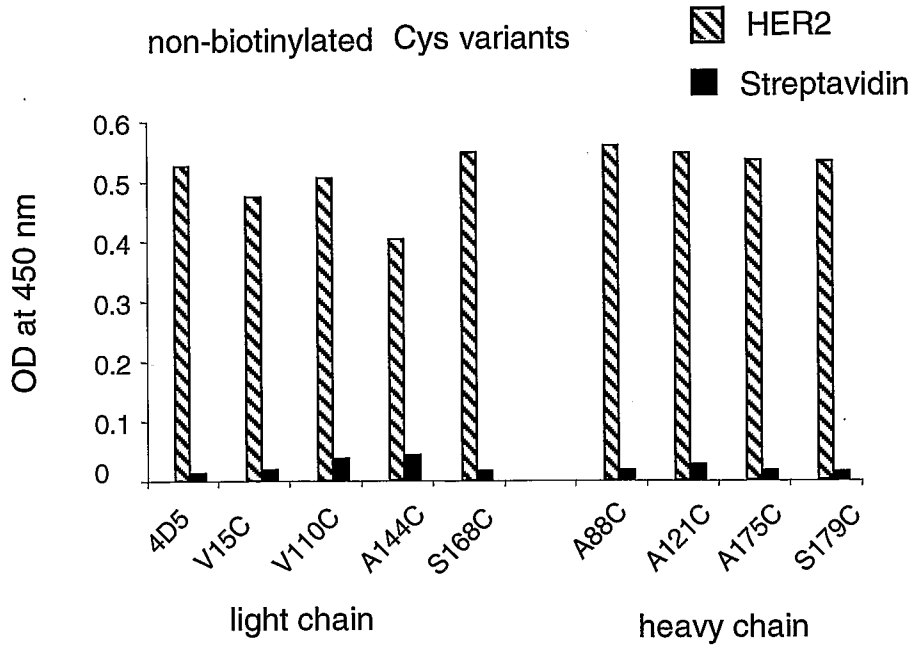


Figure 3A

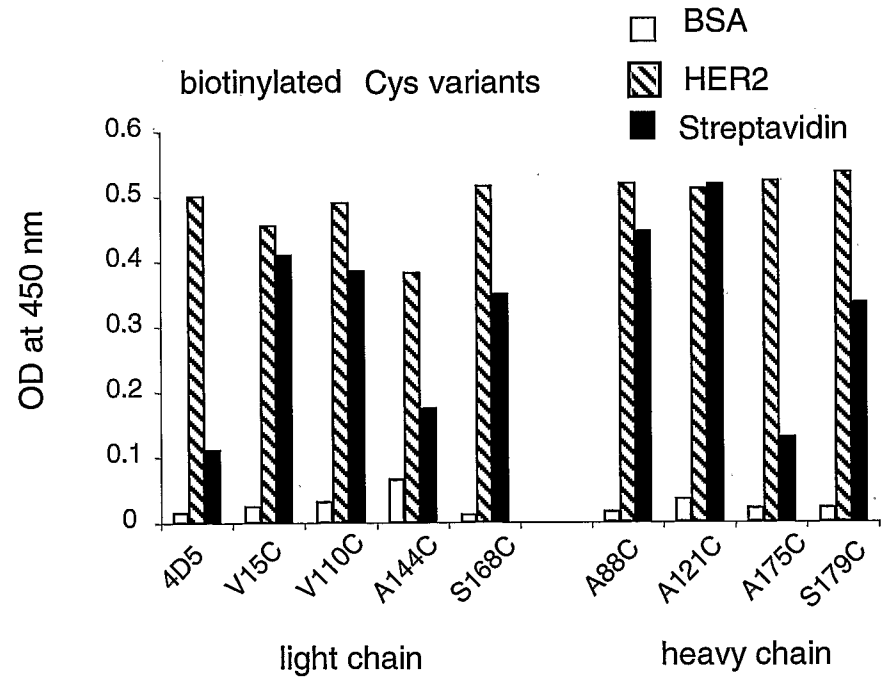


Figure 3B

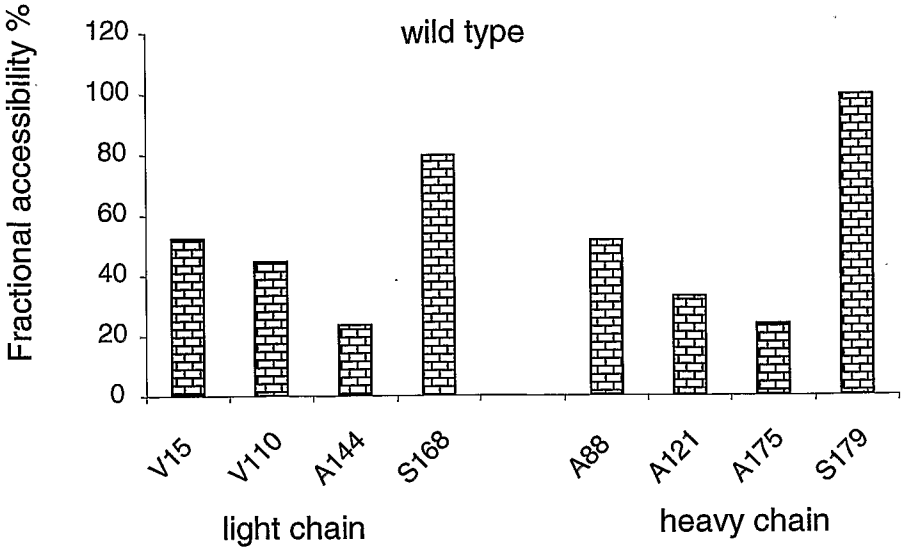


Figure 4A

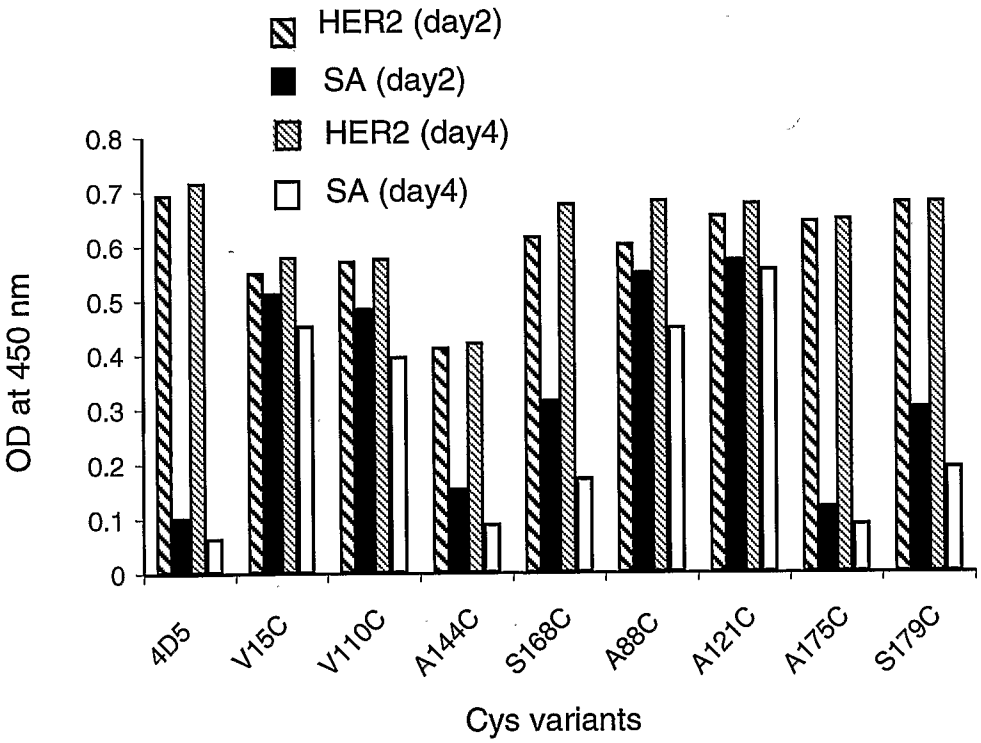


Figure 4B

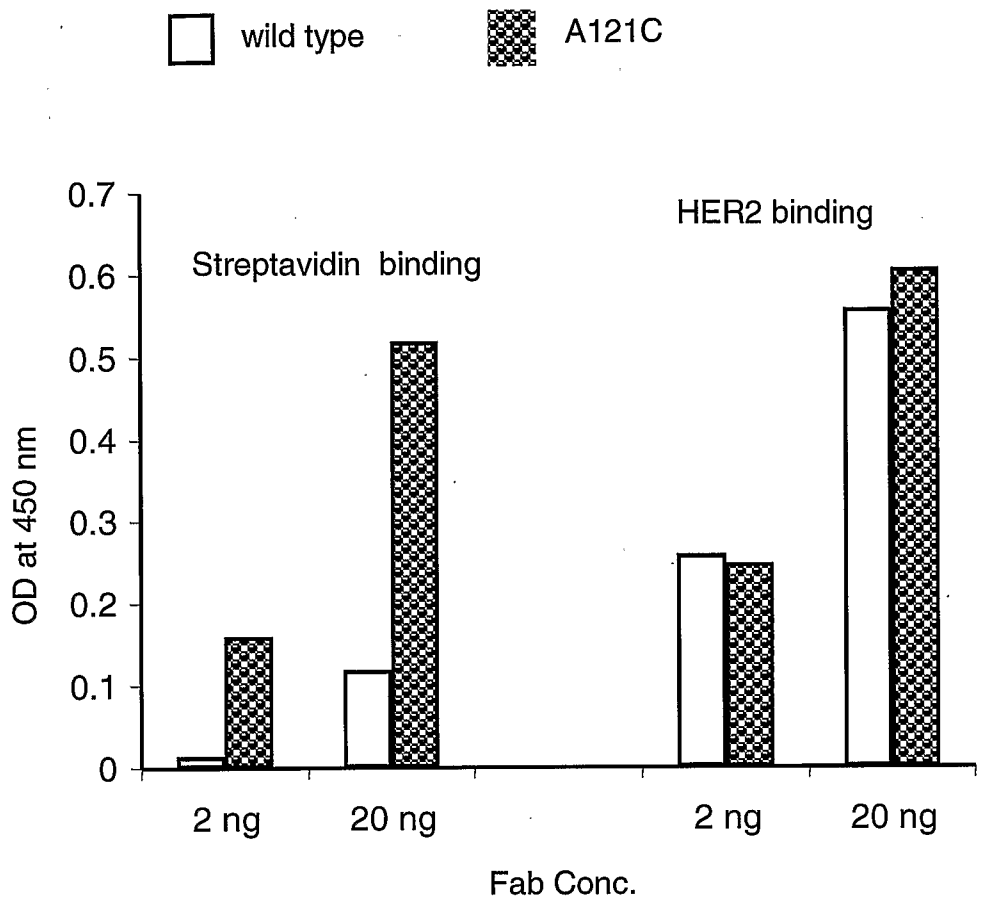

wild type
A121C
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
OD at 450 nm
Streptavidin binding
HER2 binding
2 ng
20 ng
2 ng
20 ng
Fab Conc.

Figure 5

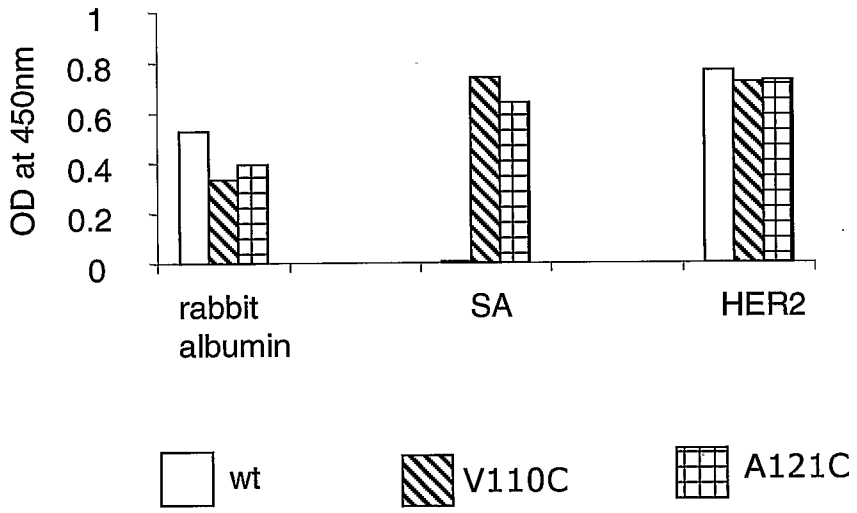

OD at 450nm
1
0.8
0.6
0.4
0.2
0
rabbit albumin
SA
HER2
wt
V110C
A121C

Figure 6

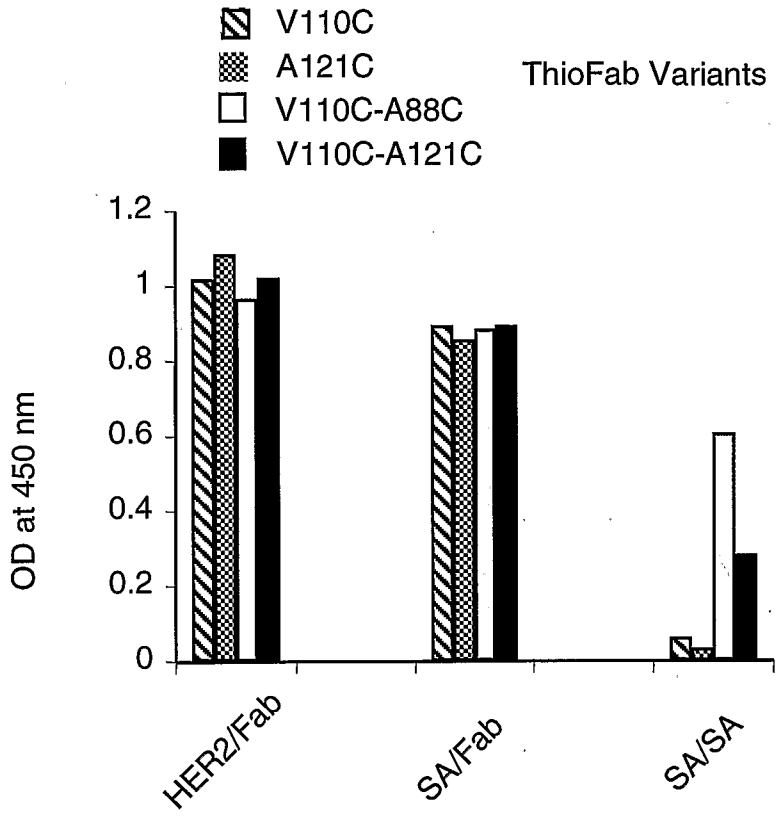
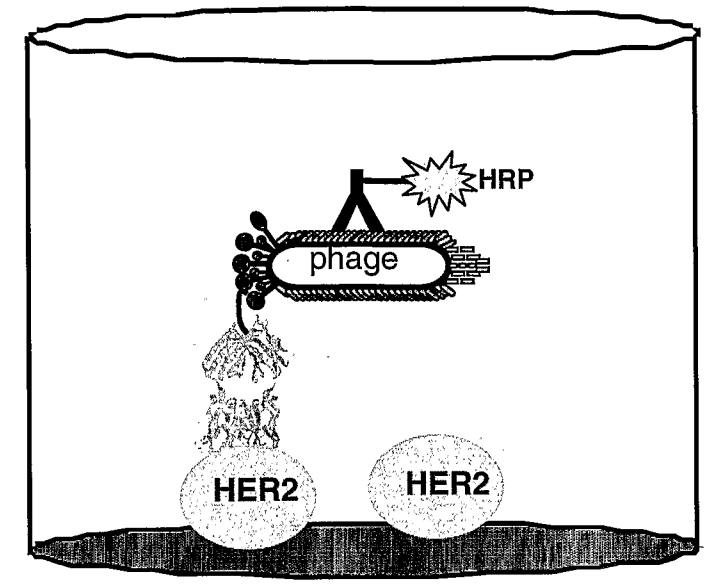

V110C
A121C
V110C-A88C
V110C-A121C
ThioFab Variants
OD at 450 nm
1.2
1
0.8
0.6
0.4
0.2
0
HER2/Fab
SA/Fab
SA/SA

Figure 7



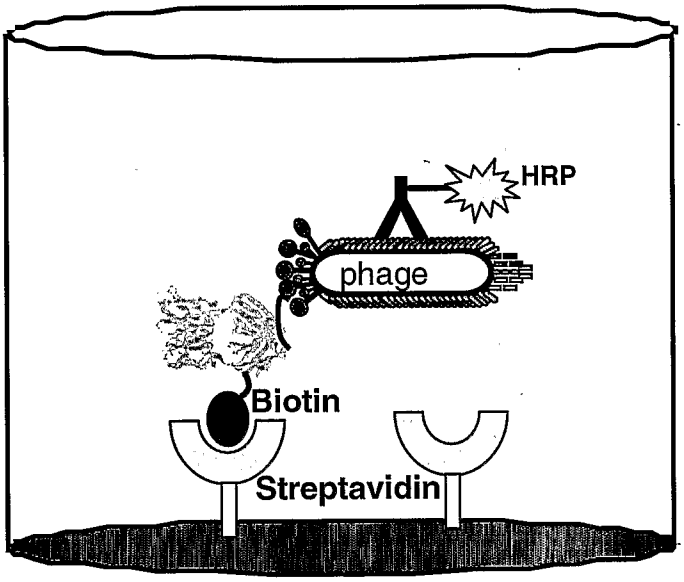


Figure 8

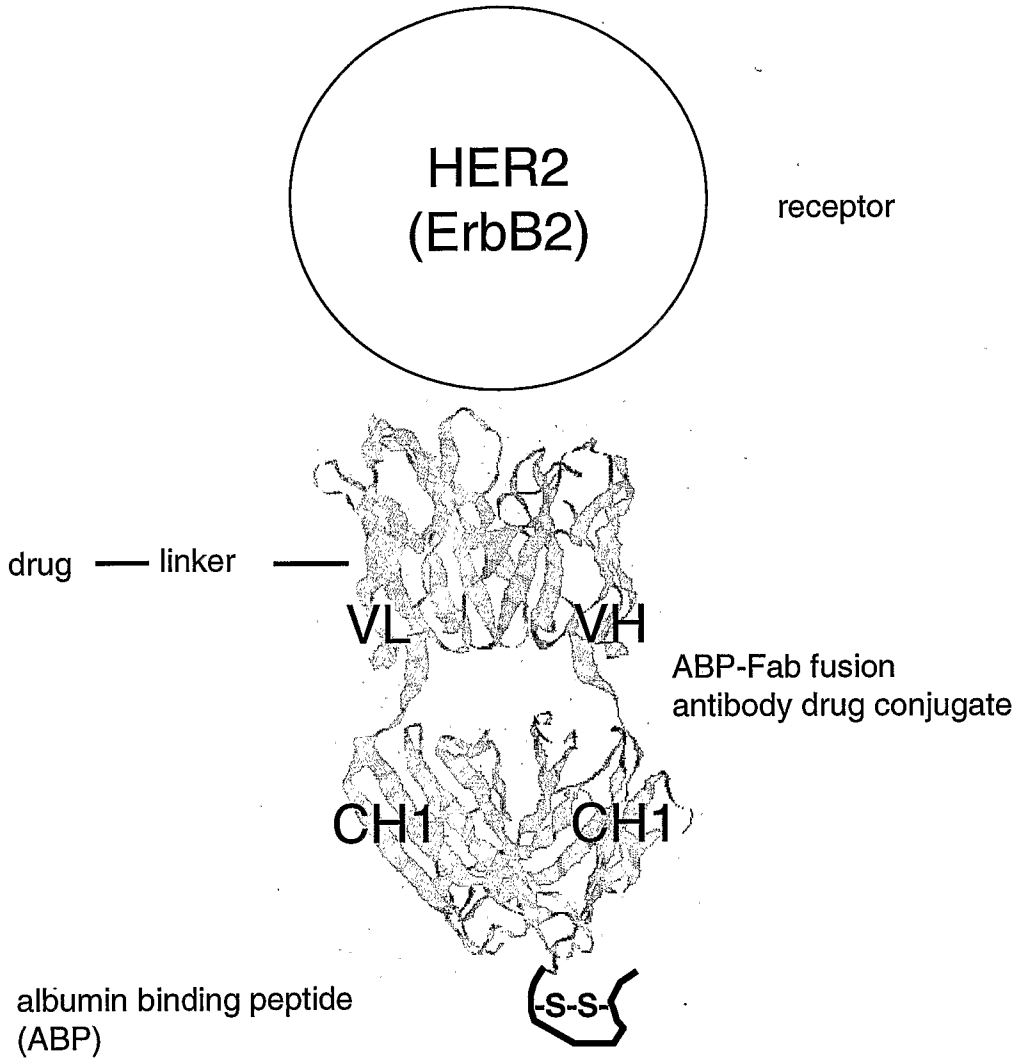

HER2
(ErbB2)
receptor
drug
linker
VL
VH
ABP-Fab fusion
antibody drug conjugate
CH1
CH1
-S-S-
albumin binding peptide
(ABP)

Figure 9

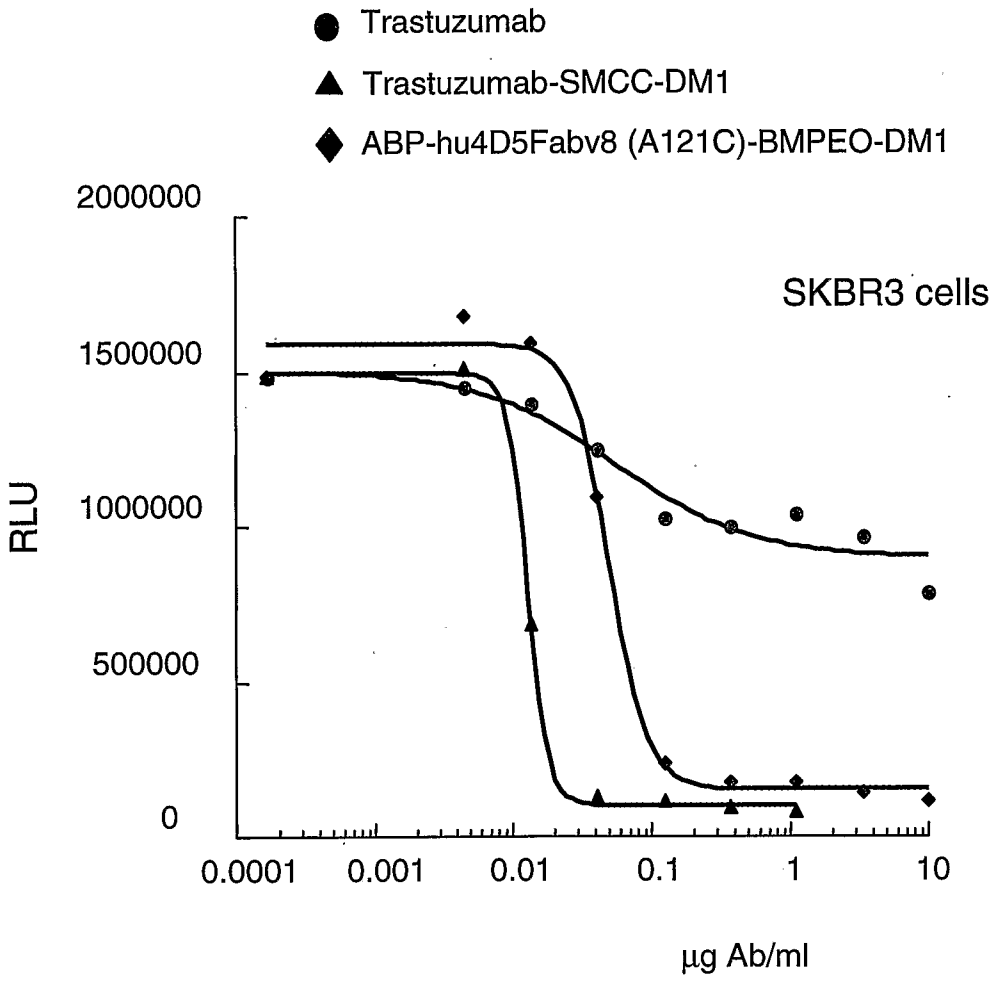

Trastuzumab
Trastuzumab-SMCC-DM1
ABP-hu4D5Fabv8 (A121C)-BMPEO-DM1
SKBR3 cells
RLU
2000000
1500000
1000000
500000
0
0.0001
0.001
0.01
0.1
1
10
µg Ab/ml

Figure 10

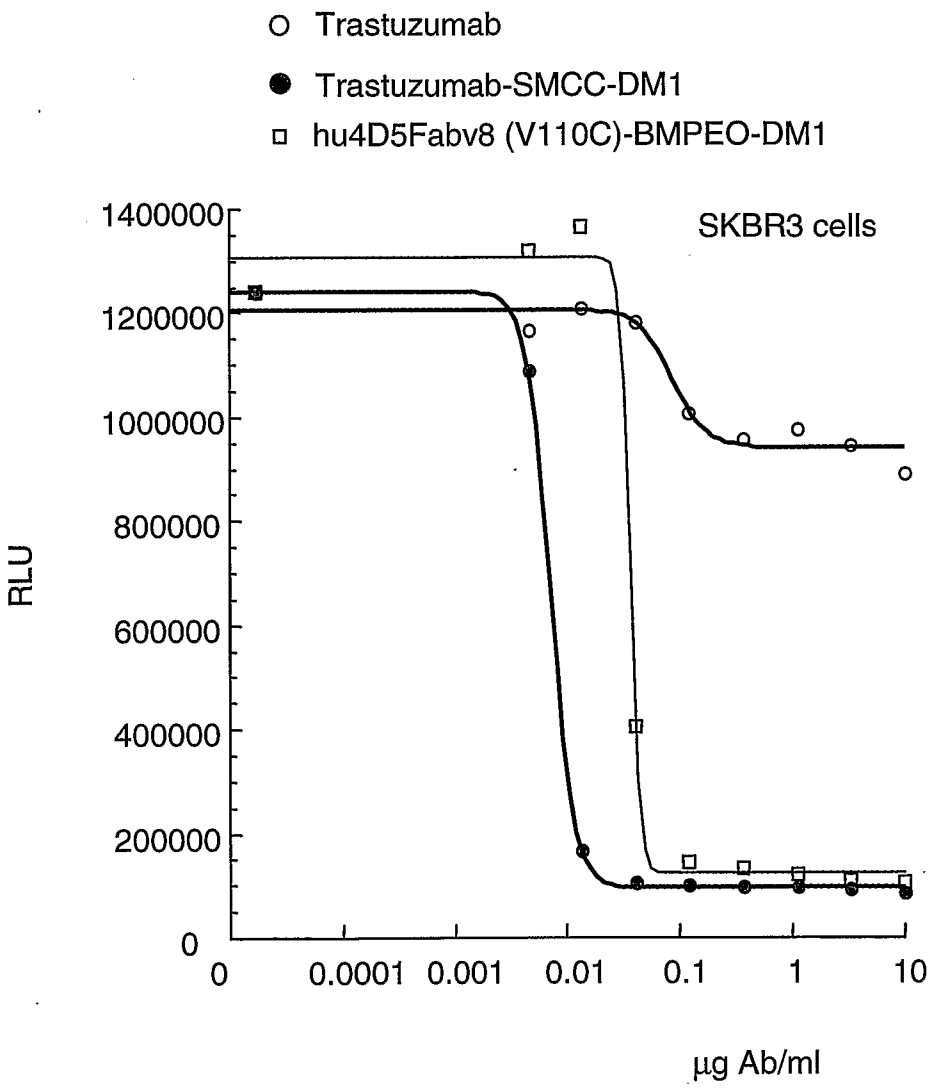

Trastuzumab
Trastuzumab-SMCC-DM1
hu4D5Fabv8 (V110C)-BMPEO-DM1
SKBR3 cells
RLU
1400000
1200000
1000000
800000
600000
400000
200000
0
0
0.0001
0.001
0.01
0.1
1
10
µg Ab/ml

Figure 11

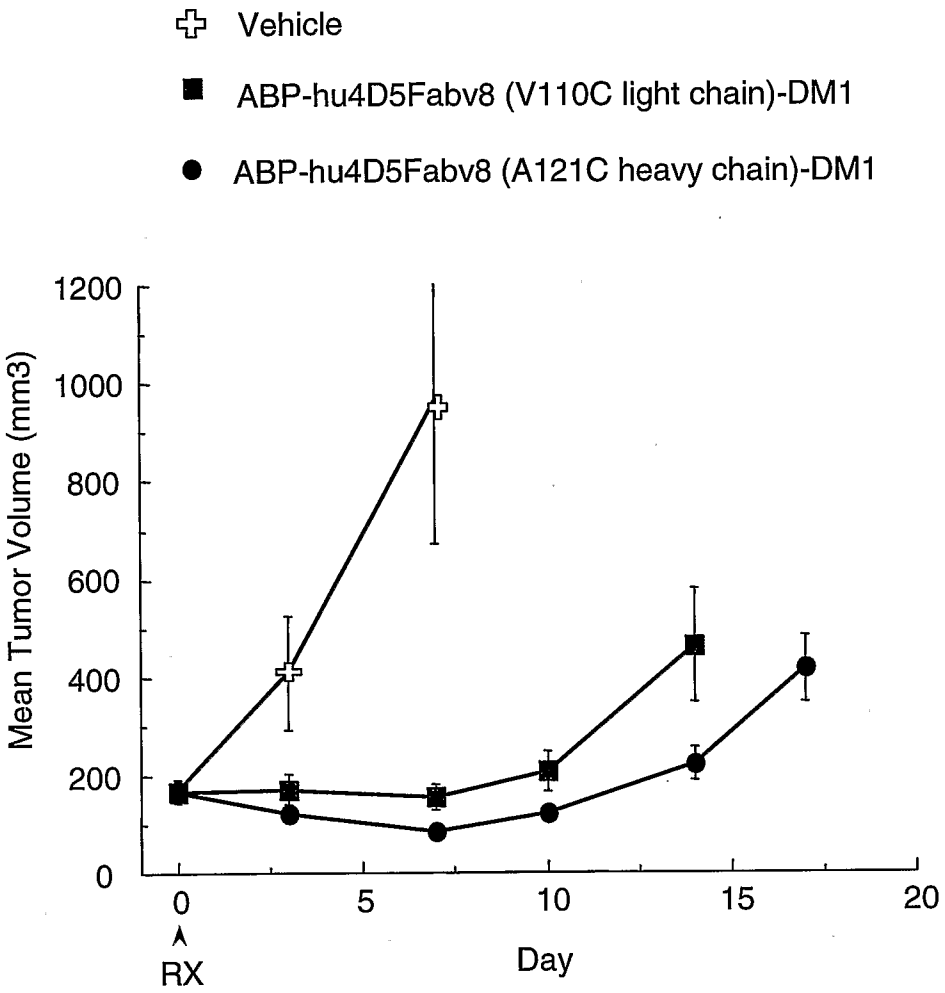

Vehicle
ABP-hu4D5Fabv8 (V110C light chain)-DM1
ABP-hu4D5Fabv8 (A121C heavy chain)-DM1
Mean Tumor Volume (mm3)
1200
1000
800
600
400
200
0
0
5
10
15
20
RX
Day

Figure 12

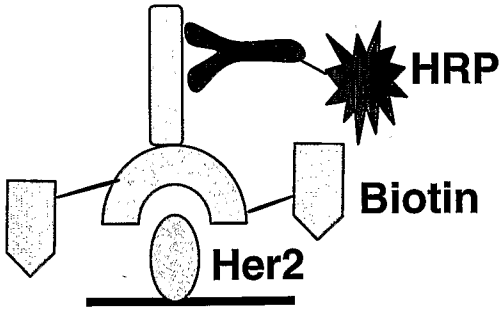


Figure 13A

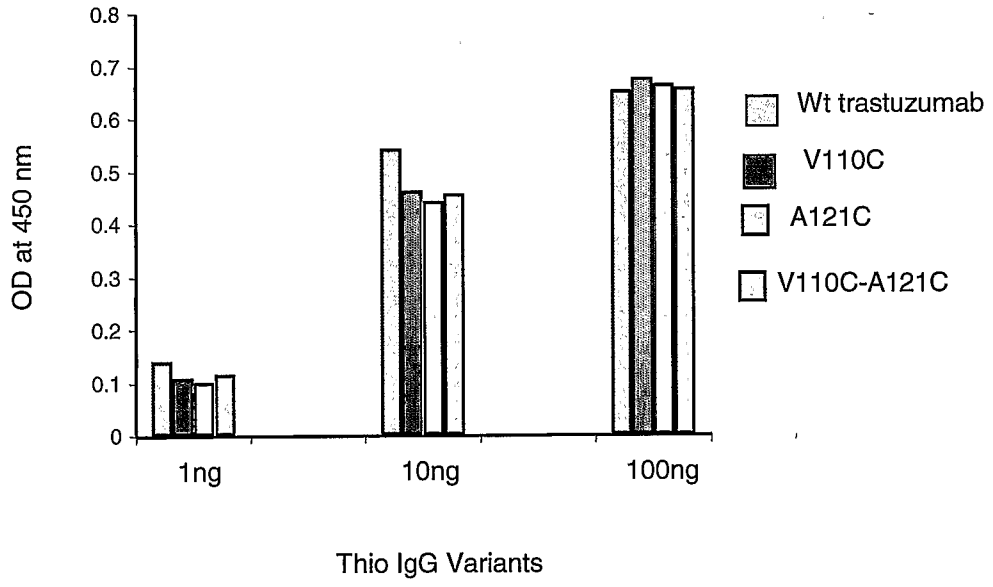


Figure 13B

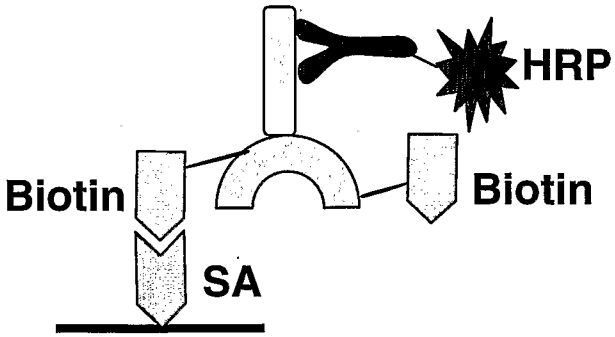


Figure 14A

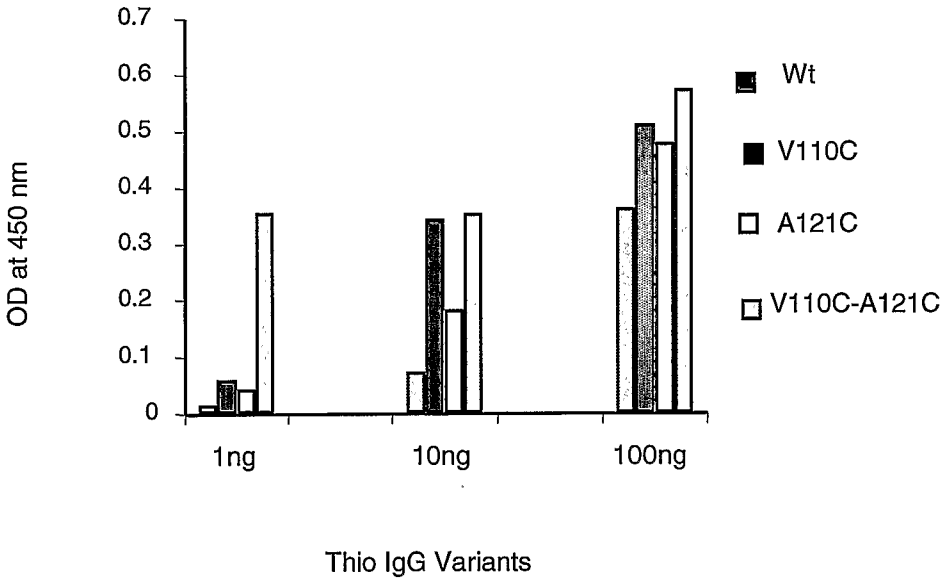


Figure 14B

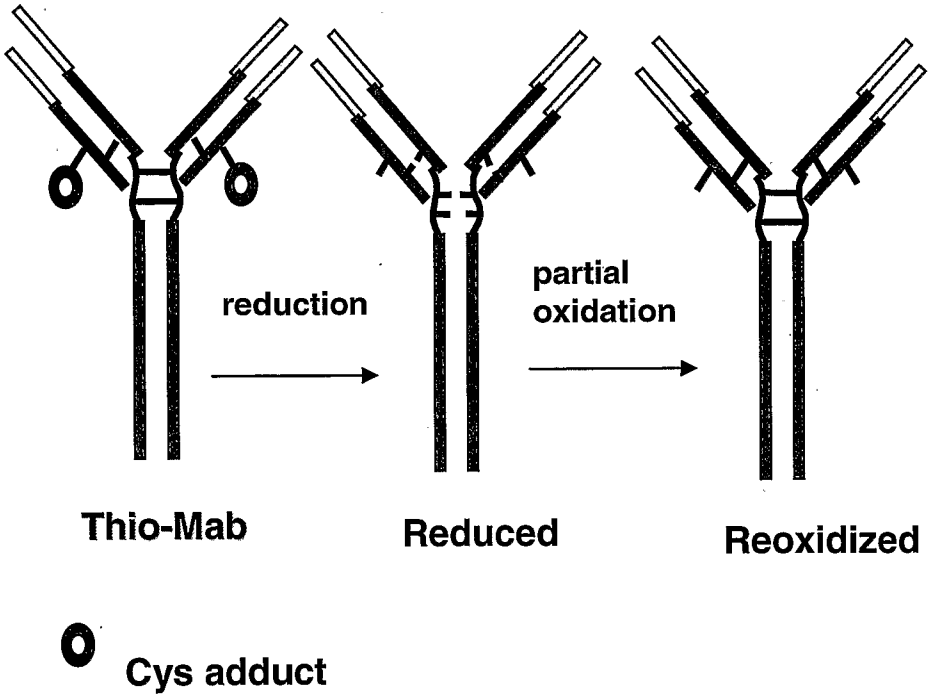
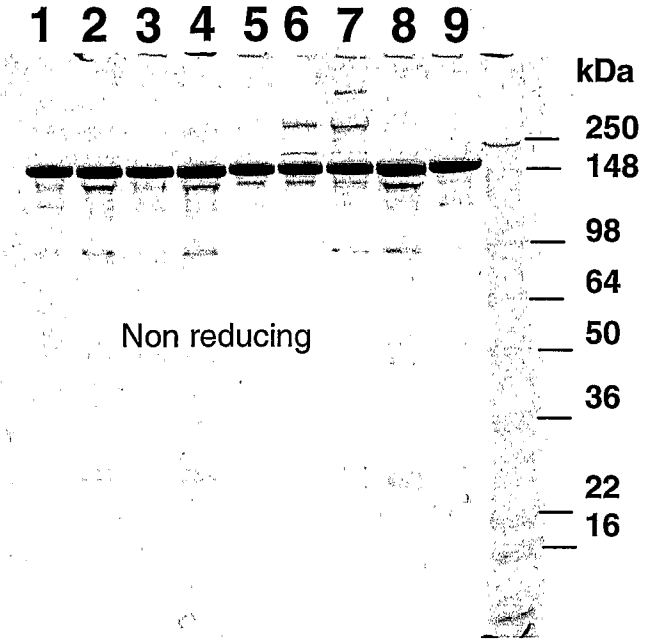

reduction
partial
oxidation
Thio-Mab
Reduced
Reoxidized
Cys adduct

Figure 15



2H9 Fc variants

1: A339C
2: S337C
3: S324C
4: A287C
5: V284C
6: V282C
7: V279C
8: V273C
9: Wt

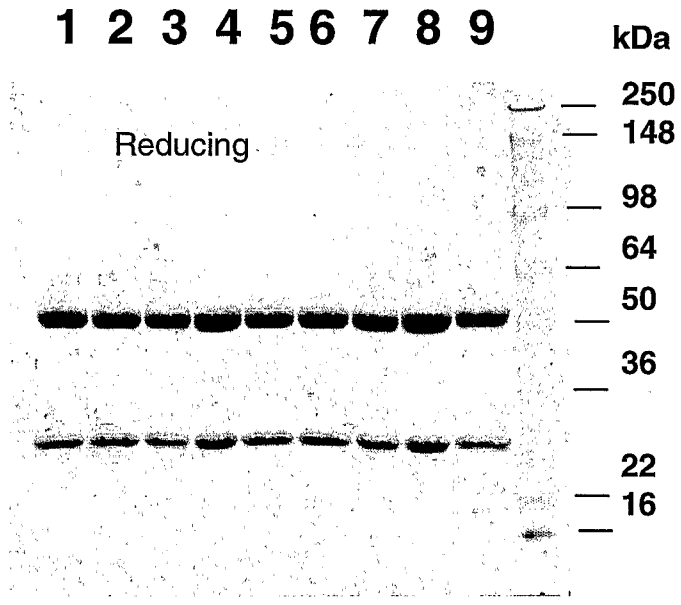


Figure 16

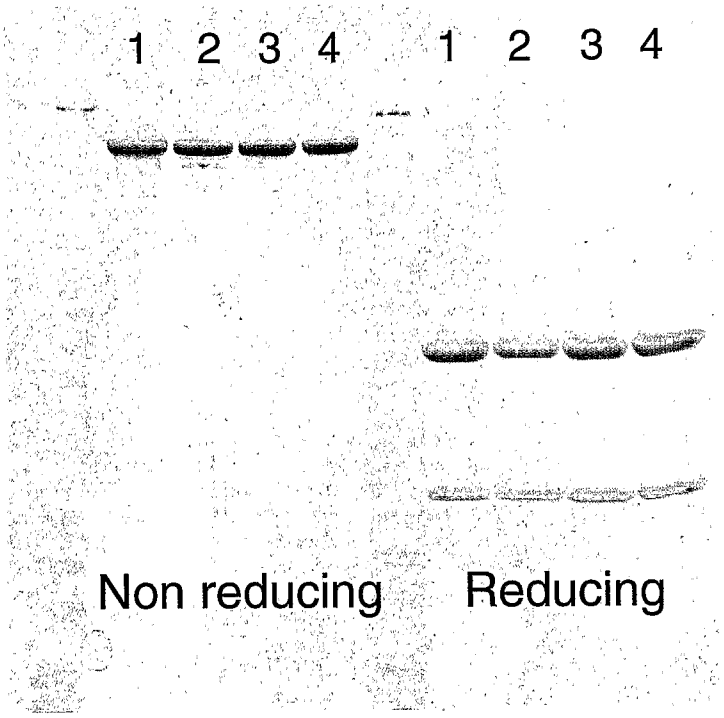


Figure 17A

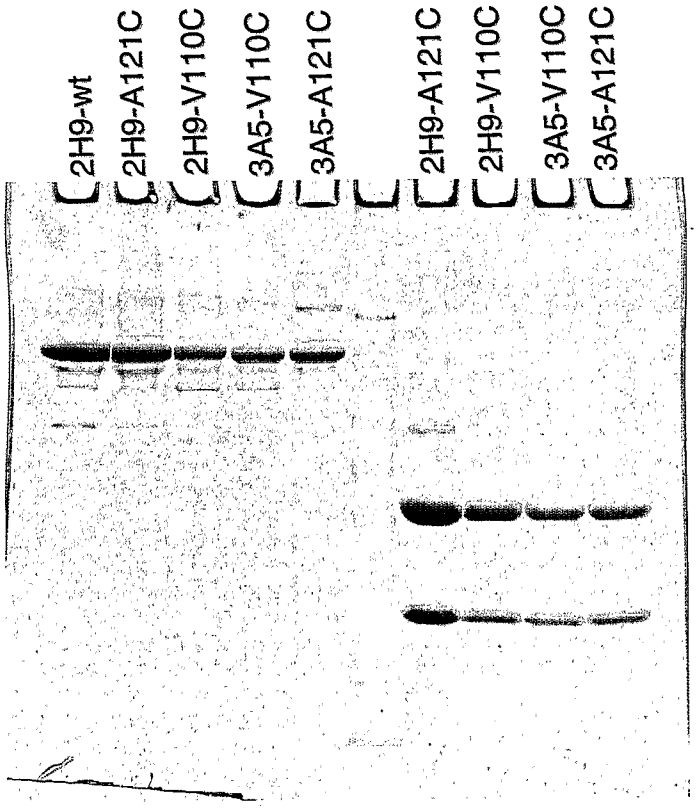


Figure 17B

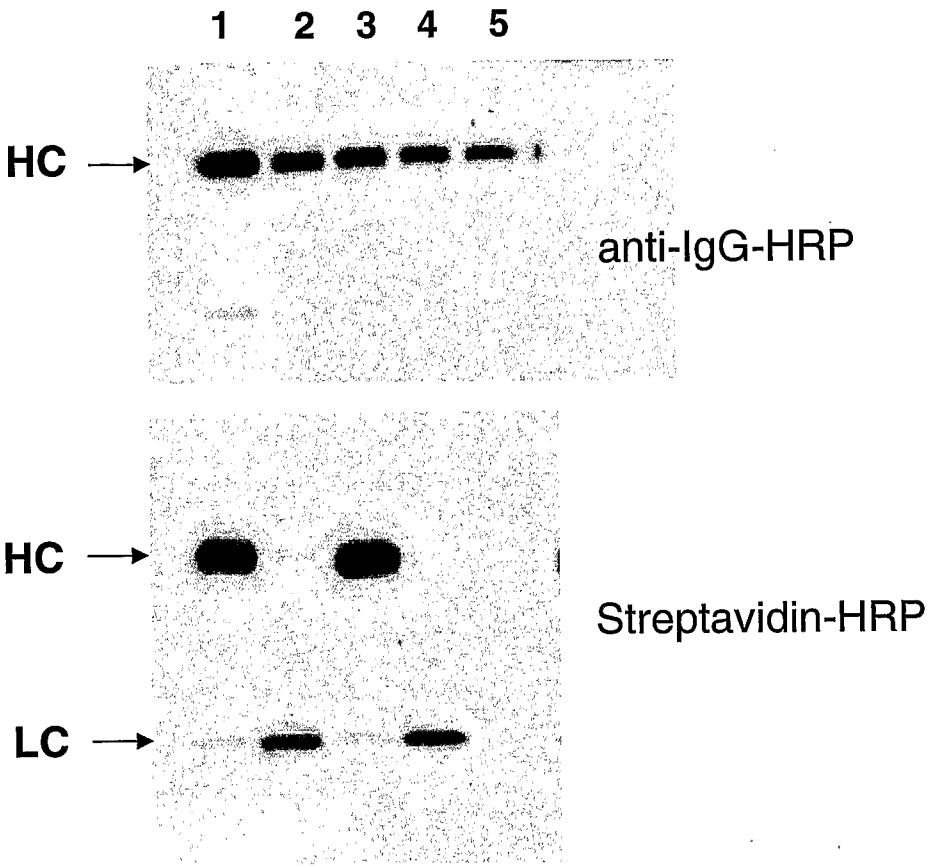


Figure 18

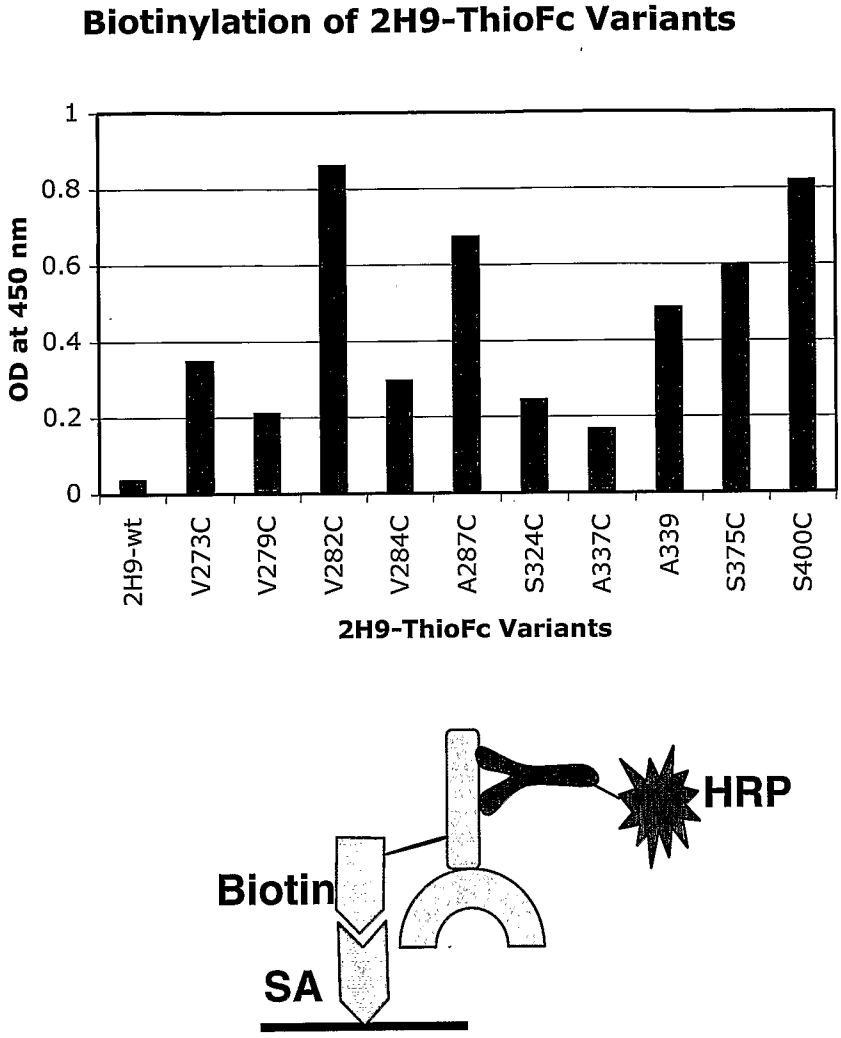

Biotinylation of 2H9-ThioFc Variants
OD at 450 nm
1
0.8
0.6
0.4
0.2
0
2H9-wt
V273C
V279C
V282C
V284C
A287C
S324C
A337C
A339
S375C
S400C
2H9-ThioFc Variants
HRP
Biotin
SA

Figure 19

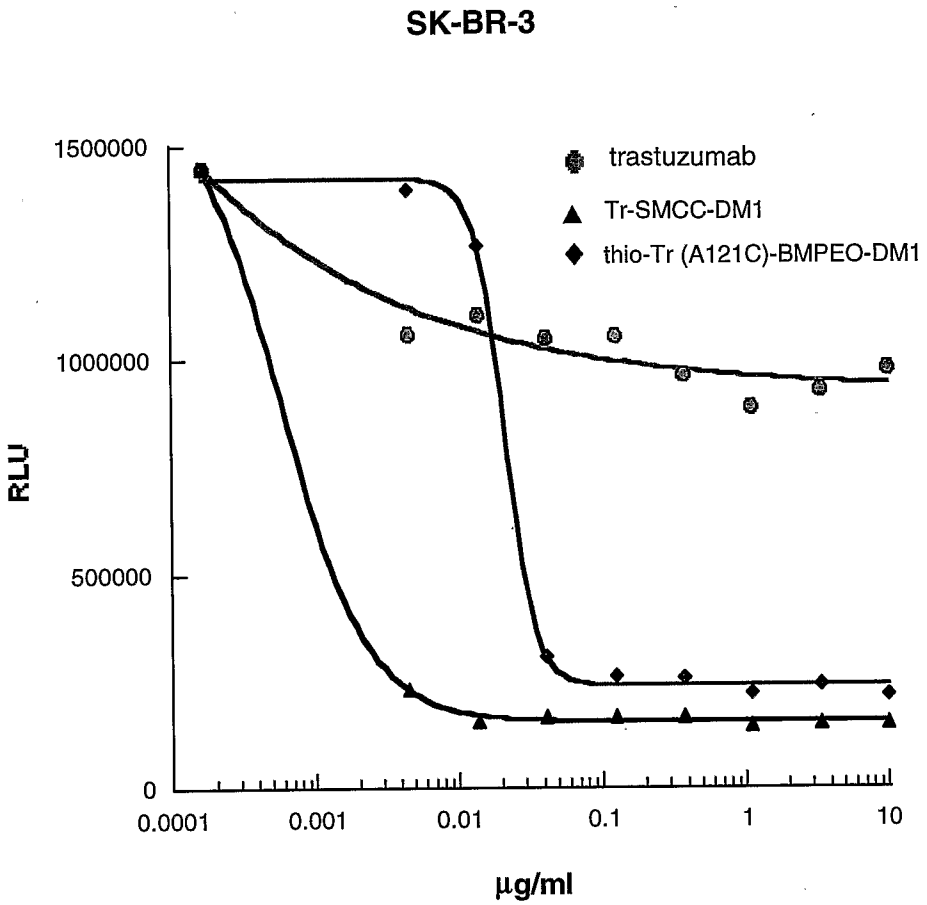

SK-BR-3
1500000
1000000
500000
0
RLU
trastuzumab
Tr-SMCC-DM1
thio-Tr (A121C)-BMPEO-DM1
0.0001
0.001
0.01
0.1
1
10
µg/ml

Figure 20

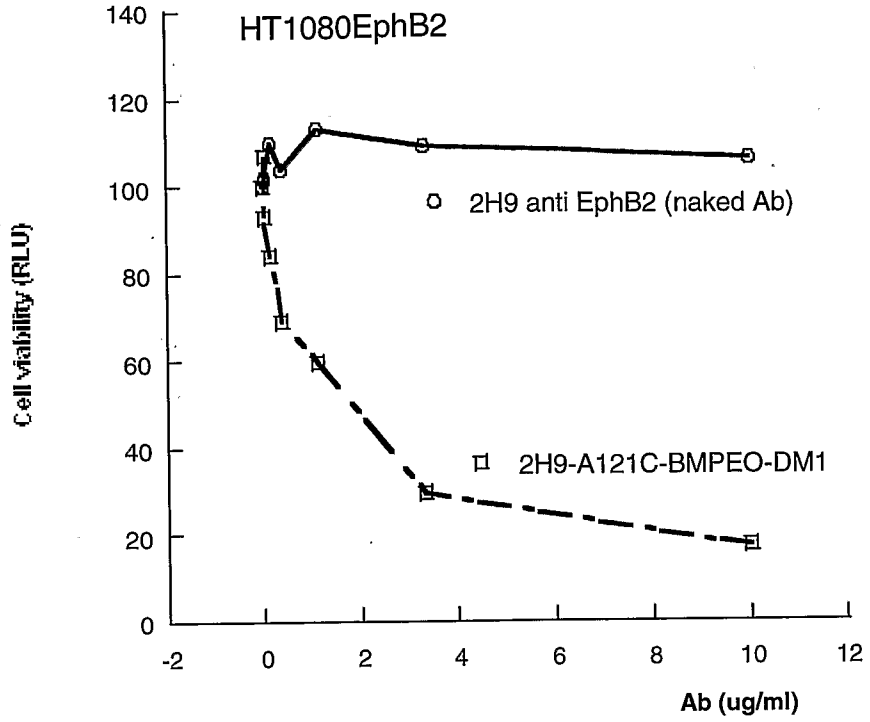


Figure 21A

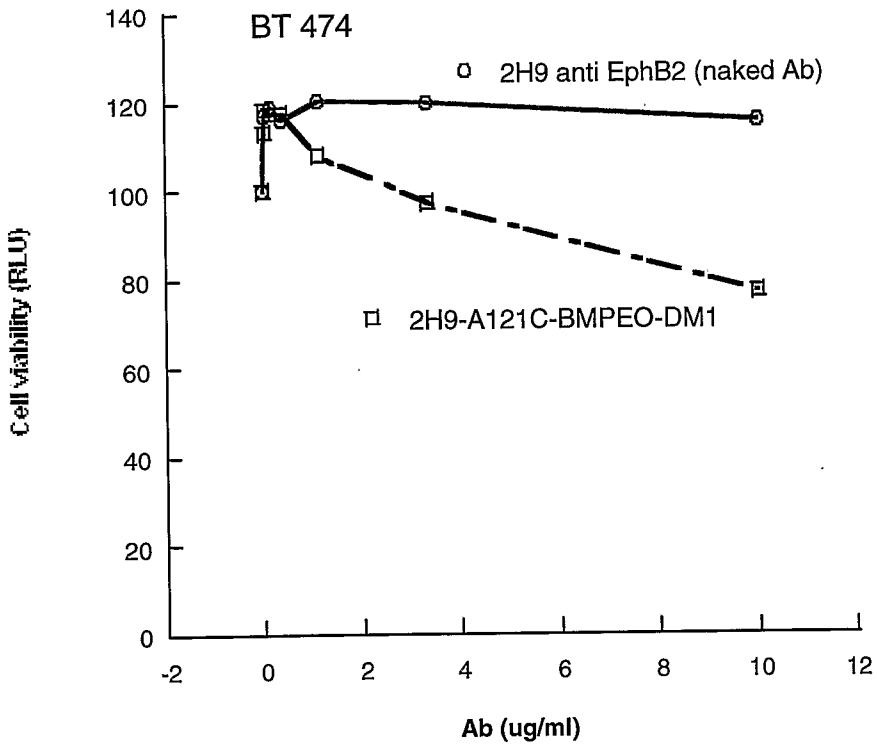


Figure 21B

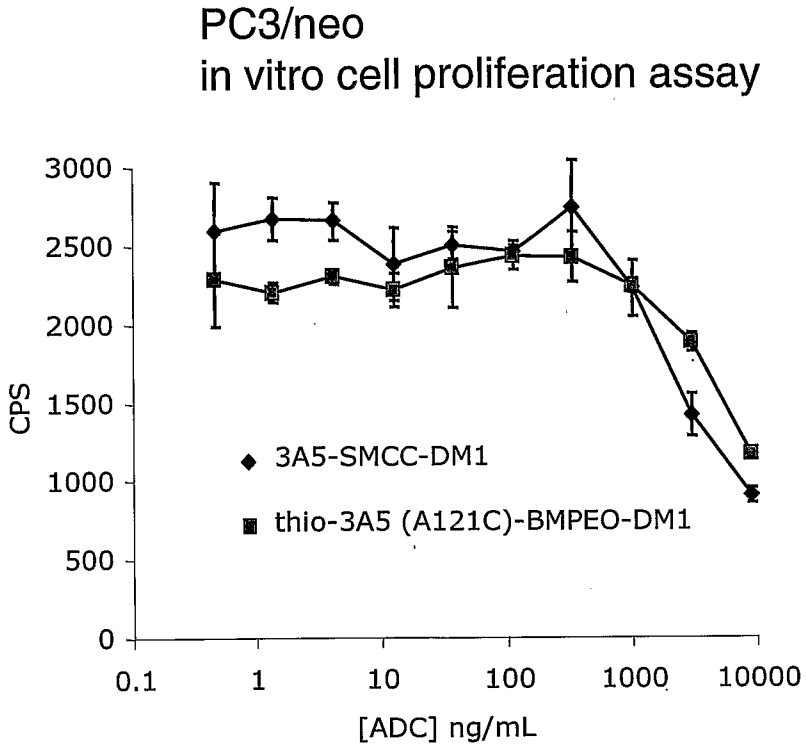

PC3/neo
in vitro cell proliferation assay
CPS
3000
2500
2000
1500
1000
500
0
0.1
1
10
100
1000
10000
[ADC] ng/mL
3A5-SMCC-DM1
thio-3A5 (A121C)-BMPEO-DM1

Figure 22

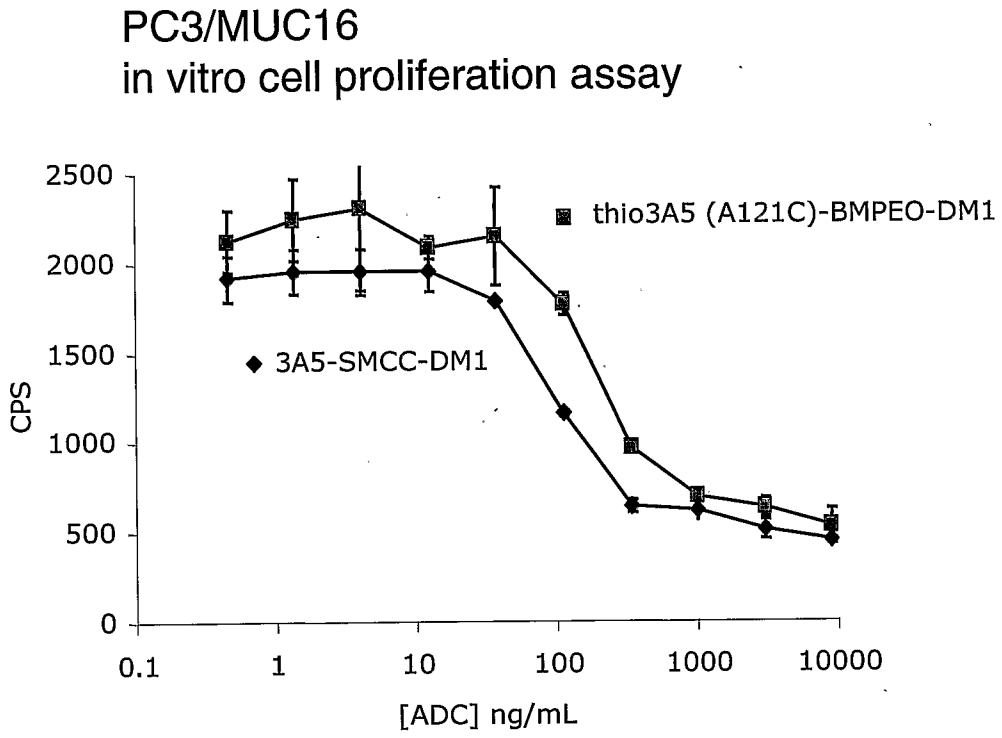

PC3/MUC16
in vitro cell proliferation assay
2500
2000
1500
1000
500
0
CPS
0.1
1
10
100
1000
10000
[ADC] ng/mL
thio3A5 (A121C)-BMPEO-DM1
3A5-SMCC-DM1

Figure 23

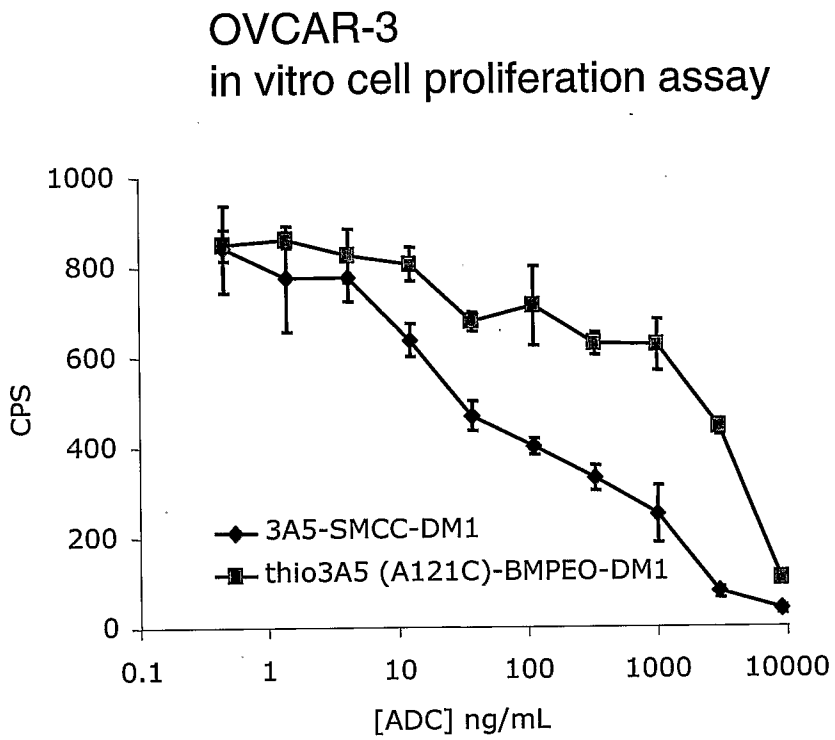

OVCAR-3
in vitro cell proliferation assay
1000
800
600
400
200
0
CPS
0.1
1
10
100
1000
10000
[ADC] ng/mL
3A5-SMCC-DM1
thio3A5 (A121C)-BMPEO-DM1

Figure 24

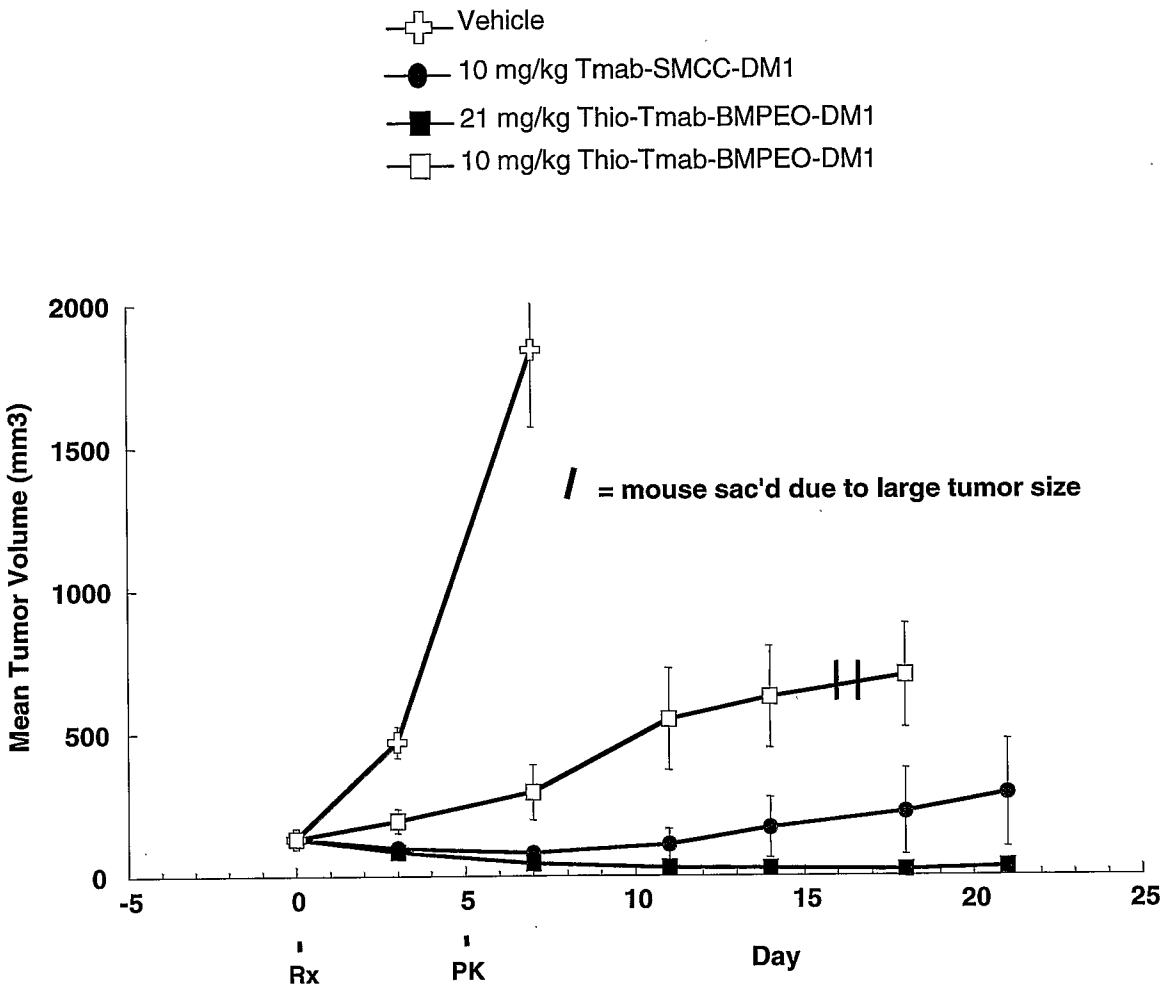

Vehicle
10 mg/kg Tmab-SMCC-DM1
21 mg/kg Thio-Tmab-BMPEO-DM1
10 mg/kg Thio-Tmab-BMPEO-DM1
Mean Tumor Volume (mm3)
2000
1500
1000
500
0
= mouse sac'd due to large tumor size
-5
0
5
10
15
20
25
Rx
PK
Day

Figure 25