

FIG. 1A

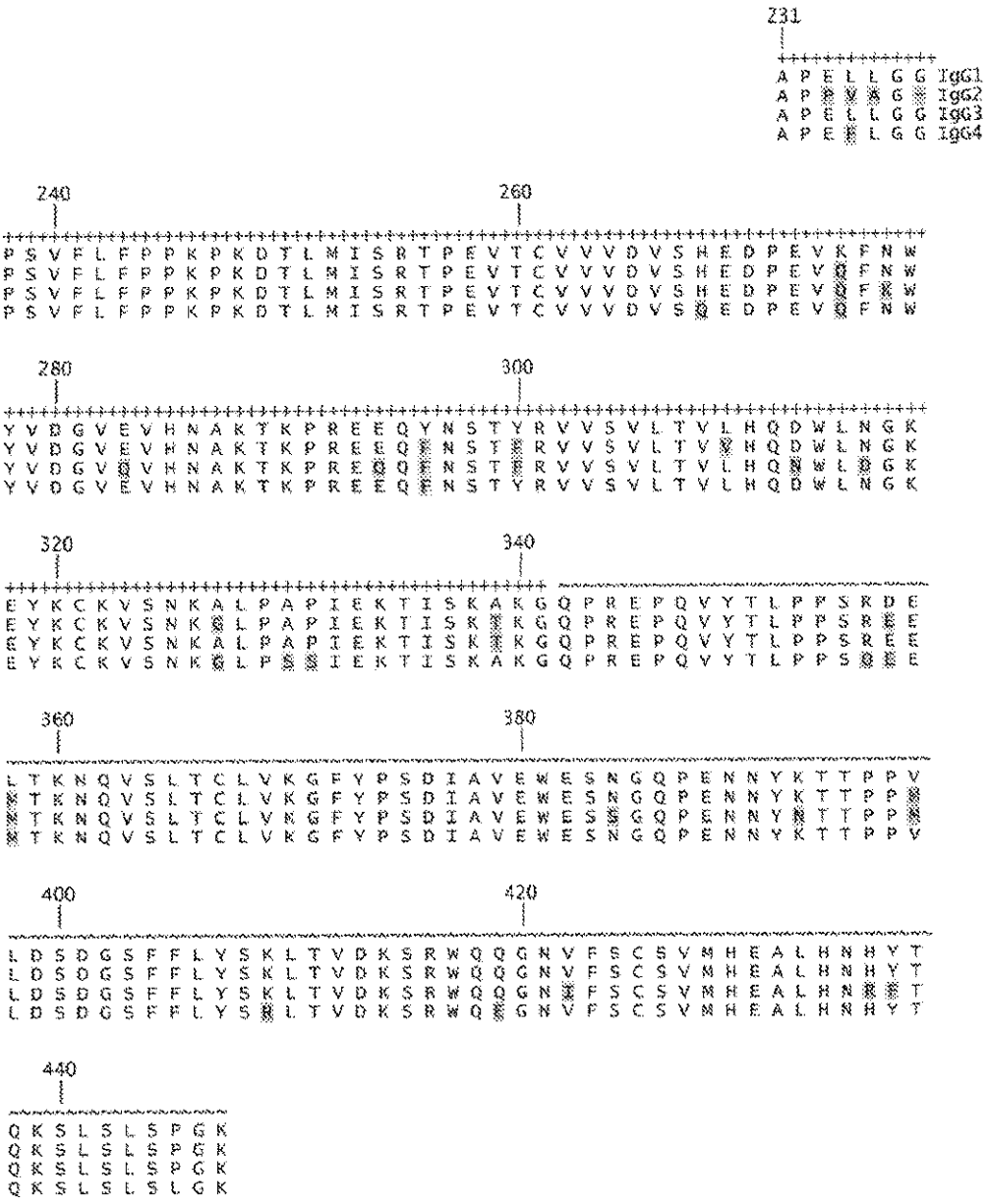

Z31
A P E L L G G IgG1
A P ▒ V ▒ G ▒ IgG2
A P E L L G G IgG3
A P E ▒ L G G IgG4
240
260
P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S H E D P E V K F N W
P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S H E D P E V Q F N W
P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S H E D P E V Q F ▒ W
P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S ▒ E D P E V ▒ F N W
280
300
Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D W L N G K
Y V D G V E V H N A K T K P R E E Q ▒ N S T ▒ R V V S V L T V ▒ H Q D W L N G K
Y V D G V ▒ V H N A K T K P R E ▒ Q ▒ N S T ▒ R V V S V L T V L H Q ▒ W L ▒ G K
Y V D G V E V H N A K T K P R E E Q ▒ N S T Y R V V S V L T V L H Q D W L N G K
320
340
E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R D E
E Y K C K V S N K ▒ L P A P I E K T I S K ▒ K G Q P R E P Q V Y T L P P S R ▒ E
E Y K C K V S N K A L P A P I E K T I S K ▒ K G Q P R E P Q V Y T L P P S R E E
E Y K C K V S N K ▒ L P ▒ ▒ I E K T I S K A K G Q P R E P Q V Y T L P P S ▒ ▒ E
360
380
L T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V
▒ T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P ▒
▒ T K N Q V S L T C L V K G F Y P S D I A V E W E S ▒ G Q P E N N Y ▒ T T P P ▒
▒ T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V
400
420
L D S D G S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T
L D S D G S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T
L D S D G S F F L Y S K L T V D K S R W Q Q G N ▒ F S C S V M H E A L H N ▒ ▒ T
L D S D G S F F L Y S ▒ L T V D K S R W Q ▒ G N V F S C S V M H E A L H N H Y T
440
Q K S L S L S P G K
Q K S L S L S P G K
Q K S L S L S P G K
Q K S L S L S L G K

FIG. 1B

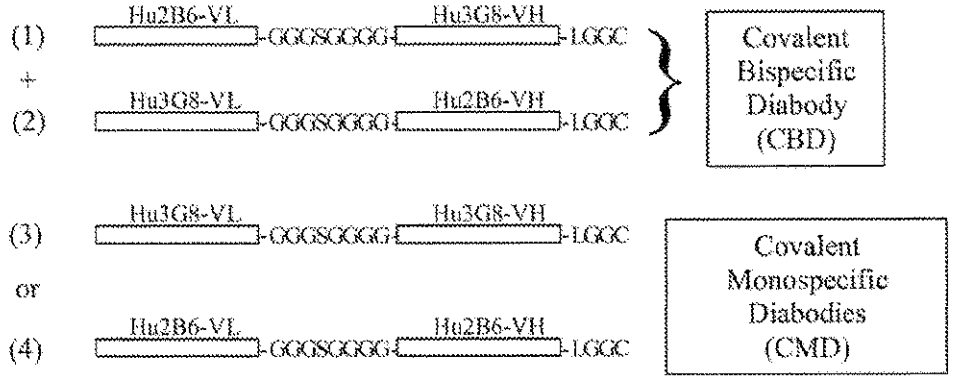

(1)
Hu2B6-VL
GGGSGGGG
Hu3G8-VH
LGGC
+
(2)
Hu3G8-VL
GGGSGGGG
Hu2B6-VH
LGGC
Covalent Bispecific Diabody (CBD)
(3)
Hu3G8-VL
GGGSGGGG
Hu3G8-VH
LGGC
or
(4)
Hu2B6-VL
GGGSGGGG
Hu2B6-VH
LGGC
Covalent Monospecific Diabodies (CMD)

FIG. 2

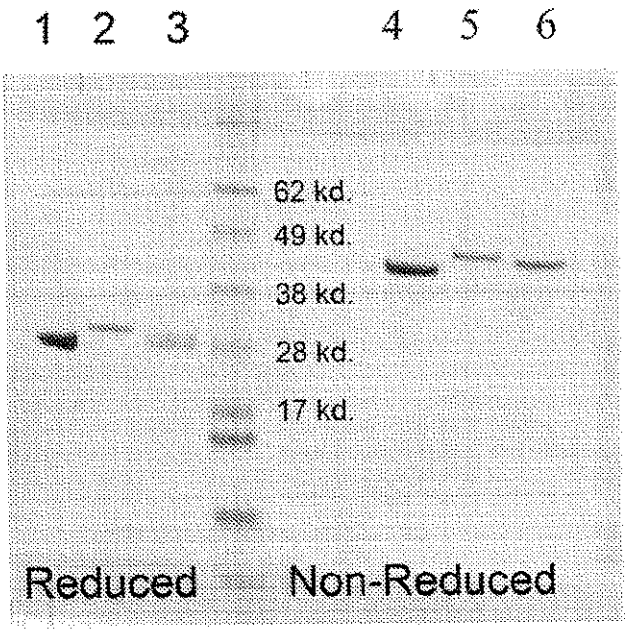


FIG. 3

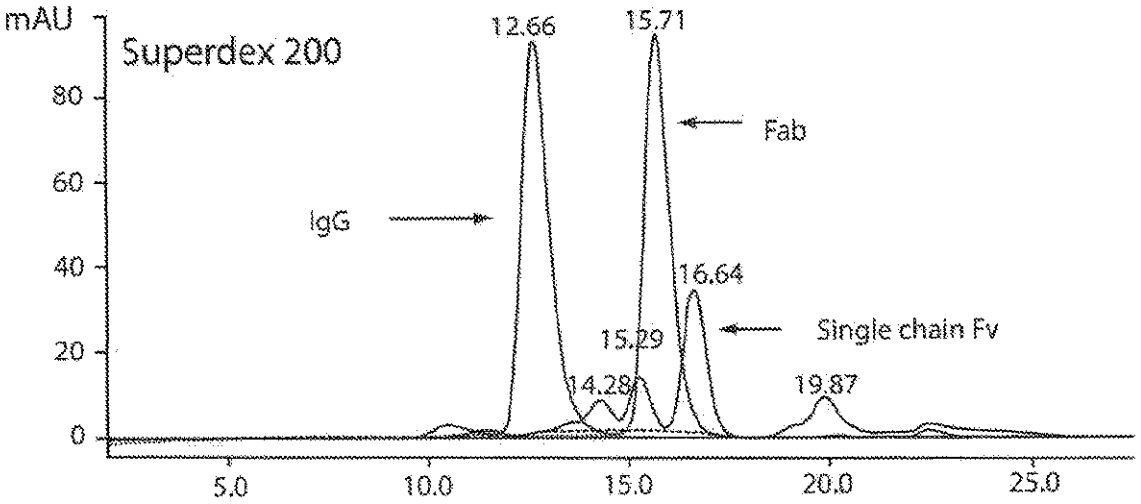

mAU
Superdex 200
12.66
15.71
Fab
IgG
16.64
15.29
Single chain Fv
14.28
19.87
80
60
40
20
0
5.0
10.0
15.0
20.0
25.0

FIG. 4A

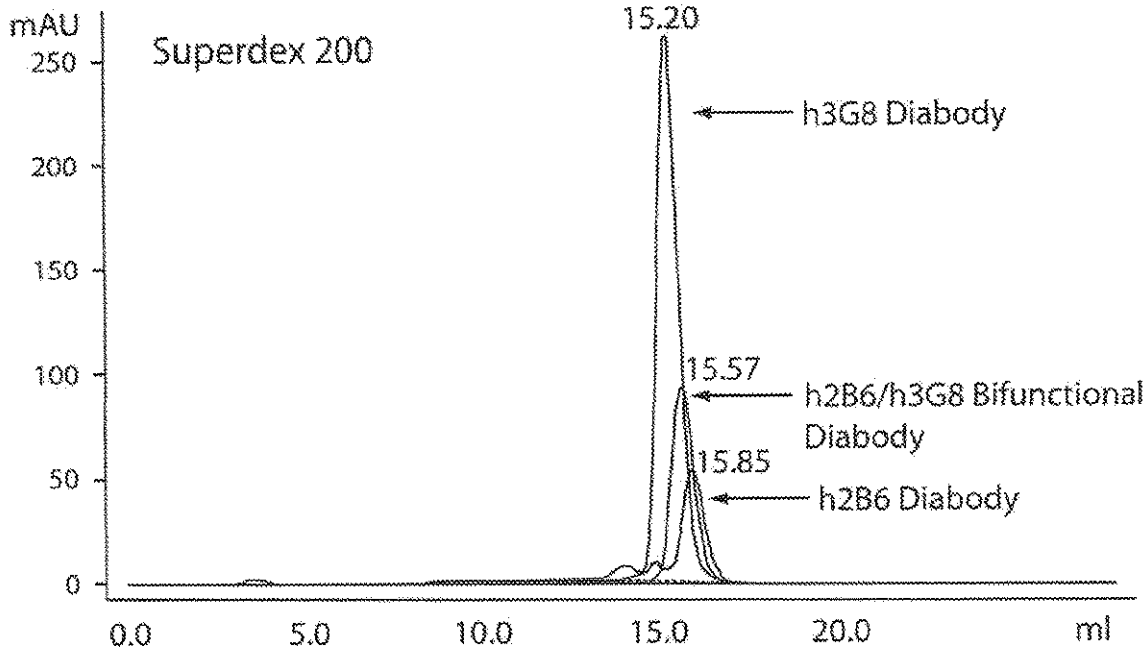

mAU
Superdex 200
15.20
h3G8 Diabody
15.57
h2B6/h3G8 Bifunctional
Diabody
15.85
h2B6 Diabody
250
200
150
100
50
0
0.0
5.0
10.0
15.0
20.0
ml

FIG. 4B

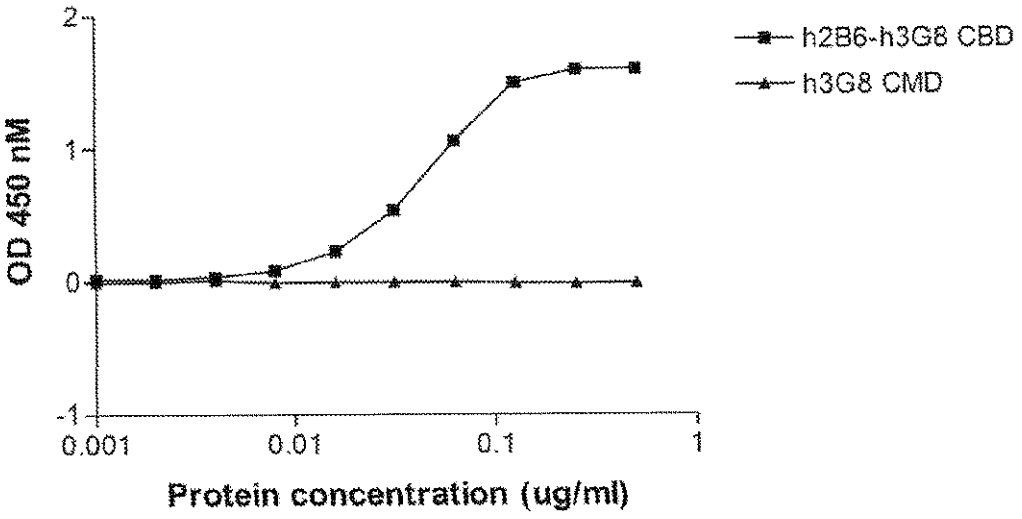

2
1
0
-1
OD 450 nM
0.001
0.01
0.1
1
Protein concentration (ug/ml)
h2B6-h3G8 CBD
h3G8 CMD

FIG. 5

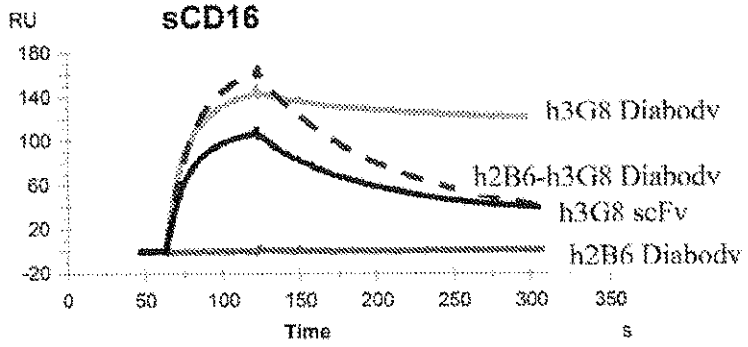


FIG. 6A

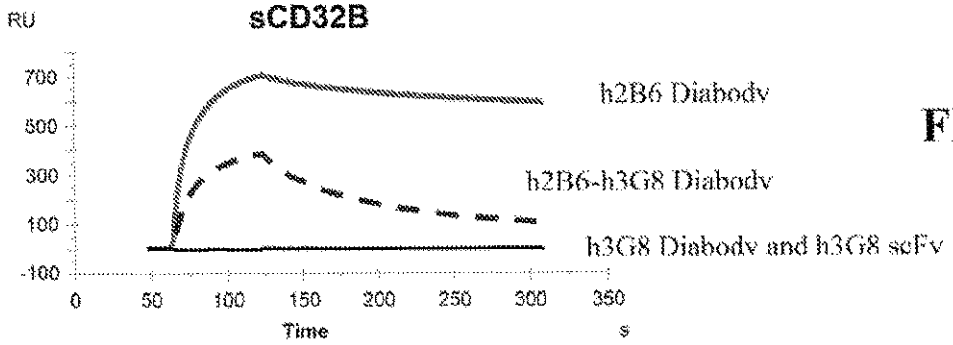


FIG. 6B

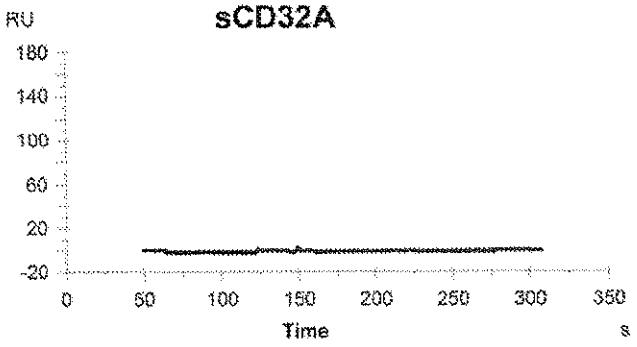


FIG. 6C

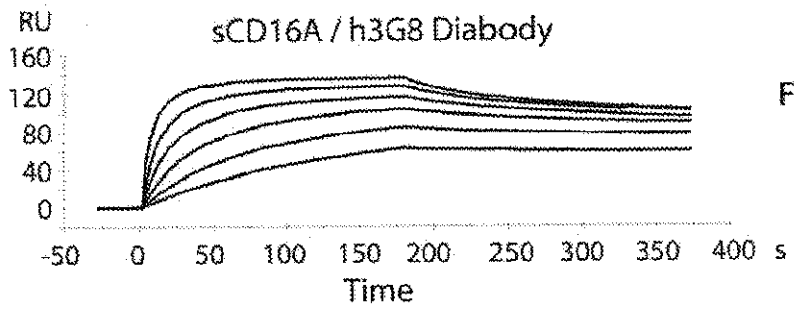


FIG. 7A

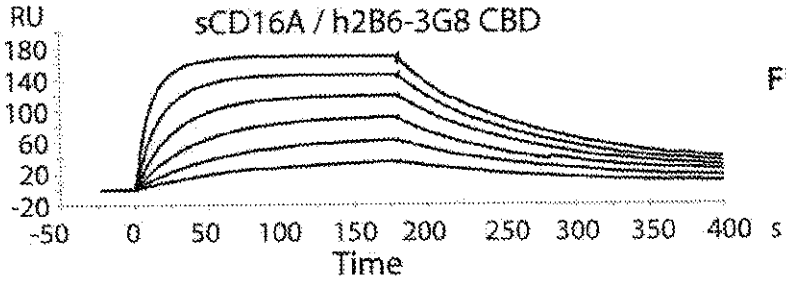


FIG. 7B

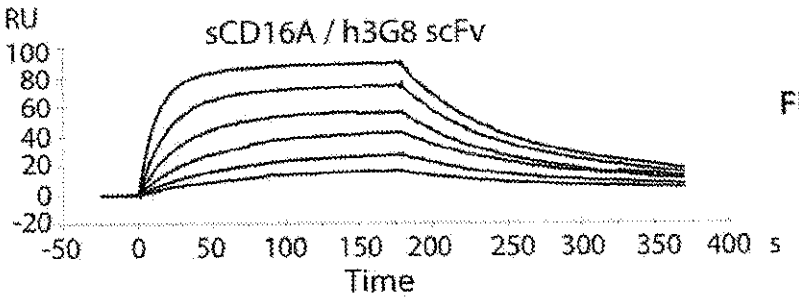


FIG. 7C

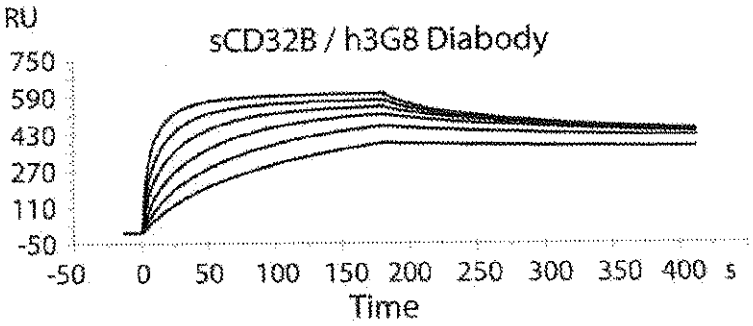


FIG. 7D

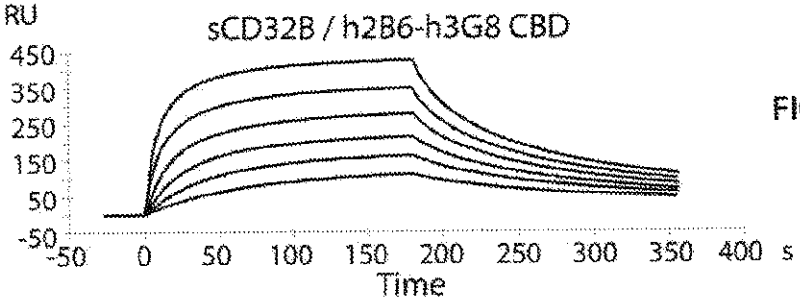


FIG. 7E

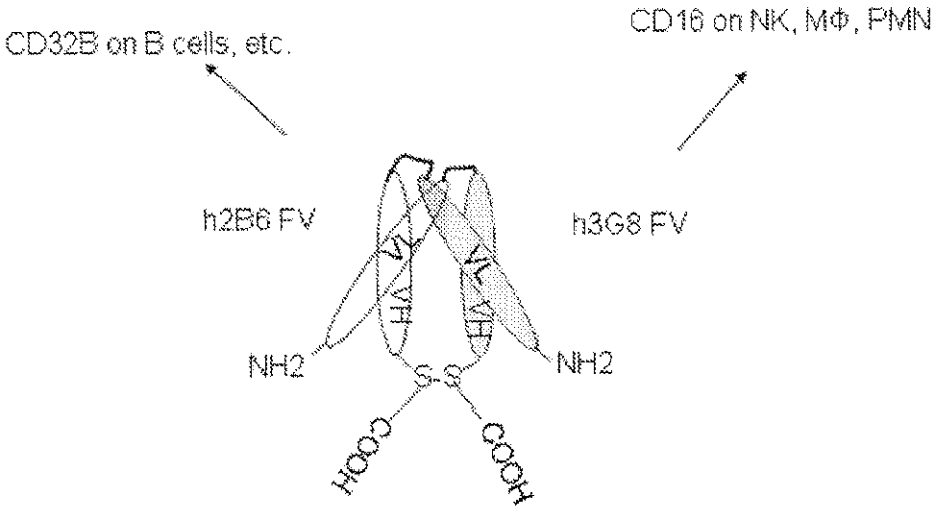

CD32B on B cells, etc.
CD16 on NK, MΦ, PMN
h2B6 FV
h3G8 FV
VL
VH
VL
VH
NH2
NH2
S-S
COOH
COOH

FIG. 8

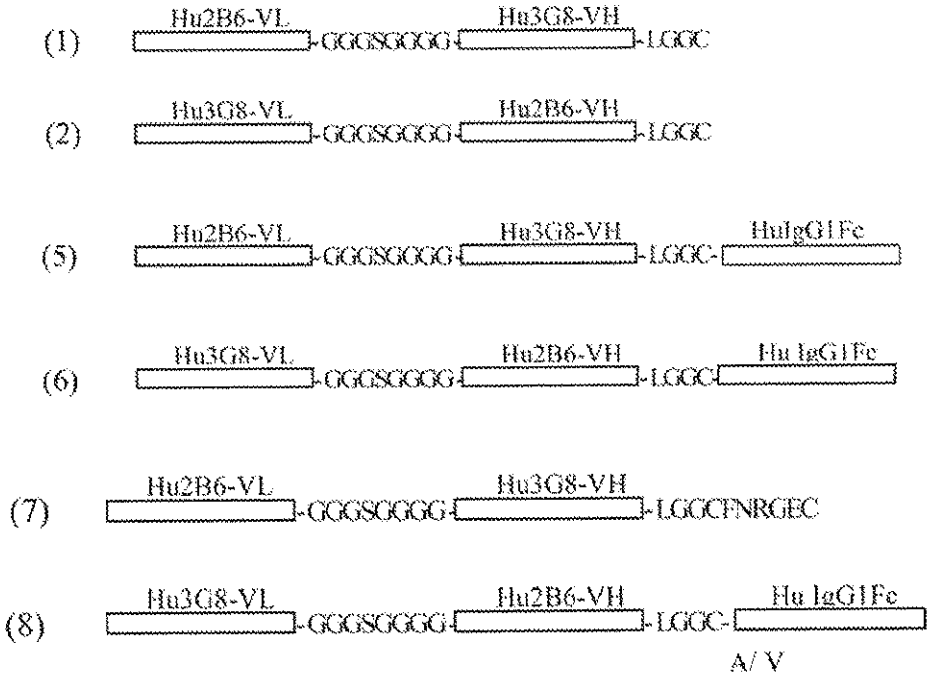

(1)
Hu2B6-VL
GGGSGGGG
Hu3G8-VH
LGGC
(2)
Hu3G8-VL
GGGSGGGG
Hu2B6-VH
LGGC
(5)
Hu2B6-VL
GGGSGGGG
Hu3G8-VH
LGGC
HuIgG1Fc
(6)
Hu3G8-VL
GGGSGGGG
Hu2B6-VH
LGGC
Hu IgG1Fc
(7)
Hu2B6-VL
GGGSGGGG
Hu3G8-VH
LGGCFNRGEC
(8)
Hu3G8-VL
GGGSGGGG
Hu2B6-VH
LGGC
Hu IgG1Fc
A/ V

FIG. 9

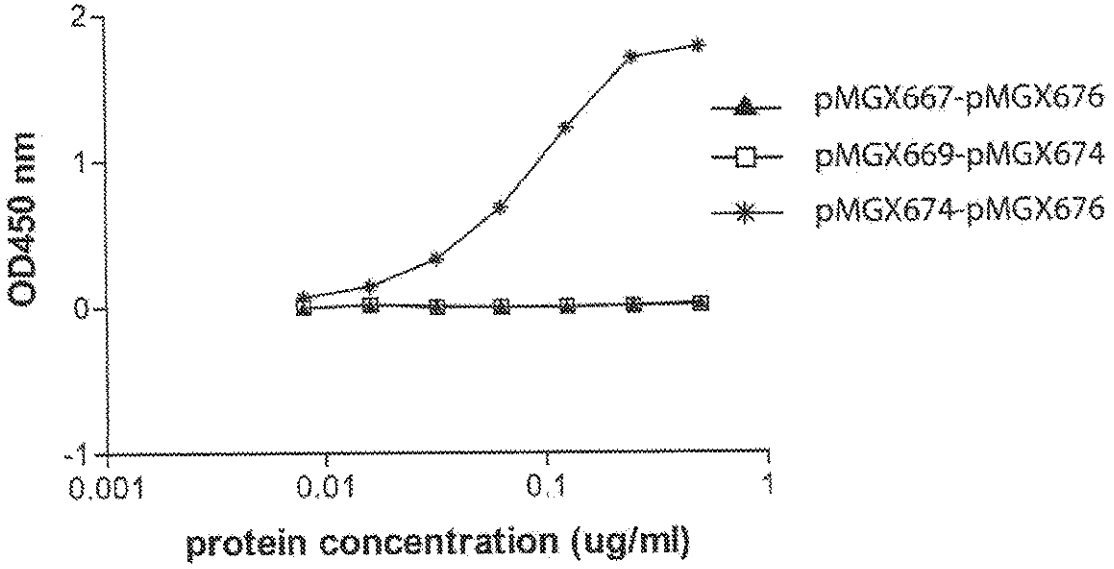

2
1
0
-1
OD450 nm
0.001
0.01
0.1
1
protein concentration (ug/ml)
pMGX667-pMGX676
pMGX669-pMGX674
pMGX674-pMGX676

FIG. 10

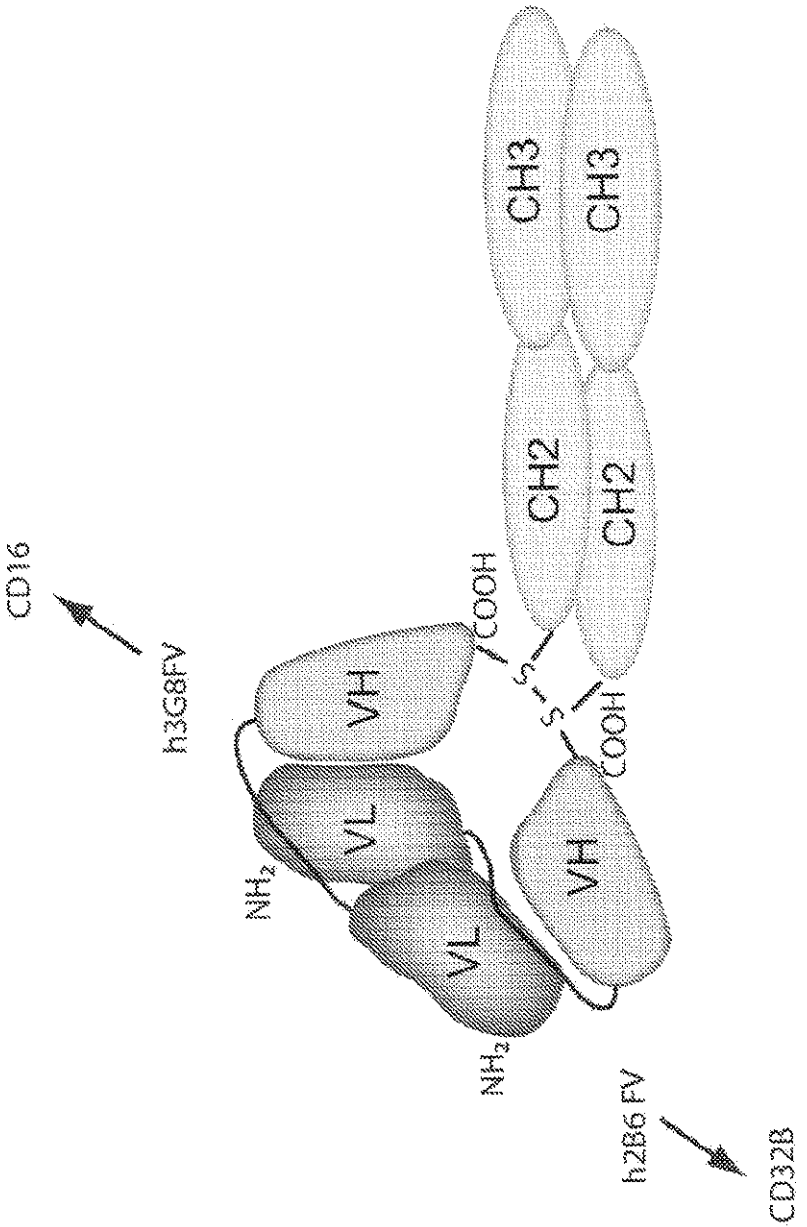

CD16
h3G8FV
VH
VL
NH2
COOH
S
S
CH3
CH3
CH2
CH2
VL
VH
NH2
COOH
h2B6 FV
CD32B

FIG. 11

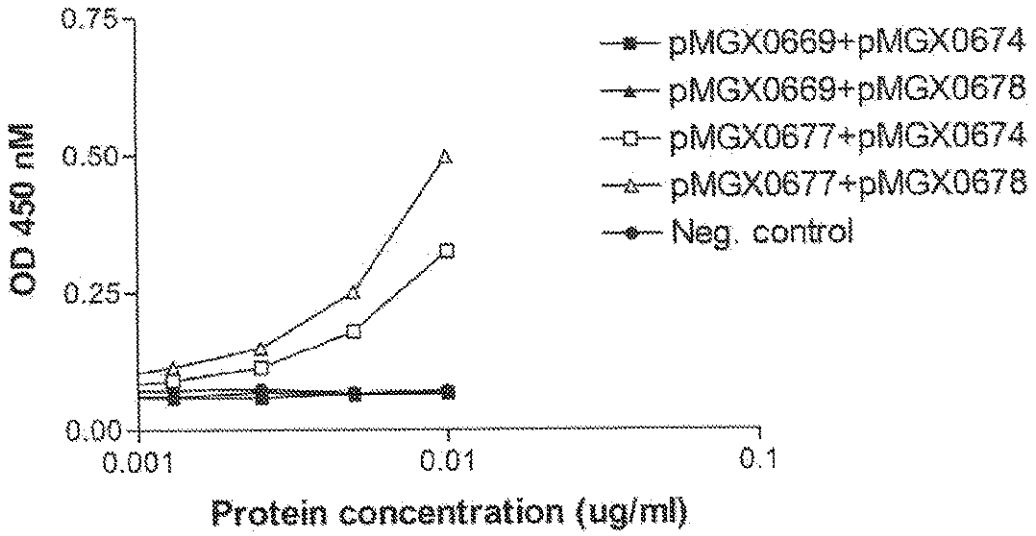

0.75
0.50
0.25
0.00
OD 450 nM
0.001
0.01
0.1
Protein concentration (ug/ml)
pMGX0669+pMGX0674
pMGX0669+pMGX0678
pMGX0677+pMGX0674
pMGX0677+pMGX0678
Neg. control

FIG. 12

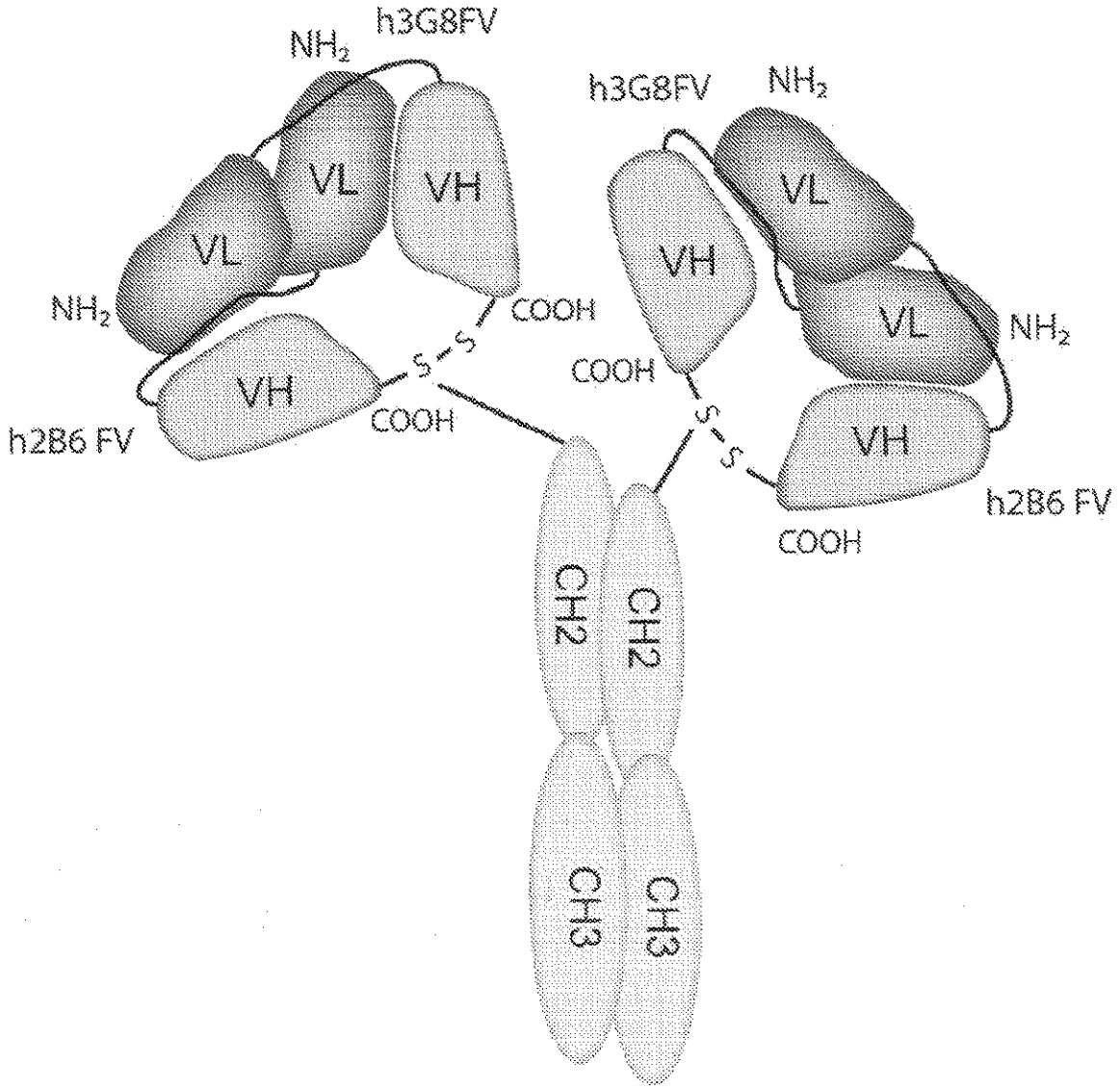

h3G8FV
NH2
VL
VH
VL
NH2
COOH
S
S
VH
COOH
h2B6 FV
h3G8FV
NH2
VL
VH
VL
NH2
COOH
S
S
VH
h2B6 FV
COOH
CH2
CH2
CH3
CH3

FIG. 13

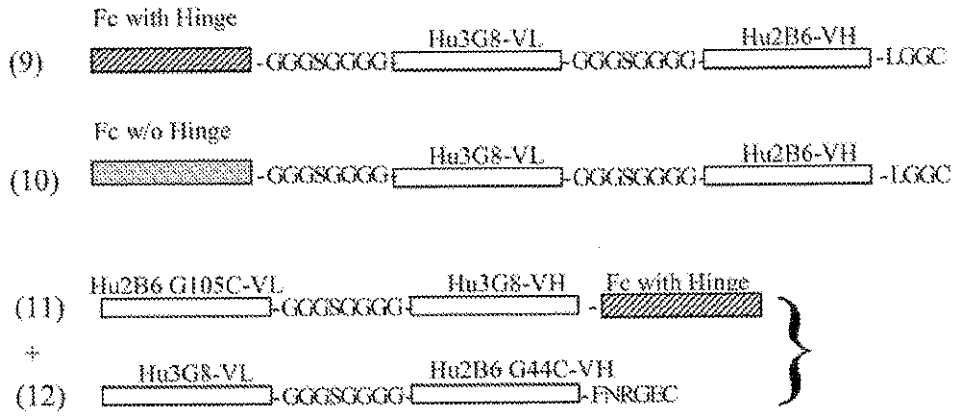

Fc with Hinge
(9)
Hu3G8-VL
Hu2B6-VH
-GGGSGGGG-
-GGGSGGGG-
-LGGC
Fc w/o Hinge
(10)
Hu3G8-VL
Hu2B6-VH
-GGGSGGGG-
-GGGSGGGG-
-LGGC
Hu2B6 G105C-VL
Hu3G8-VH
Fc with Hinge
(11)
-GGGSGGGG-
-
+
Hu3G8-VL
Hu2B6 G44C-VH
(12)
-GGGSGGGG-
-FNRGEC

FIG. 14

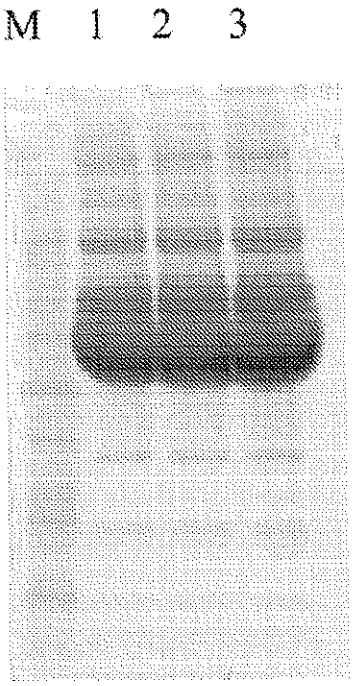


FIG. 15A

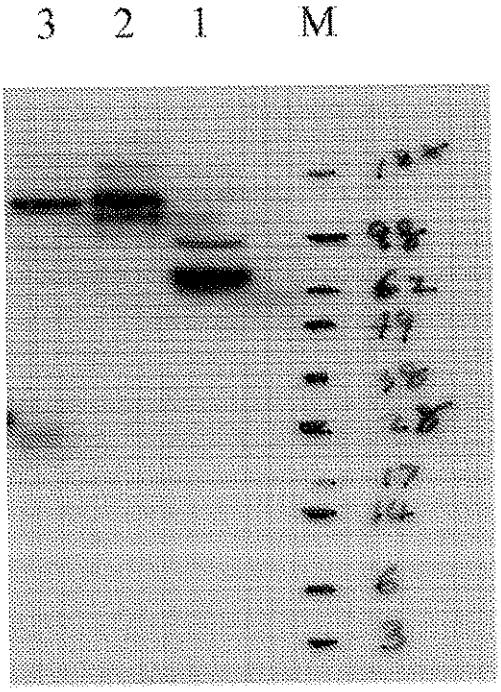


FIG. 15B

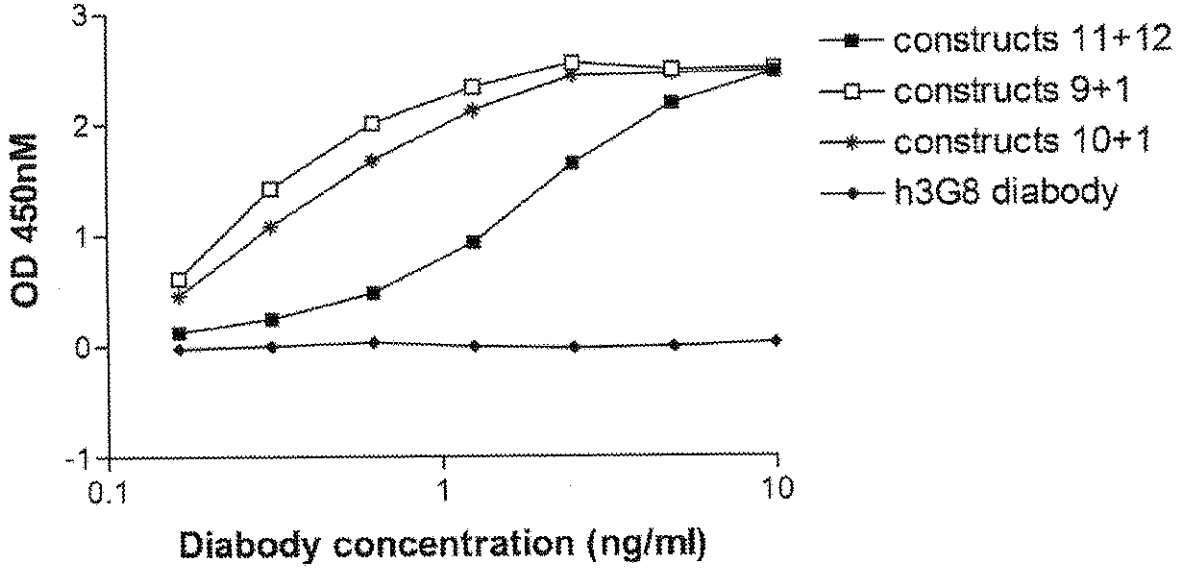

3
2
1
0
-1
OD 450nM
0.1
1
10
Diabody concentration (ng/ml)
constructs 11+12
constructs 9+1
constructs 10+1
h3G8 diabody

FIG. 16

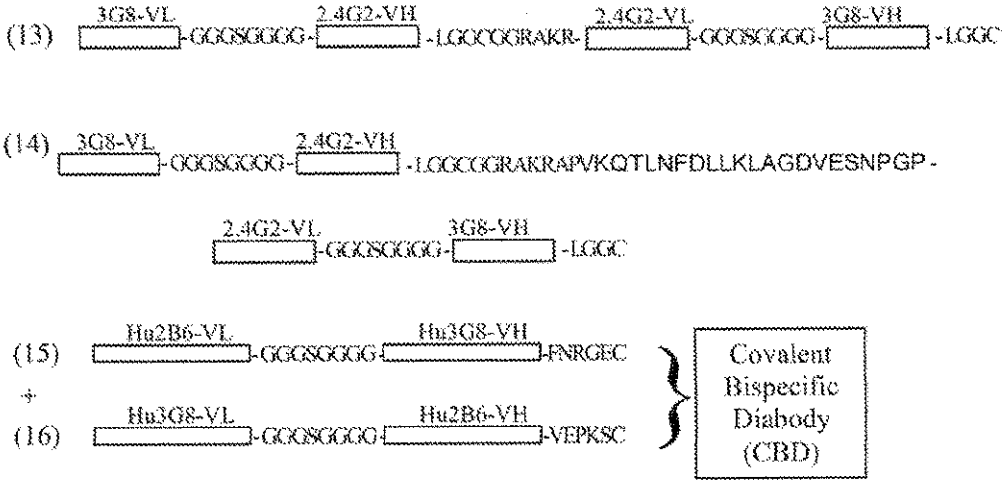

(13) 3G8-VL -GGGSGGGG- 2.4G2-VH -LGGCGGRAKR- 2.4G2-VL -GGGSGGGG- 3G8-VH -LGGC
(14) 3G8-VL -GGGSGGGG- 2.4G2-VH -LGGCGGRAKRAPVKQTLNFDLLKLAGDVESNPGP-
2.4G2-VL -GGGSGGGG- 3G8-VH -LGGC
(15) Hu2B6-VL -GGGSGGGG- Hu3G8-VH -FNRGEC
+
(16) Hu3G8-VL -GGGSGGGG- Hu2B6-VH -VEPKSC
Covalent Bispecific Diabody (CBD)

FIG. 17

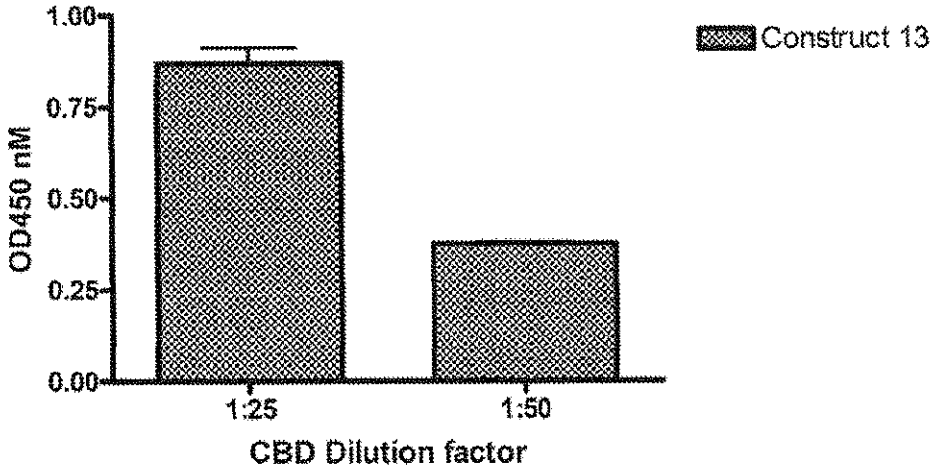

1.00
0.75
0.50
0.25
0.00
OD450 nM
1:25
1:50
CBD Dilution factor
Construct 13

FIG. 18

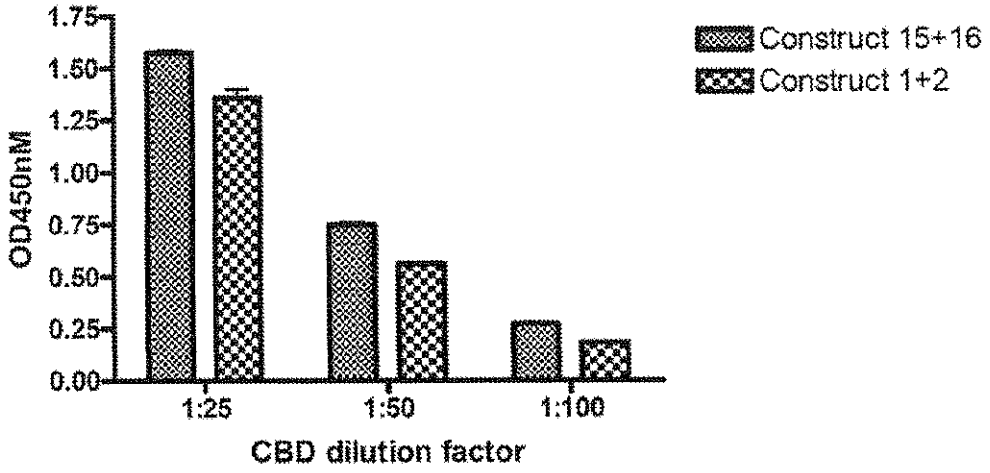

1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
OD450nM
1:25
1:50
1:100
CBD dilution factor
Construct 15+16
Construct 1+2

FIG. 19

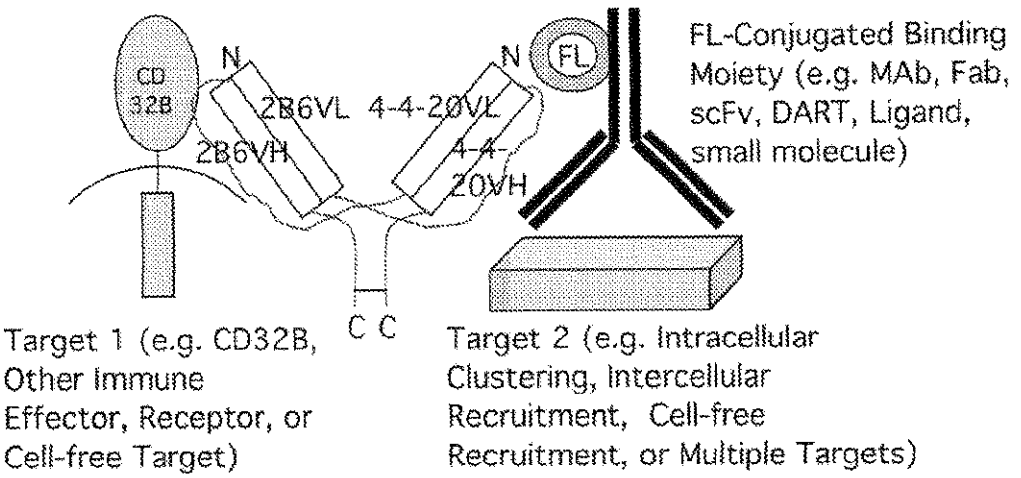

CD 32B
N
2B6VL
2B6VH
4-4-20VL
4-4-20VH
N
FL
FL-Conjugated Binding Moiety (e.g. MAb, Fab, scFv, DART, Ligand, small molecule)
C C
Target 1 (e.g. CD32B, Other Immune Effector, Receptor, or Cell-free Target)
Target 2 (e.g. Intracellular Clustering, Intercellular Recruitment, Cell-free Recruitment, or Multiple Targets)

FIG. 20

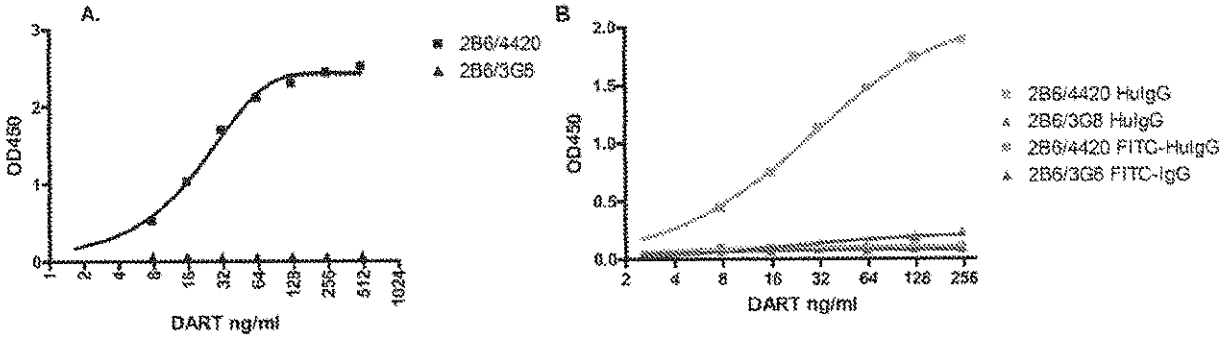


FIG. 21

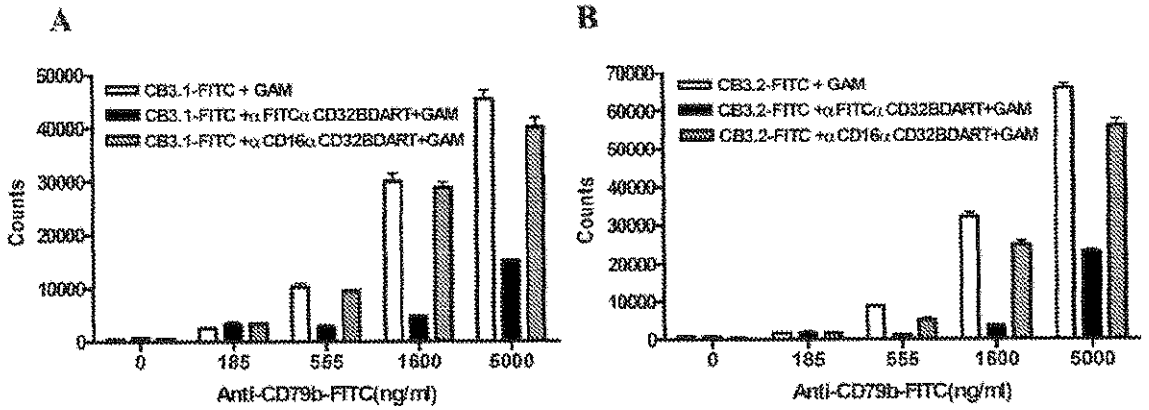


FIG. 22

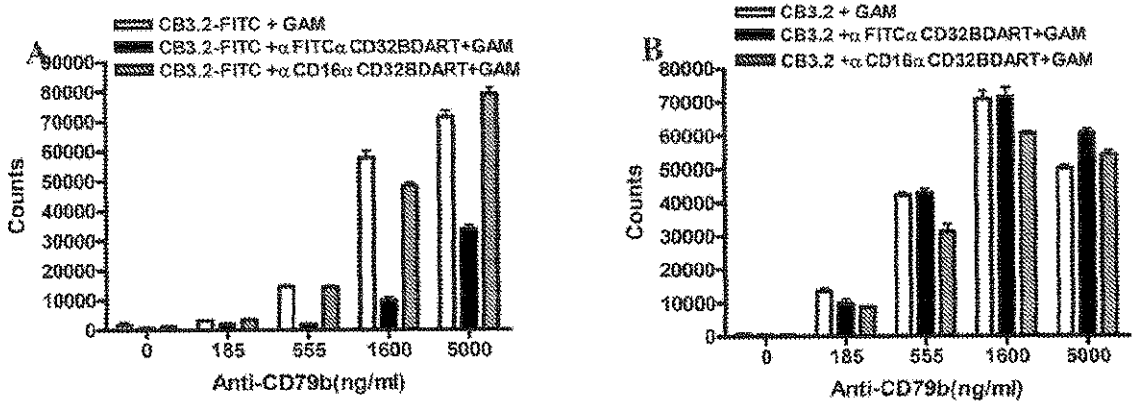


FIG. 23

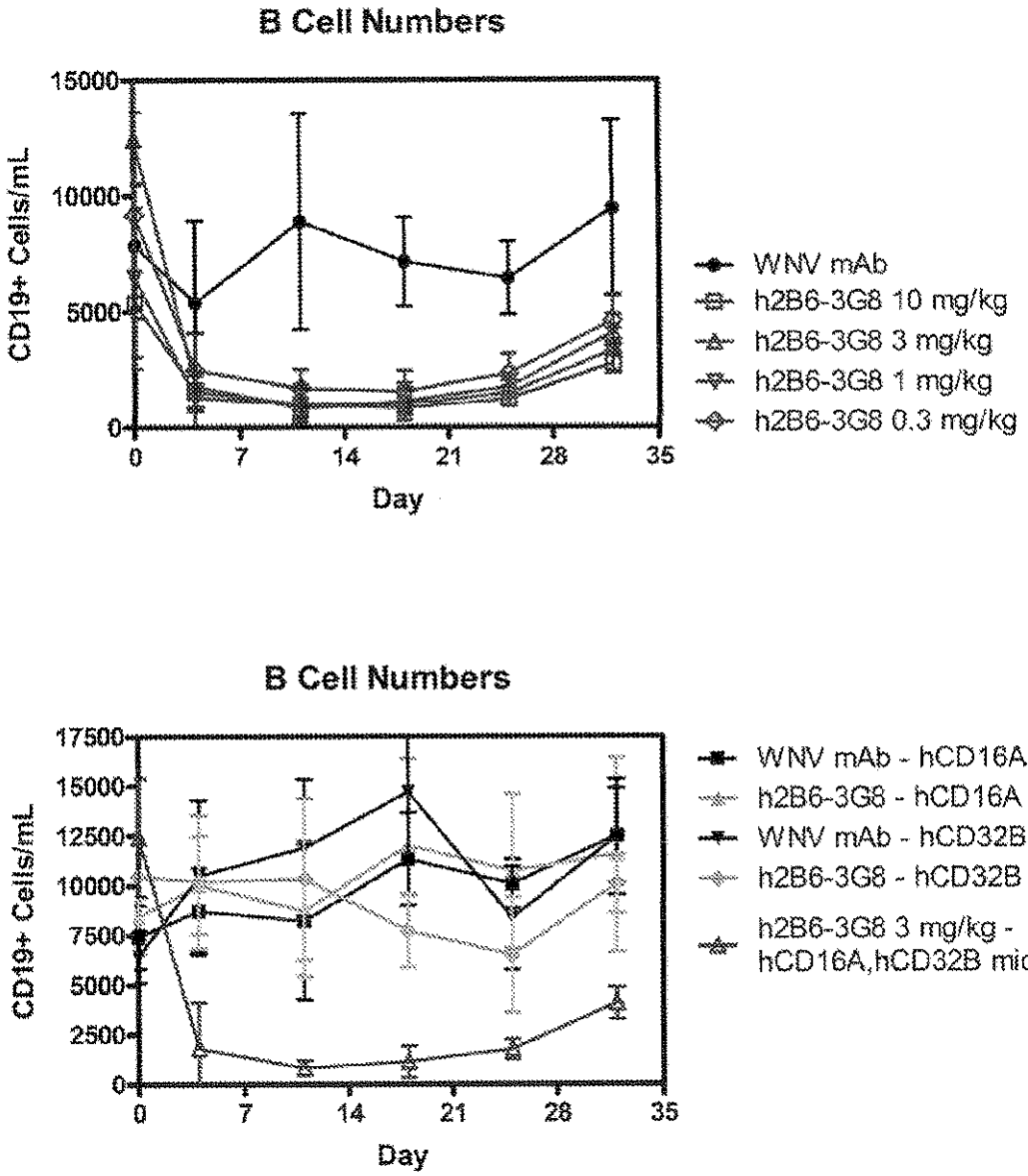

B Cell Numbers
CD19+ Cells/mL
15000
10000
5000
0
0
7
14
21
28
35
Day
WNV mAb
h2B6-3G8 10 mg/kg
h2B6-3G8 3 mg/kg
h2B6-3G8 1 mg/kg
h2B6-3G8 0.3 mg/kg
B Cell Numbers
CD19+ Cells/mL
17500
15000
12500
10000
7500
5000
2500
0
0
7
14
21
28
35
Day
WNV mAb - hCD16A mice
h2B6-3G8 - hCD16A mice
WNV mAb - hCD32B mice
h2B6-3G8 - hCD32B mice
h2B6-3G8 3 mg/kg - hCD16A,hCD32B mice

FIG. 24

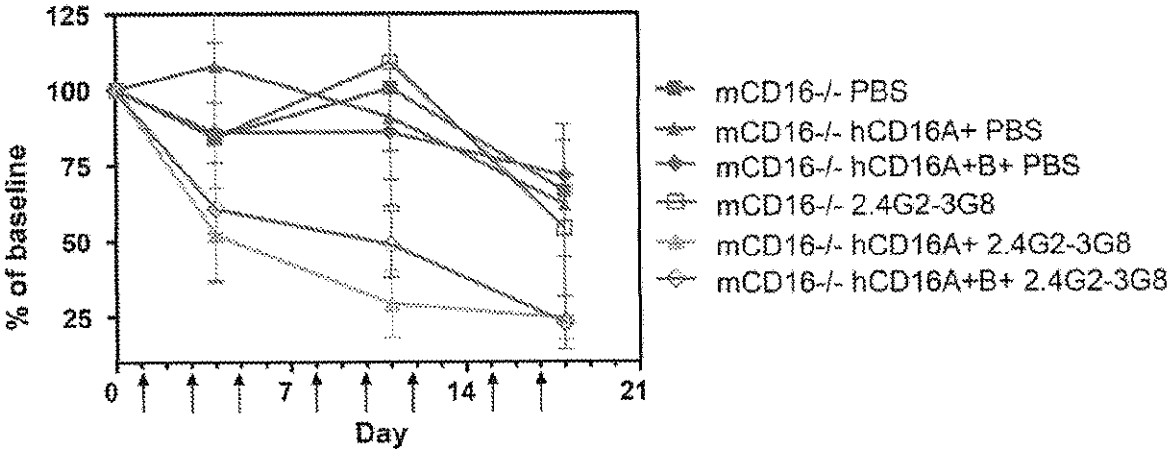

125
100
75
50
25
% of baseline
0
7
14
21
Day
mCD16-/- PBS
mCD16-/- hCD16A+ PBS
mCD16-/- hCD16A+B+ PBS
mCD16-/- 2.4G2-3G8
mCD16-/- hCD16A+ 2.4G2-3G8
mCD16-/- hCD16A+B+ 2.4G2-3G8

FIG. 25

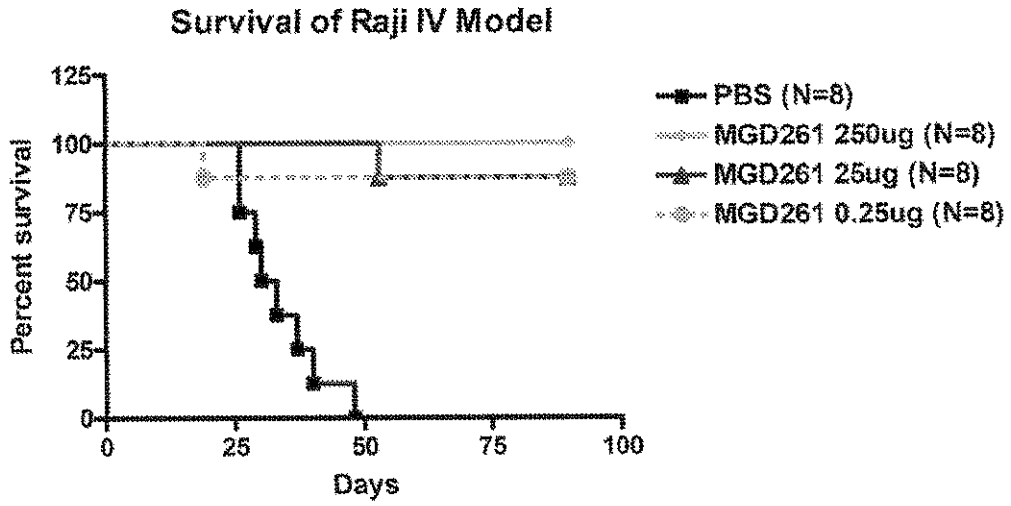

Survival of Raji IV Model
125
100
75
50
25
0
Percent survival
0
25
50
75
100
Days
PBS (N=8)
MGD261 250ug (N=8)
MGD261 25ug (N=8)
MGD261 0.25ug (N=8)

FIG. 26

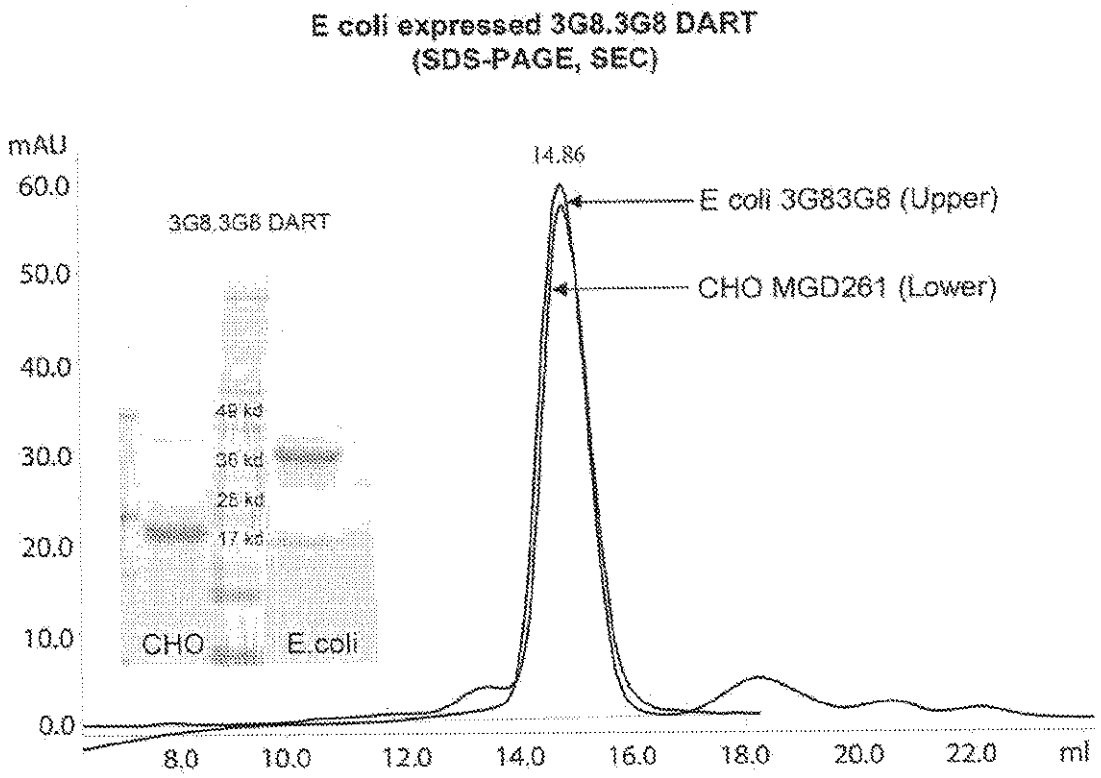

E coli expressed 3G8.3G8 DART
(SDS-PAGE, SEC)
mAU
60.0
50.0
40.0
30.0
20.0
10.0
0.0
14.86
E coli 3G83G8 (Upper)
CHO MGD261 (Lower)
3G8.3G8 DART
49 kd
36 kd
28 kd
17 kd
CHO
E.coli
8.0
10.0
12.0
14.0
16.0
18.0
20.0
22.0
ml

FIG. 27

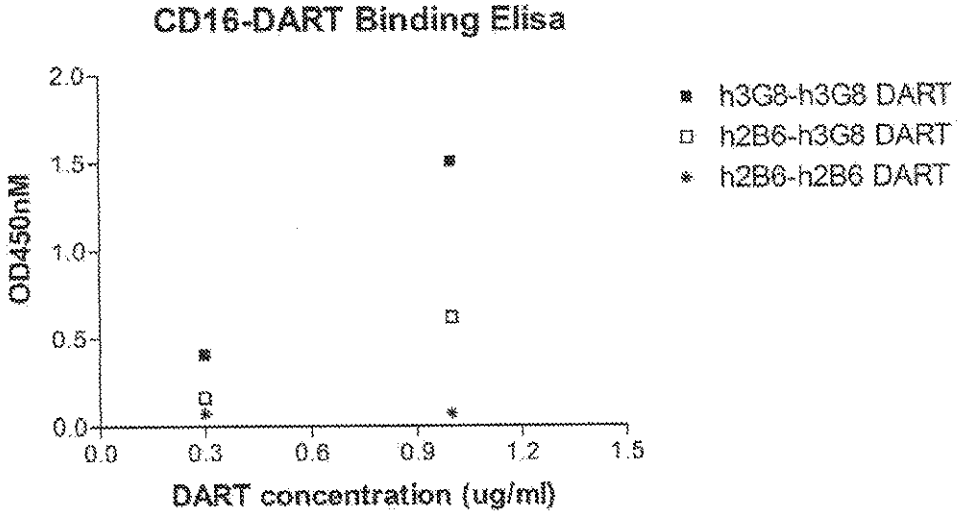

CD16-DART Binding Elisa
OD450nM
2.0
1.5
1.0
0.5
0.0
0.0
0.3
0.6
0.9
1.2
1.5
DART concentration (ug/ml)
h3G8-h3G8 DART
h2B6-h3G8 DART
h2B6-h2B6 DART

FIG. 28

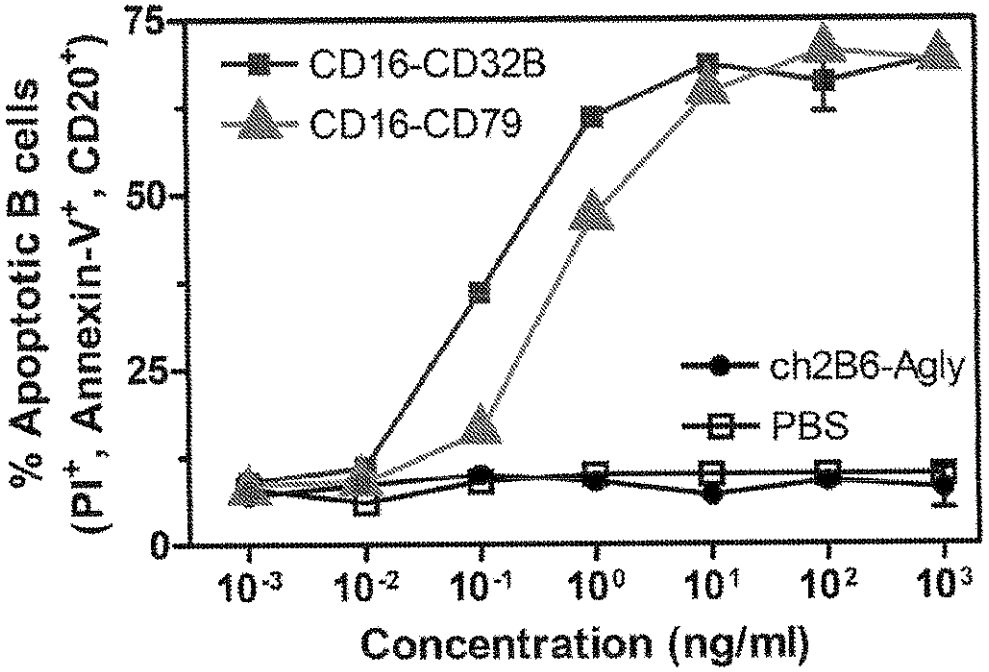

% Apoptotic B cells
(PI+, Annexin-V+, CD20+)
75
50
25
0
CD16-CD32B
CD16-CD79
ch2B6-Agly
PBS
10-3
10-2
10-1
100
101
102
103
Concentration (ng/ml)

FIG. 29

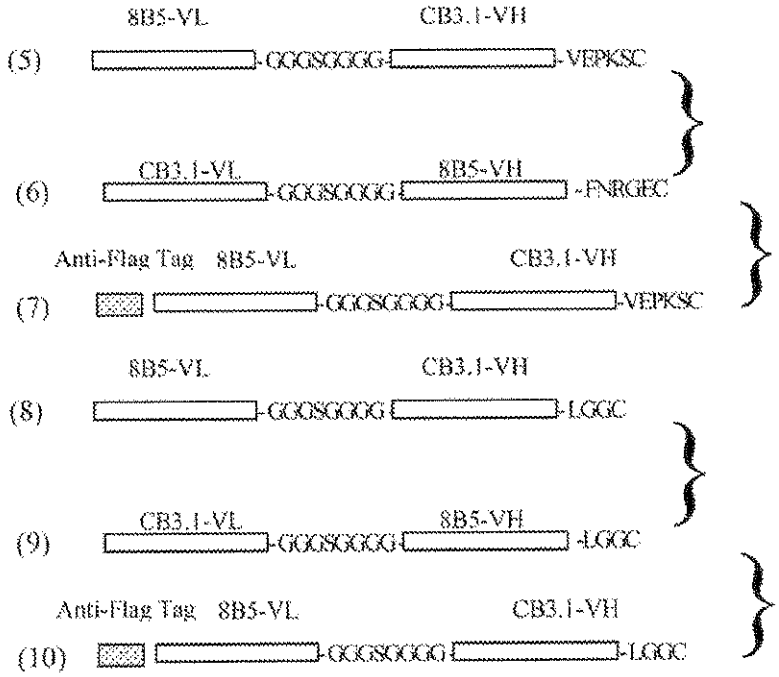

8B5-VL
CB3.1-VH
(5)
-GGGSGGGG-
-VEPKSC
CB3.1-VL
8B5-VH
(6)
-GGGSGGGG-
-FNRGEC
Anti-Flag Tag 8B5-VL
CB3.1-VH
(7)
-GGGSGGGG-
-VEPKSC
8B5-VL
CB3.1-VH
(8)
-GGGSGGGG-
-LGGC
CB3.1-VL
8B5-VH
(9)
-GGGSGGGG-
-LGGC
Anti-Flag Tag 8B5-VL
CB3.1-VH
(10)
-GGGSGGGG-
-LGGC

FIG. 30

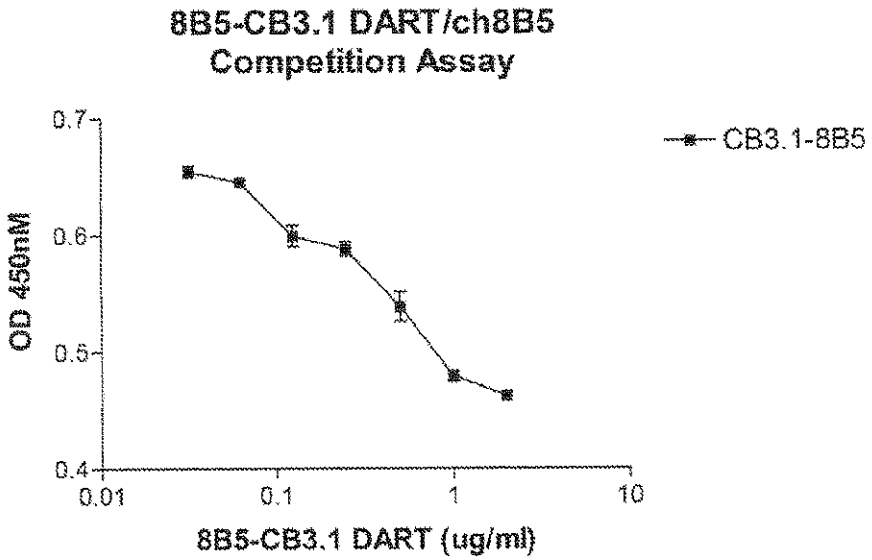

8B5-CB3.1 DART/ch8B5
Competition Assay
CB3.1-8B5
OD 450nM
0.7
0.6
0.5
0.4
0.01
0.1
1
10
8B5-CB3.1 DART (ug/ml)

FIG. 31

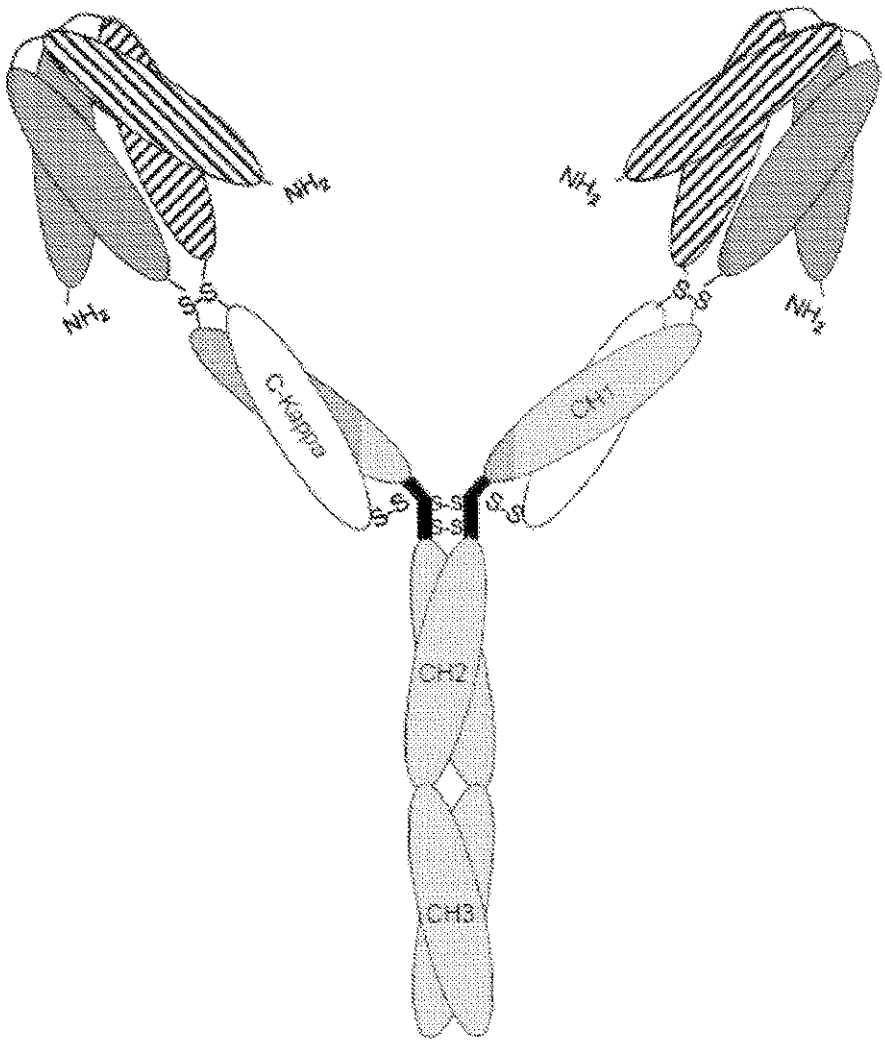
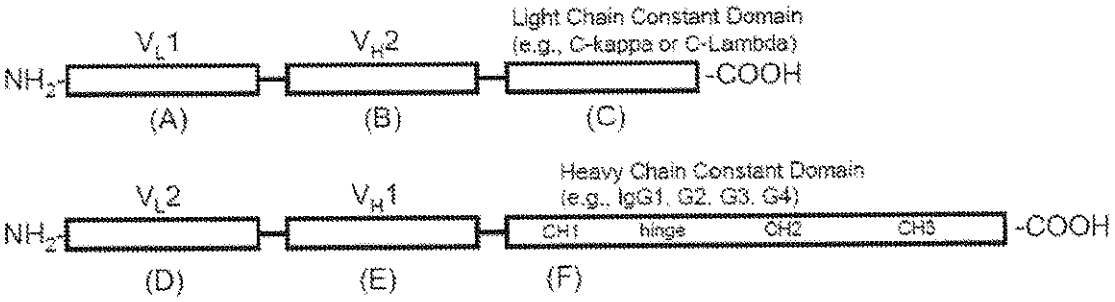


FIG. 32

Ig-Like Tetravalent DART



(A) + (E) = epitope 1 binding site
(D) + (B) = epitope 2 binding site
(C) + (F) = C-kappa/lambda + CH_1 (similar association as a traditional Ig)
hinge-CH2-CH3 will associate to form an Fc

FIG. 33

mCD32-hCD16A Binding ELISA

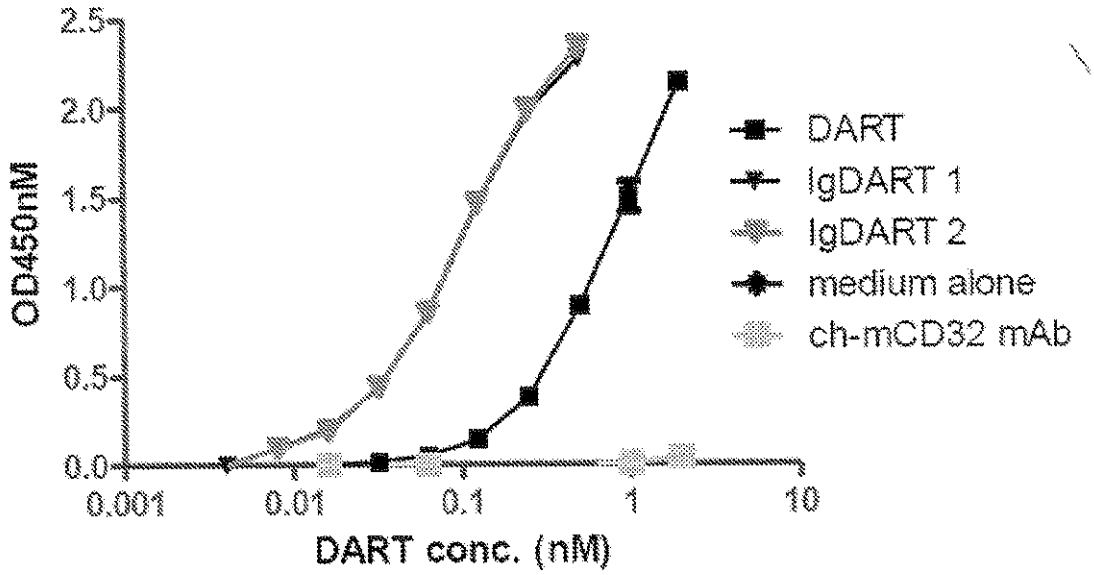


FIG. 34

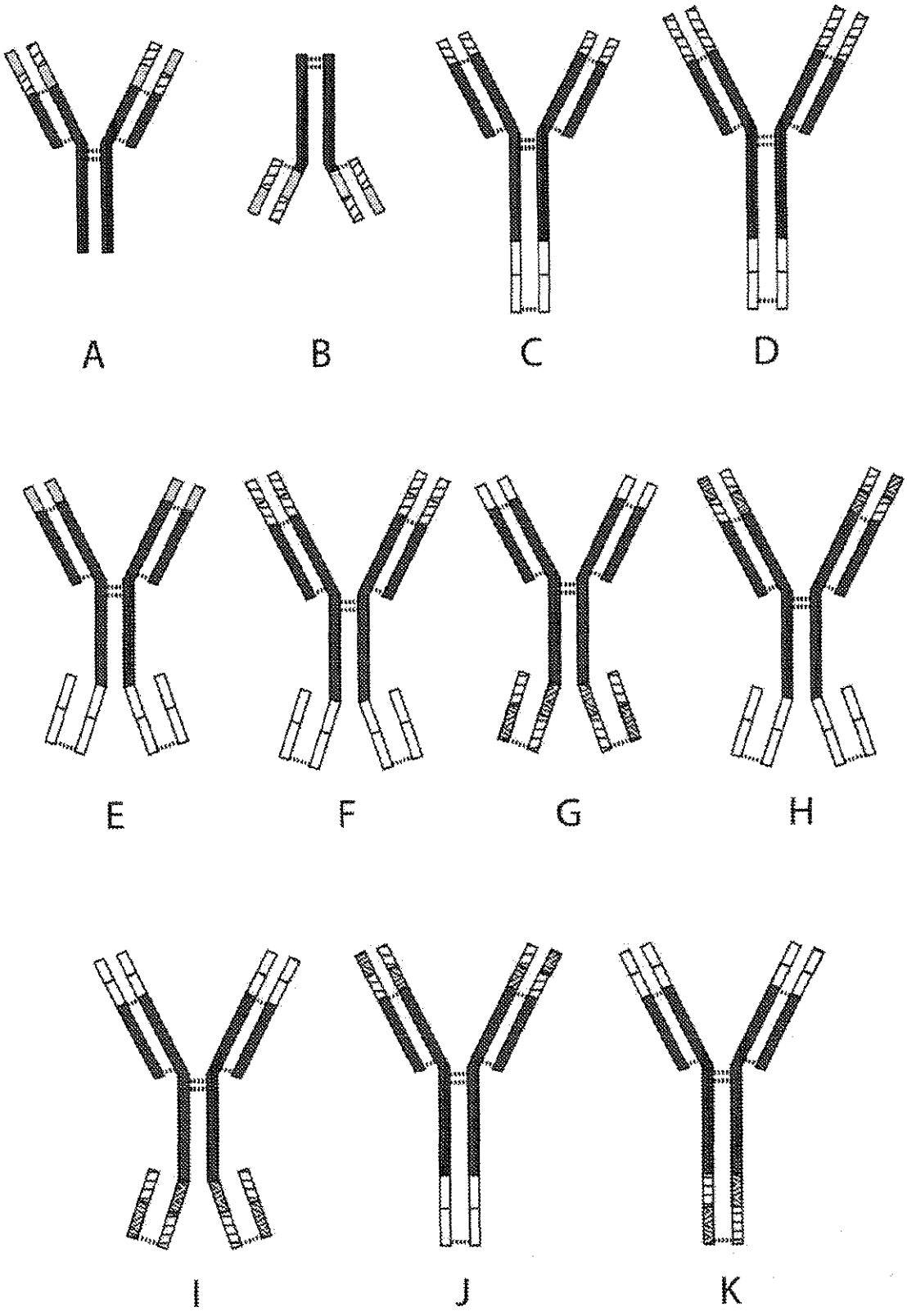


FIG. 35

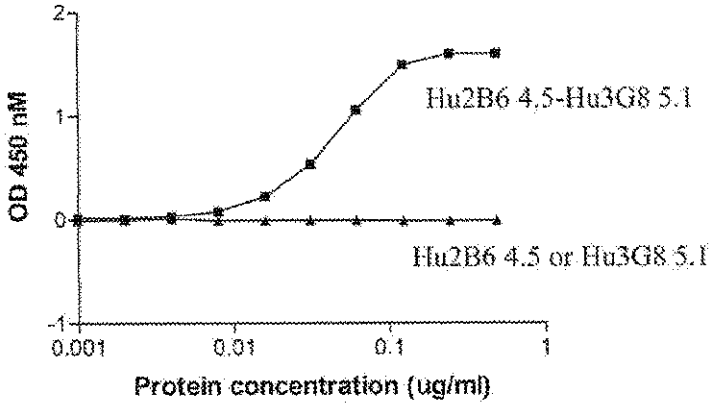

2
1
0
-1
OD 450 nM
Hu2B6 4.5-Hu3G8 5.1
Hu2B6 4.5 or Hu3G8 5.1
0.001
0.01
0.1
1
Protein concentration (ug/ml)

FIG. 36

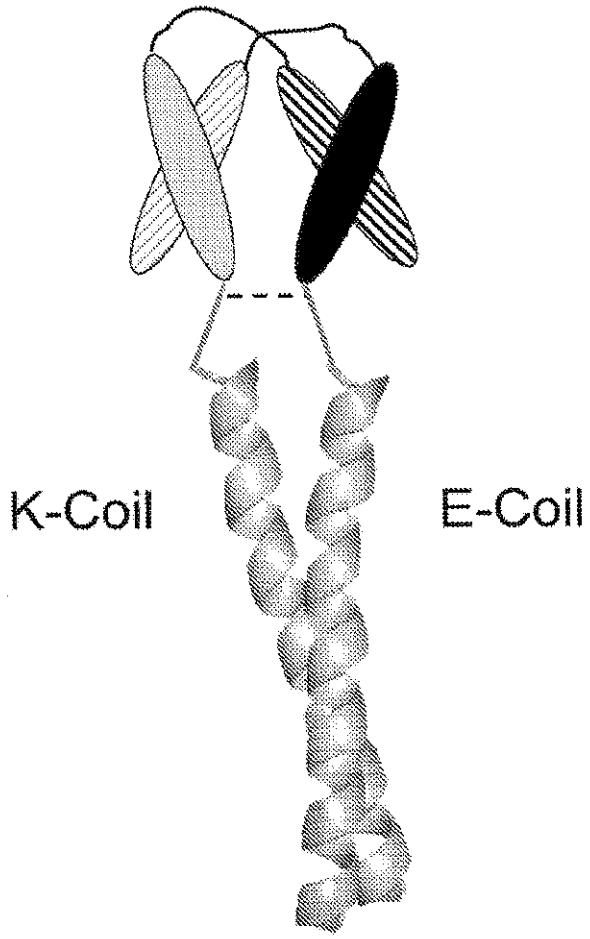


FIG. 37

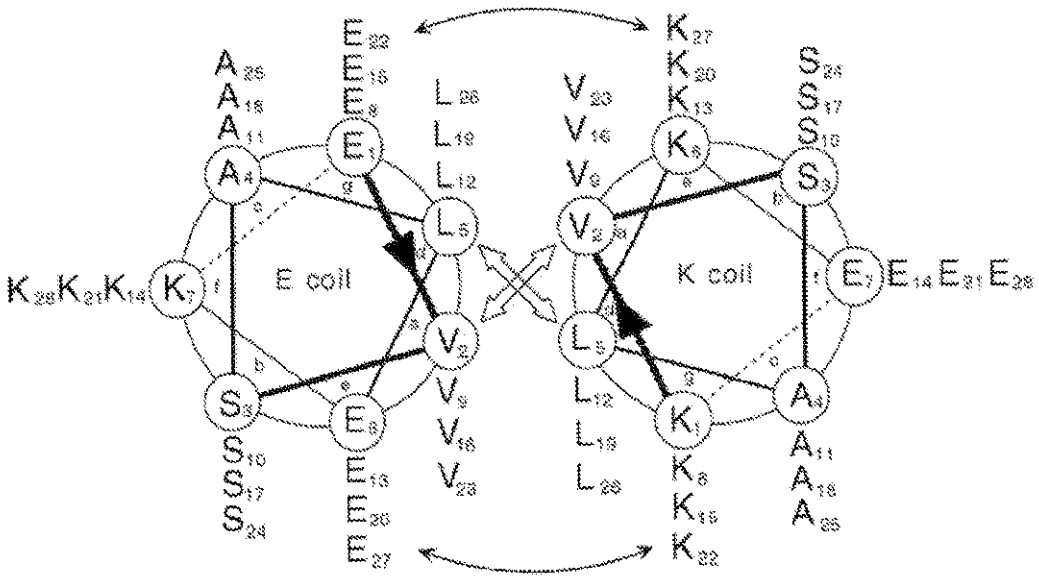


FIG. 38

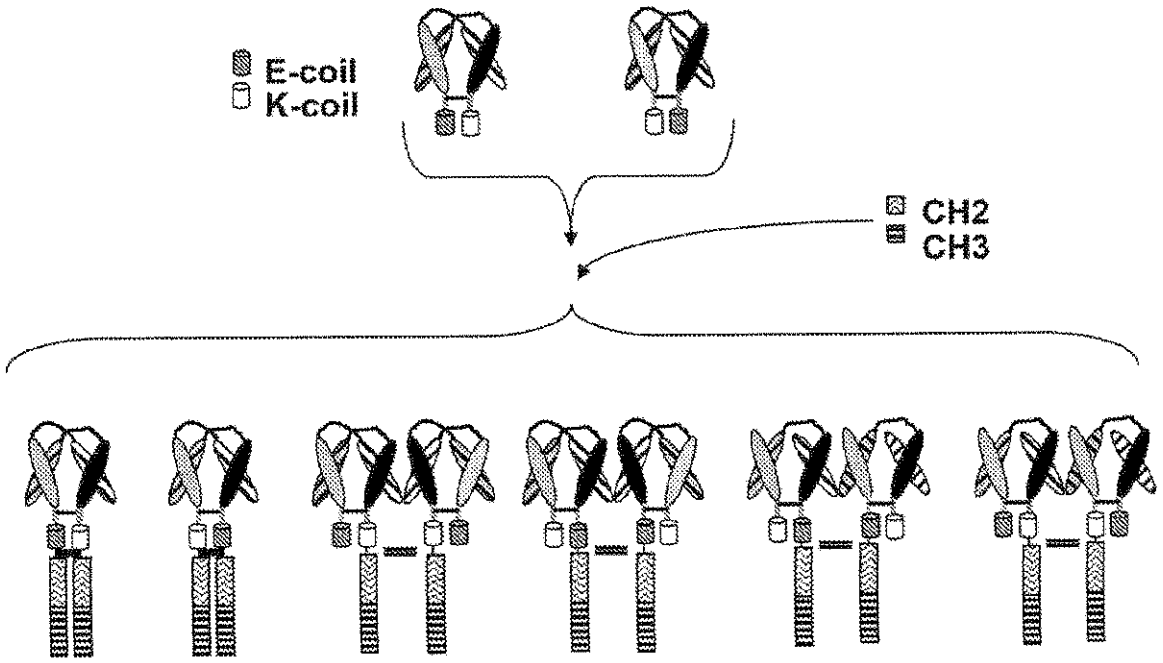


Figure 39

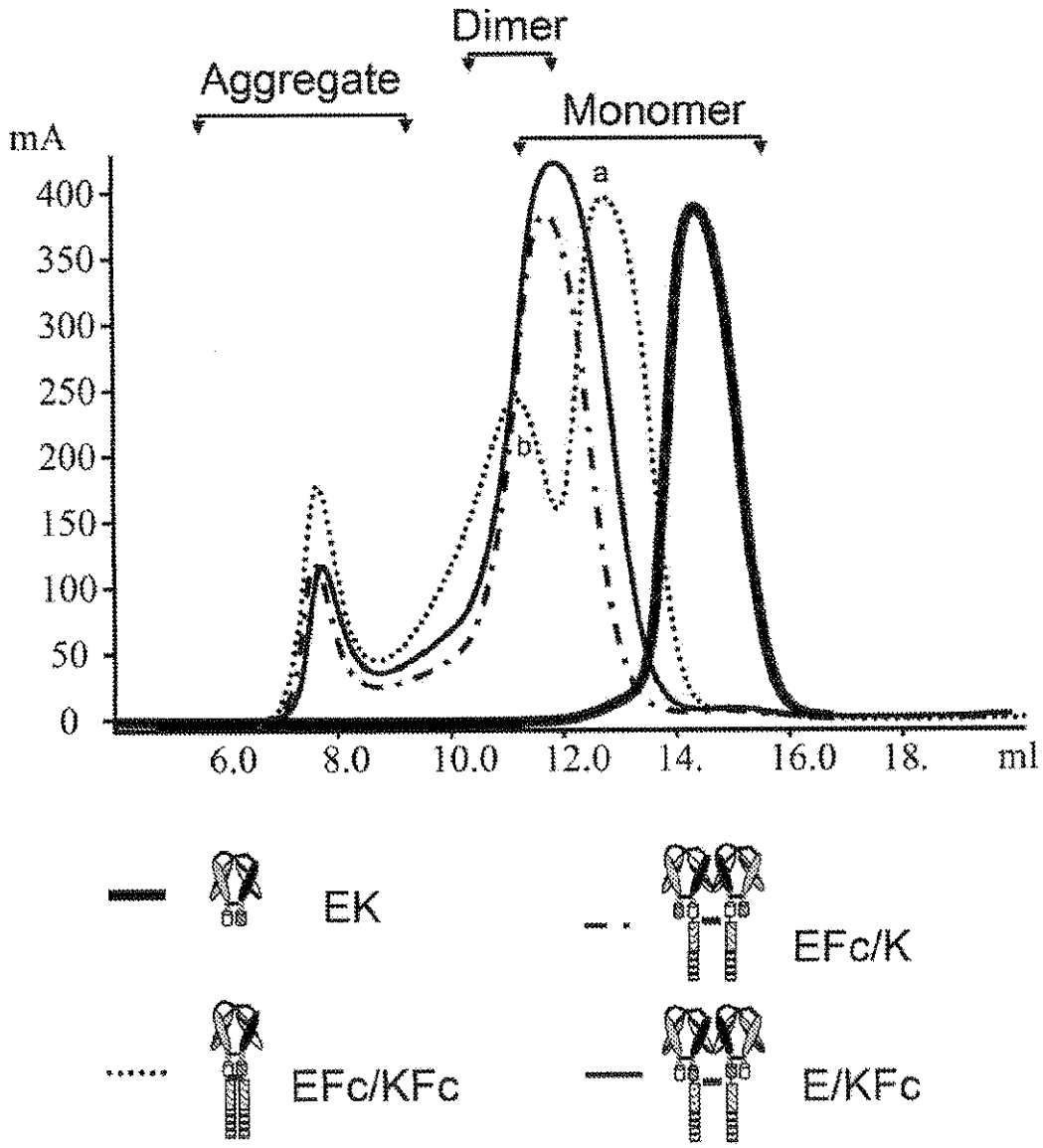

Dimer
Aggregate
Monomer
mA
400
350
300
250
200
150
100
50
0
a
b
6.0
8.0
10.0
12.0
14.
16.0
18.
ml
EK
EFc/K
EFc/KFc
E/KFc

Figure 40

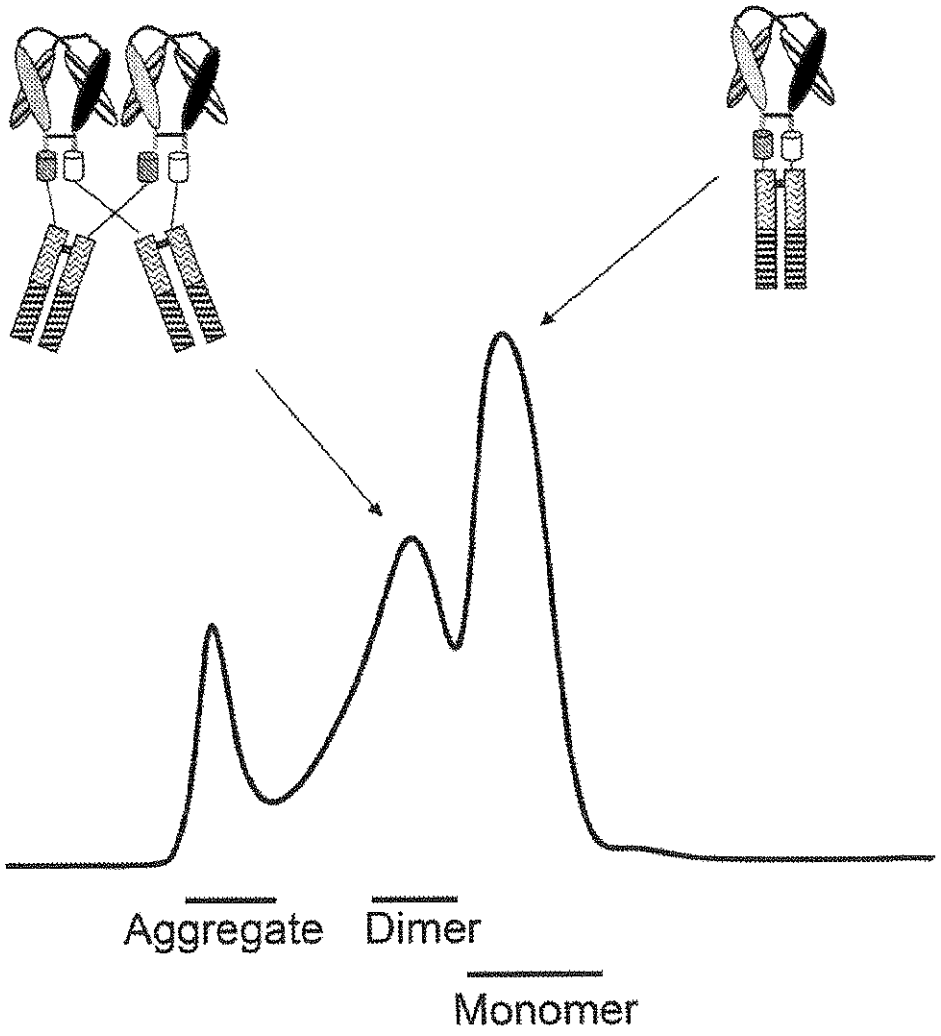


Figure 41

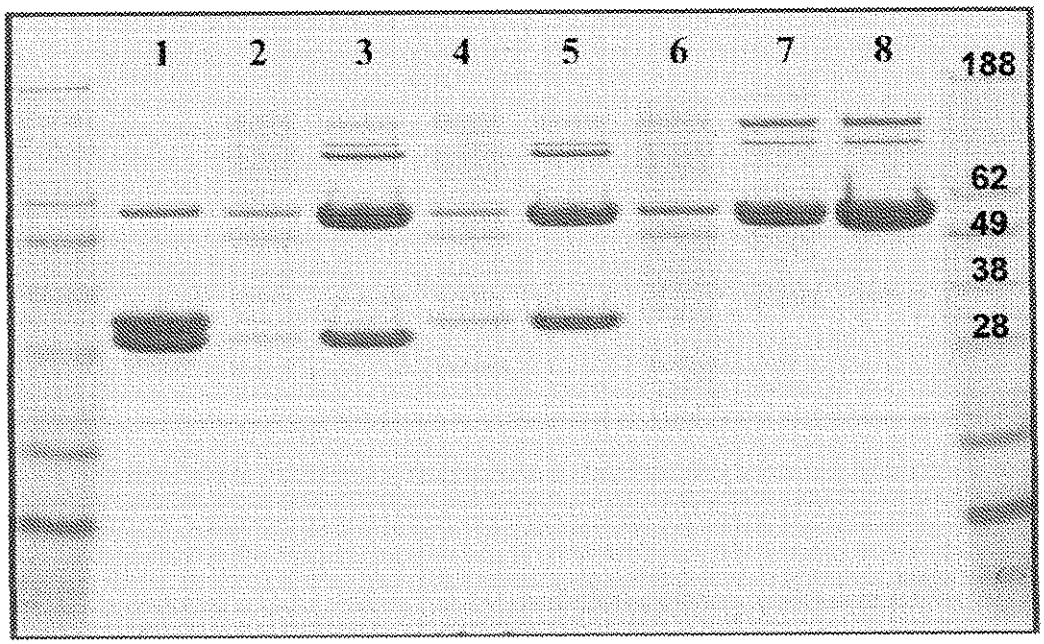


Figure 42

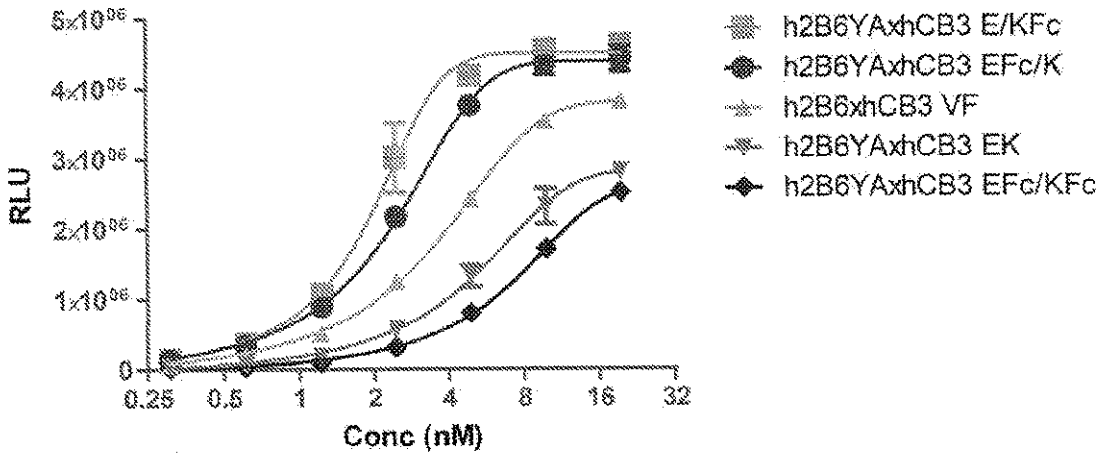

RLU
5x10^06
4x10^06
3x10^06
2x10^06
1x10^06
0
0.25
0.5
1
2
4
8
16
32
Conc (nM)
h2B6YAxhCB3 E/KFc
h2B6YAxhCB3 EFc/K
h2B6xhCB3 VF
h2B6YAxhCB3 EK
h2B6YAxhCB3 EFc/KFc

Figure 43

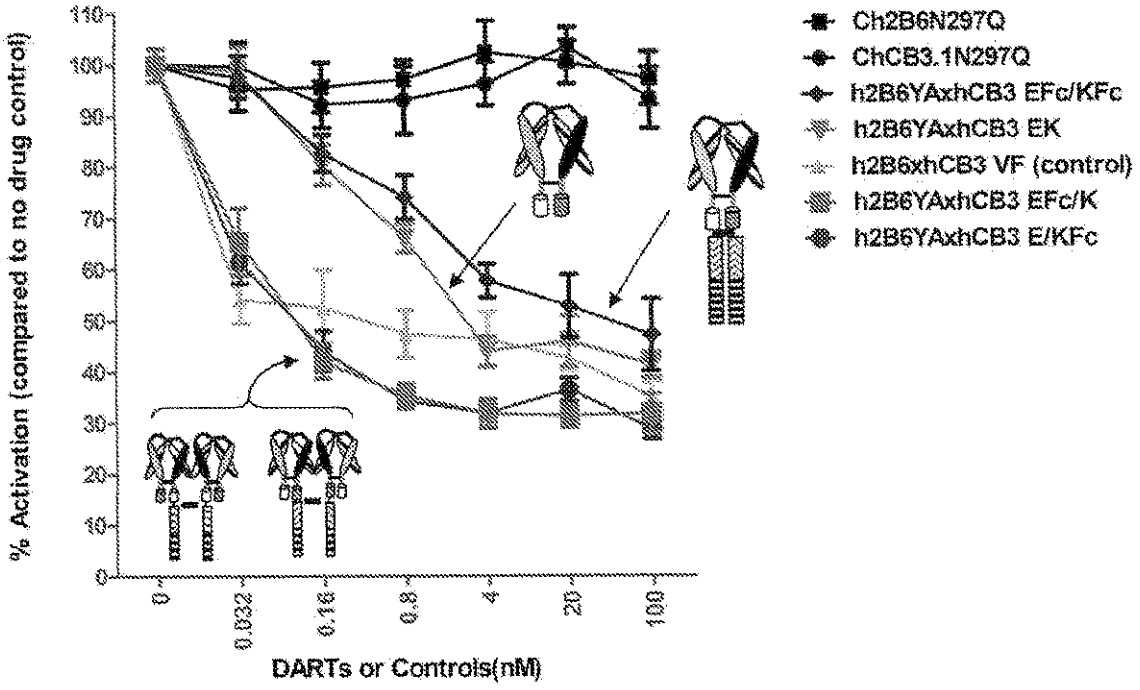

% Activation (compared to no drug control)
110
100
90
80
70
60
50
40
30
20
10
0
0
0.032
0.16
0.8
4
20
100
DARTs or Controls(nM)
Ch2B6N297Q
ChCB3.1N297Q
h2B6YAxhCB3 EFc/KFc
h2B6YAxhCB3 EK
h2B6xhCB3 VF (control)
h2B6YAxhCB3 EFc/K
h2B6YAxhCB3 E/KFc

Figure 44

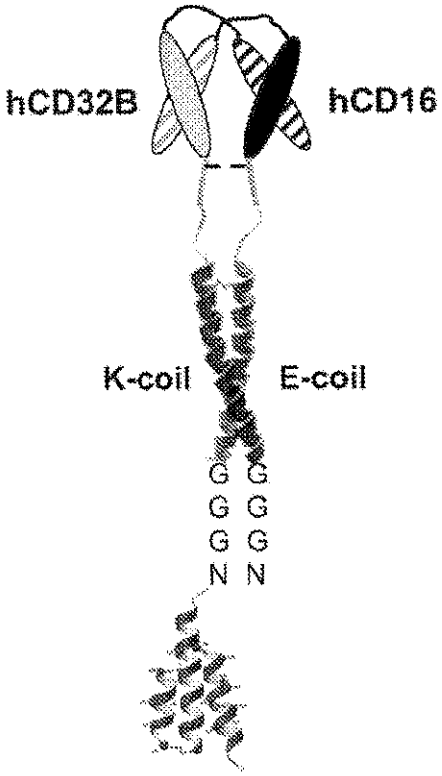


FIG. 45

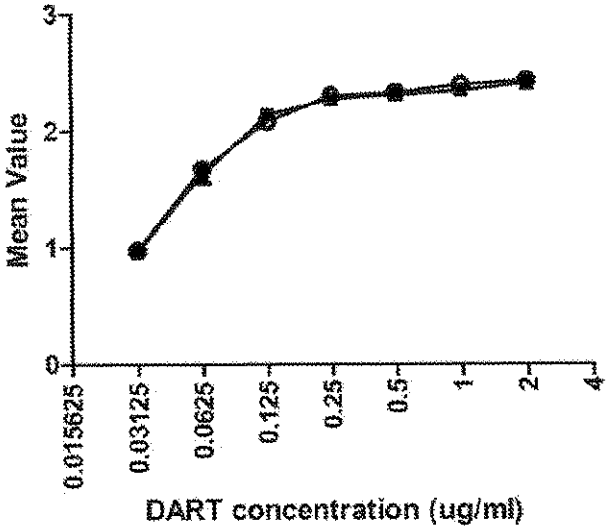


FIG. 46A

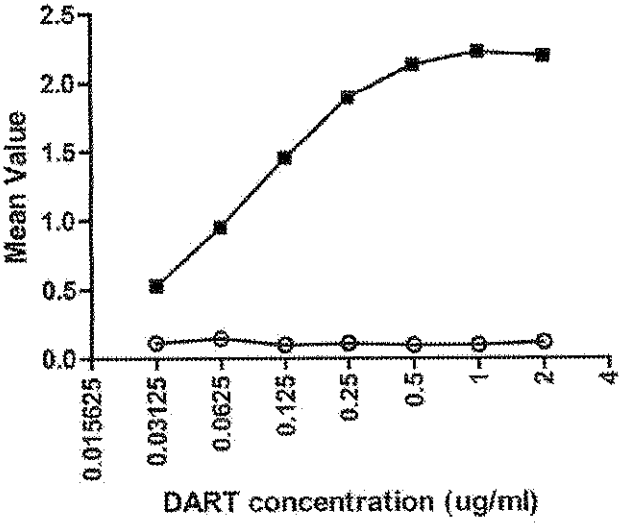


FIG. 46B

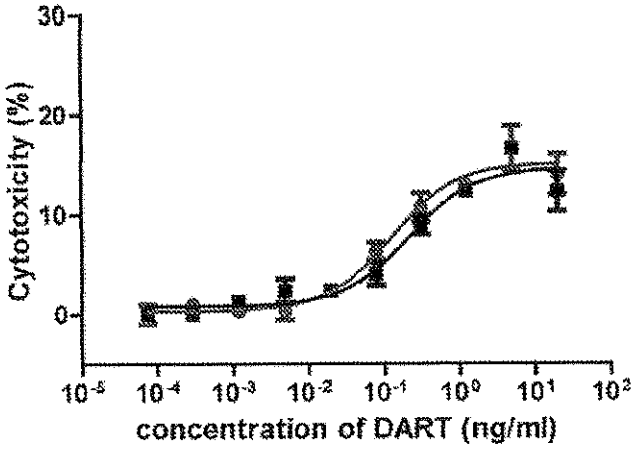

30
20
10
0
Cytotoxicity (%)
10-5 10-4 10-3 10-2 10-1 100 101 102
concentration of DART (ng/ml)

FIG. 47

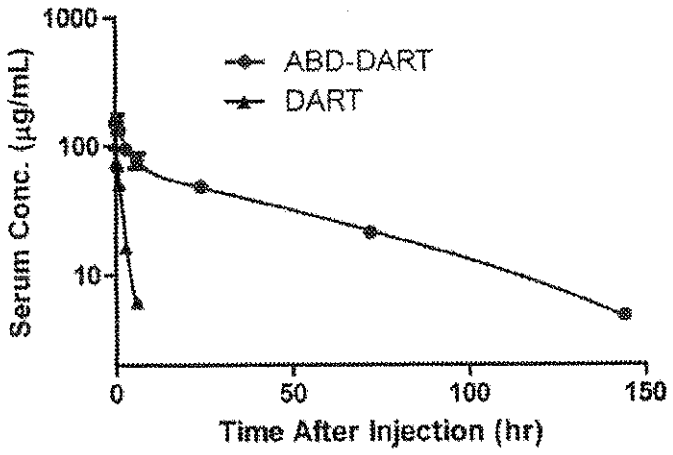

1000
100
10
Serum Conc. (µg/mL)
ABD-DART
DART
0
50
100
150
Time After Injection (hr)

FIG. 48

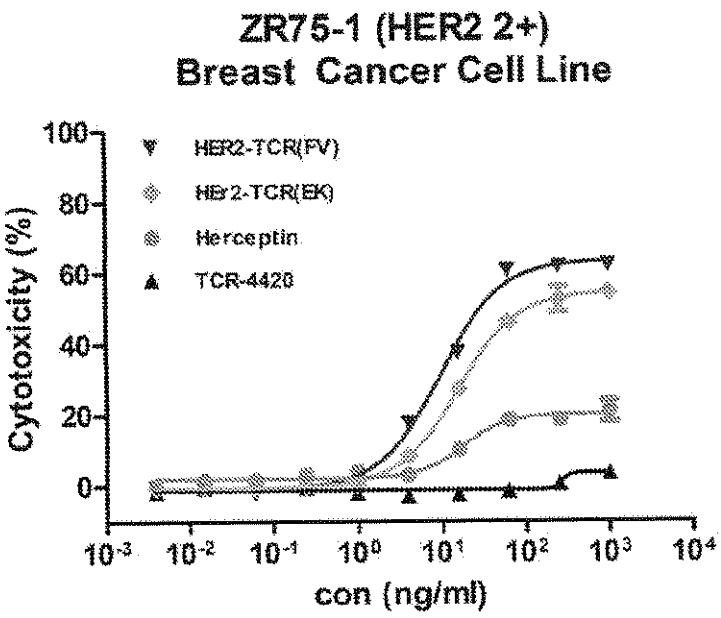


FIG. 49A

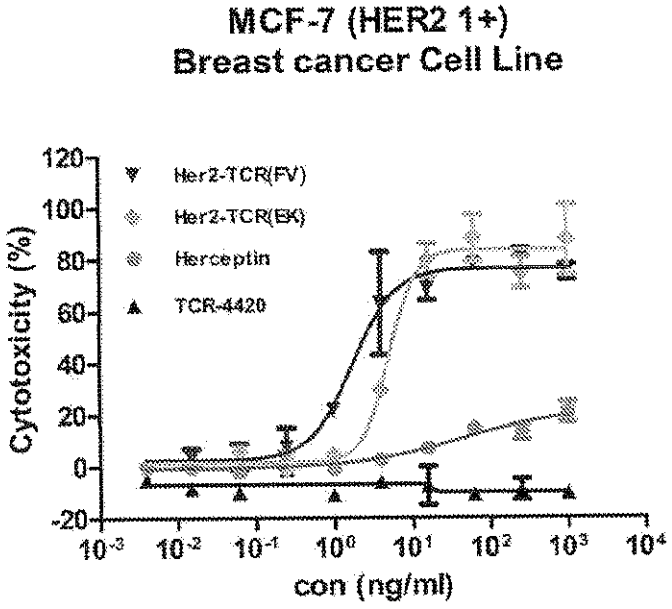


FIG. 49B

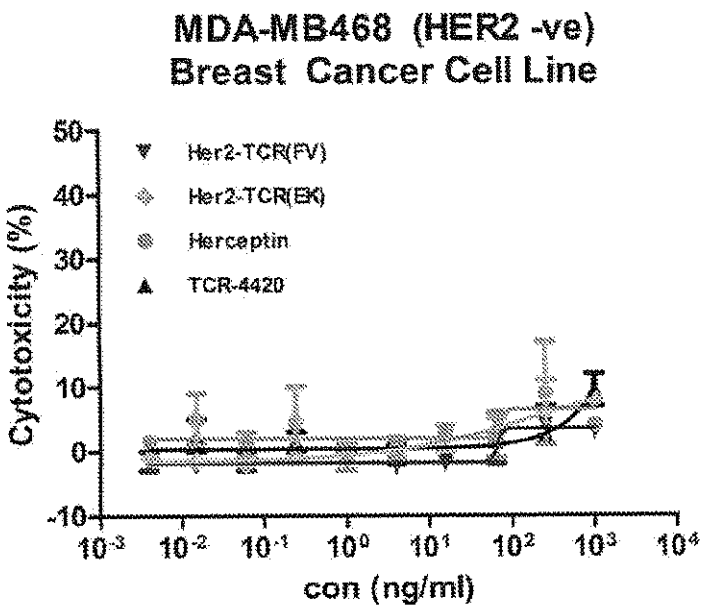


FIG. 49C

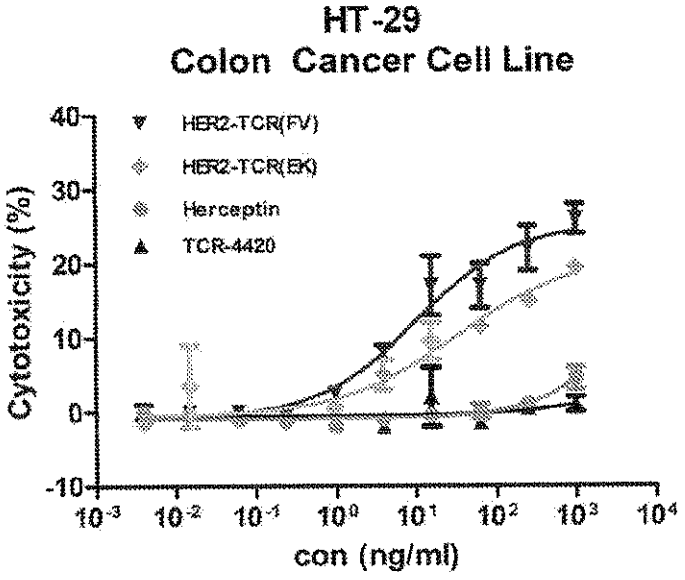


FIG. 49D

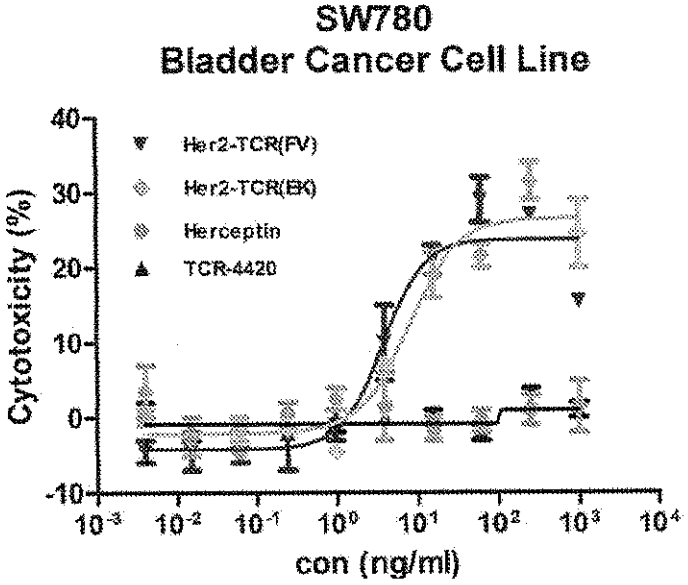

SW780
Bladder Cancer Cell Line
Cytotoxicity (%)
40
30
20
10
0
-10
Her2-TCR(FV)
Her2-TCR(BK)
Herceptin
TCR-4420
10-3
10-2
10-1
100
101
102
103
104
con (ng/ml)

FIG. 49E