

FIG. 1A

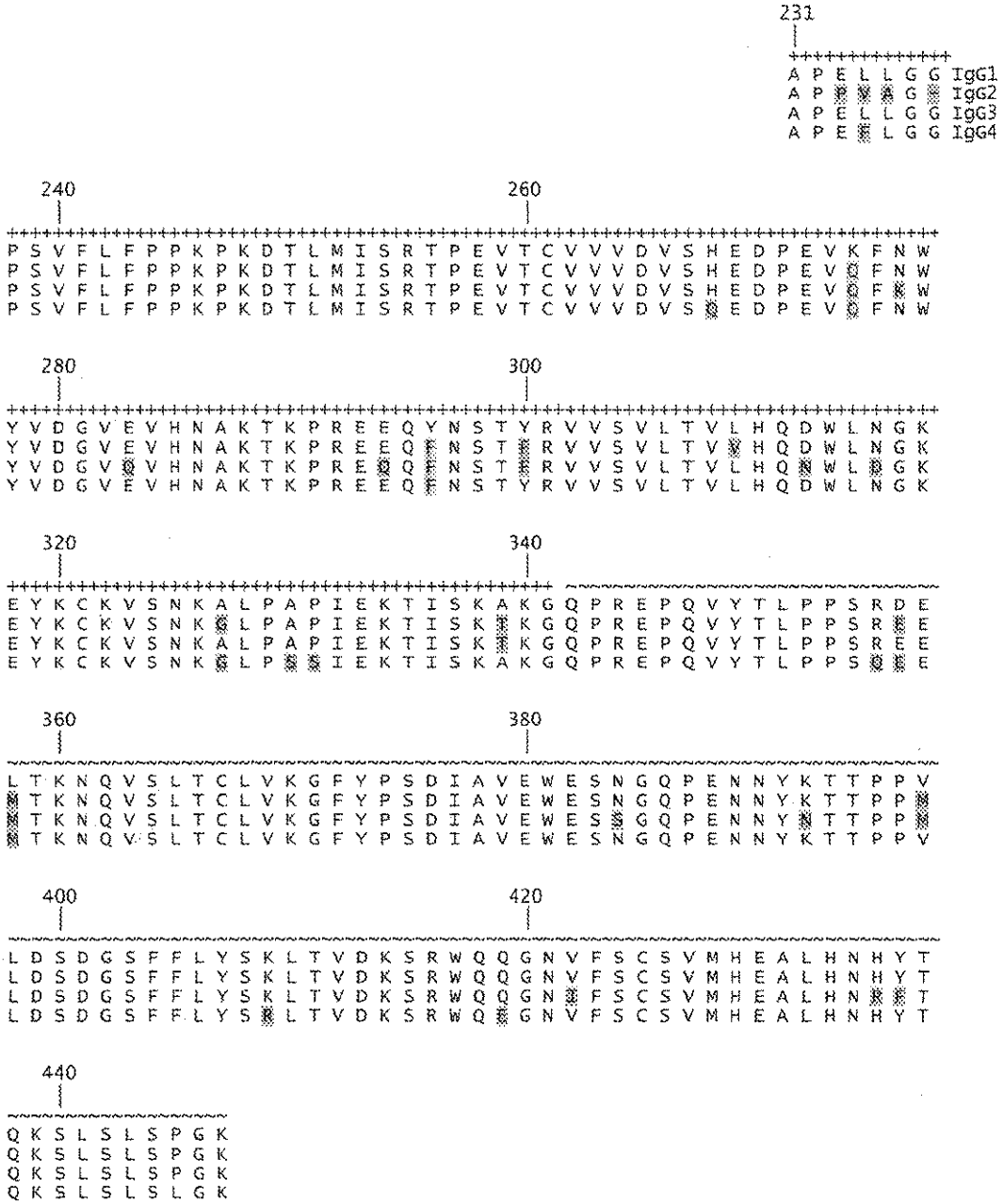

231
A P E L L G G IgG1
A P ▒ ▒ ▒ G ▒ IgG2
A P E L L G G IgG3
A P E ▒ L G G IgG4
240
260
P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S H E D P E V K F N W
P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S H E D P E V Q F N W
P S V F L F P P K P K D T L M I S R T P E V T C V V V 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 Q 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 F 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 F 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 F 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

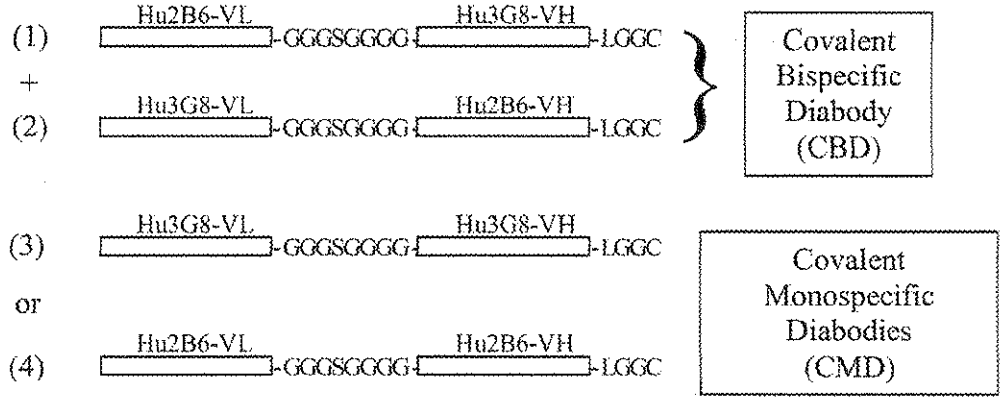

(1)
Hu2B6-VL
-GGGSGGGG-
Hu3G8-VH
-LGGC
+
(2)
Hu3G8-VL
-GGGSGGGG-
Hu2B6-VH
-LGGC
Covalent Bispecific Diabody (CBD)
(3)
Hu3G8-VL
-GGGSGGGG-
Hu3G8-VH
-LGGC
or
(4)
Hu2B6-VL
-GGGSGGGG-
Hu2B6-VH
-LGGC
Covalent Monospecific Diabodies (CMD)

FIG. 2

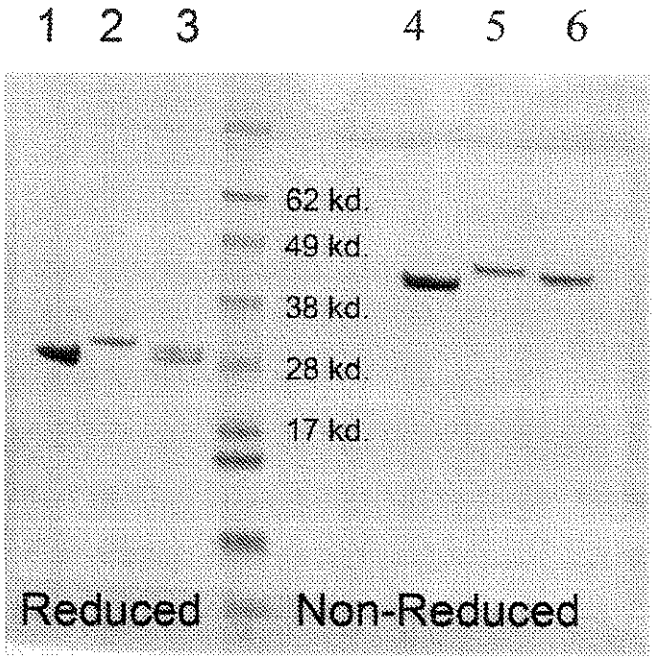


FIG. 3

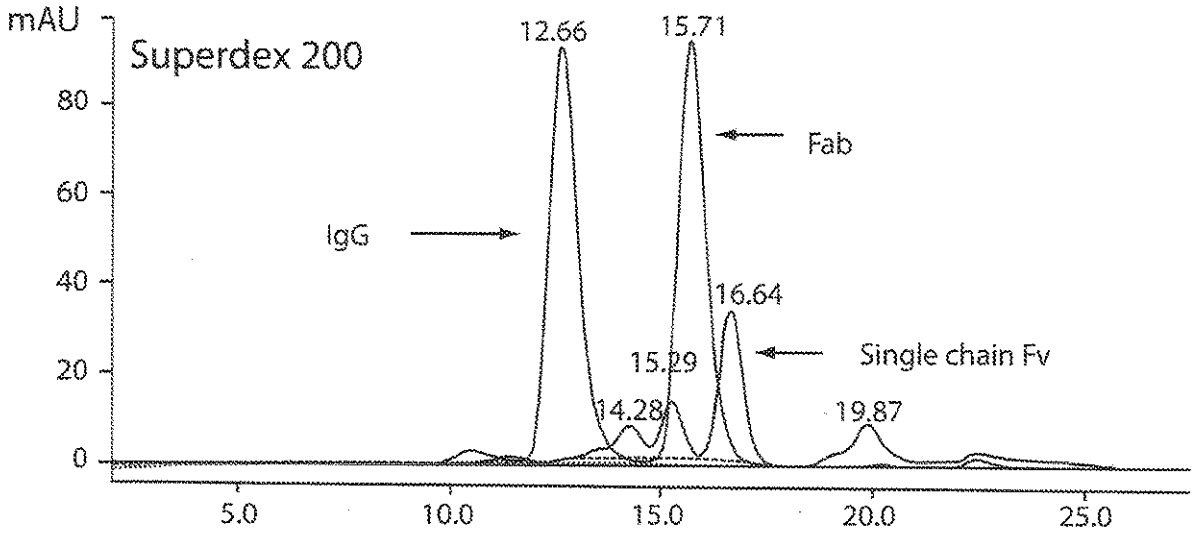

mAU
Superdex 200
12.66
15.71
Fab
IgG
16.64
15.29
Single chain Fv
14.28
19.87
80
60
40
20
0
5.0
10.0
15.0
20.0
25.0

FIG. 4A

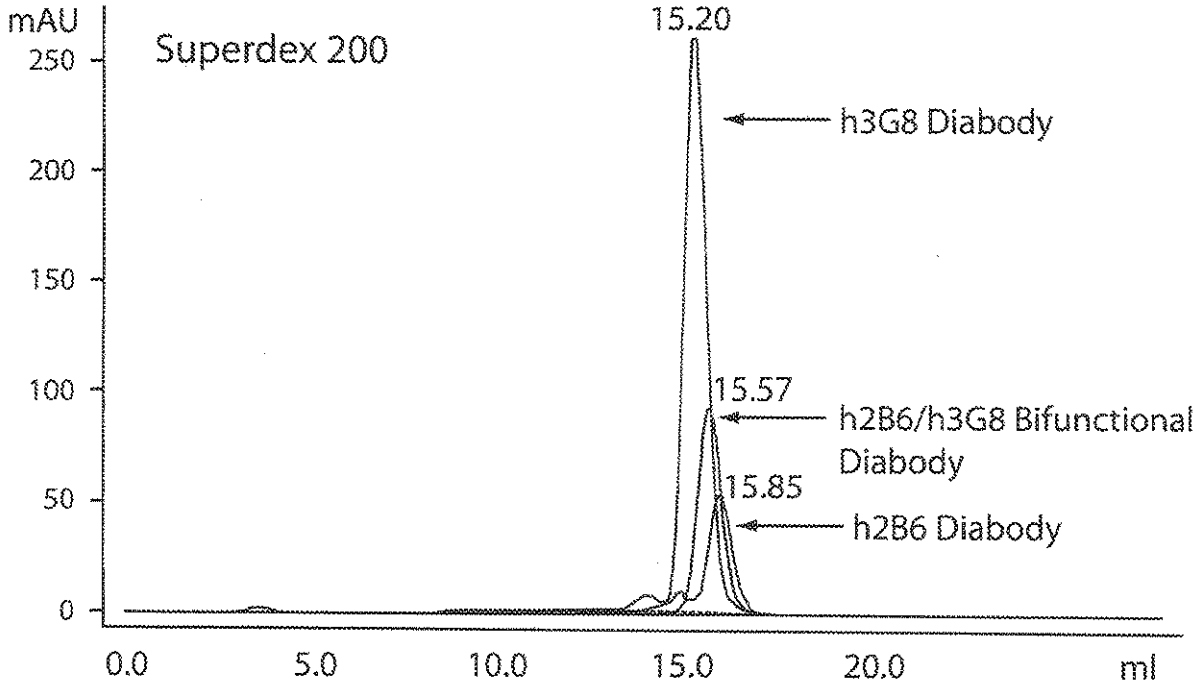

mAU
Superdex 200
15.20
h3G8 Diabody
15.57
h2B6/h3G8 Bifunctional
Diabody
15.85
h2B6 Diabody
250
200
150
100
50
0
0.0
5.0
10.0
15.0
20.0
ml

FIG. 4B

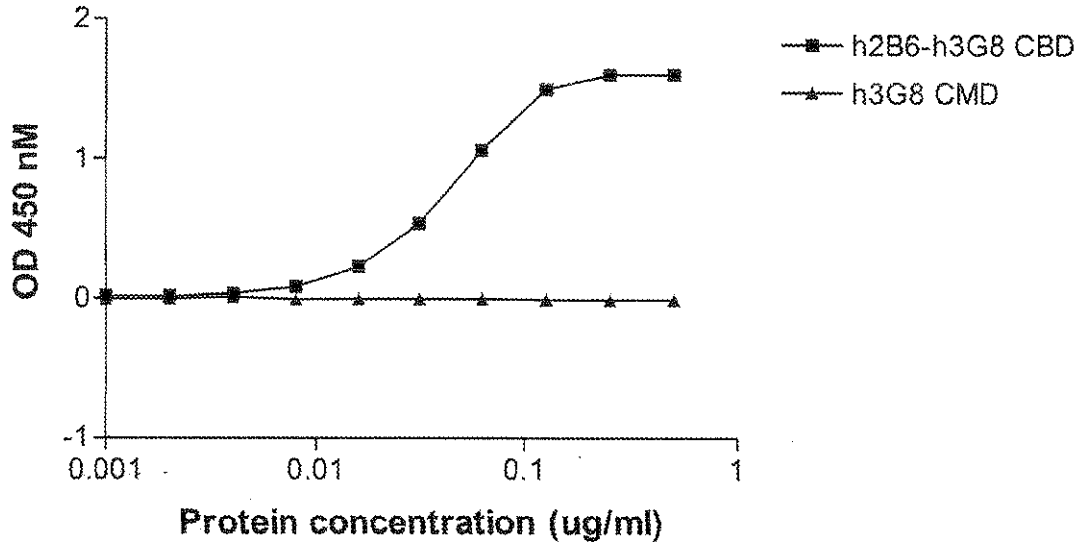

2
1
0
-1
OD 450 nM
0.001
0.01
0.1
1
Protein concentration (ug/ml)
h2B6-h3G8 CBD
h3G8 CMD

FIG. 5

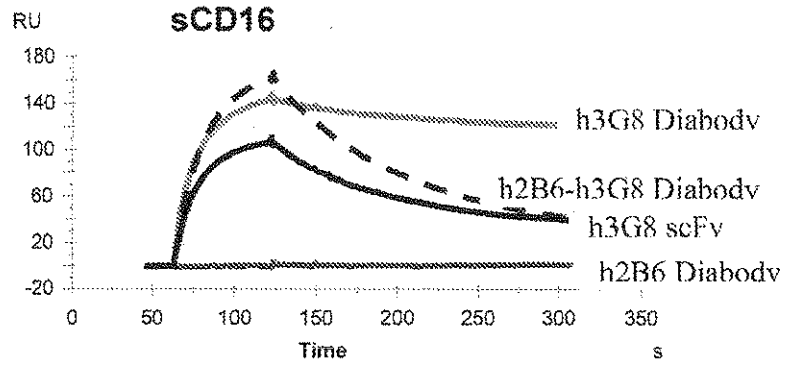


FIG. 6A

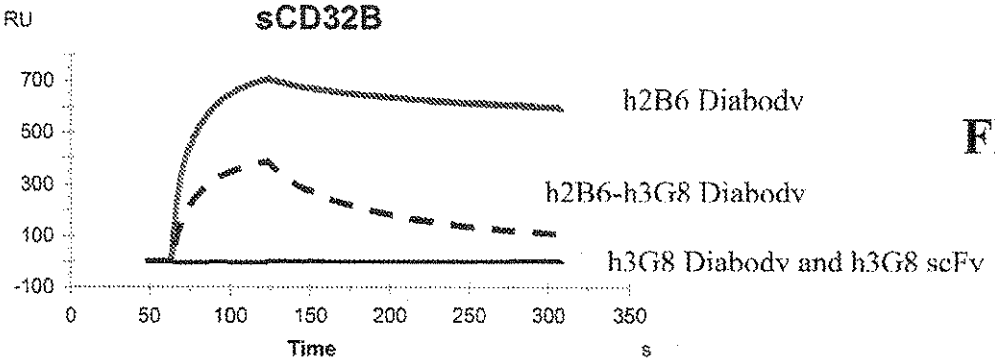


FIG. 6B

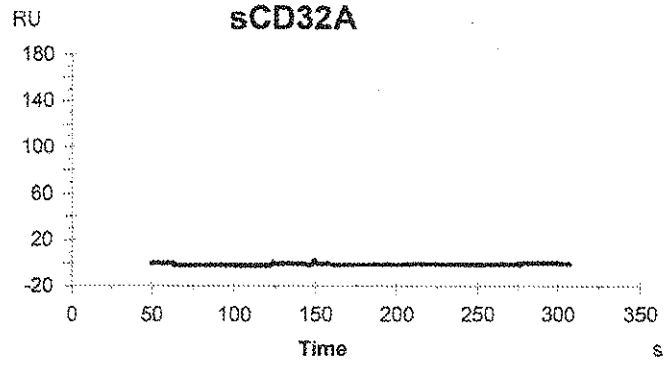


FIG. 6C

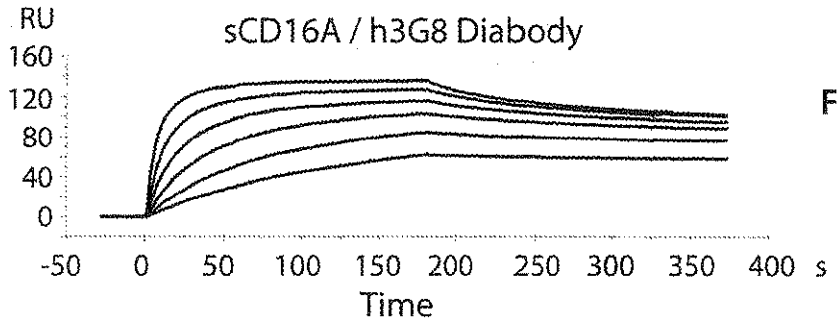


FIG. 7A

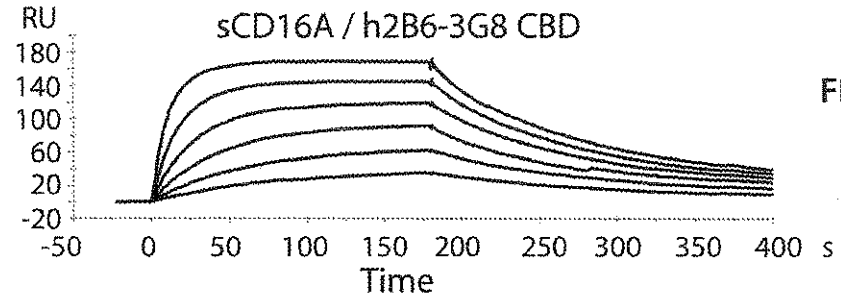


FIG. 7B

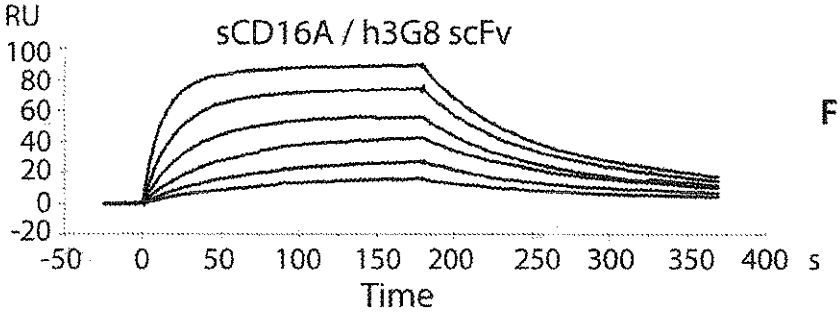


FIG. 7C

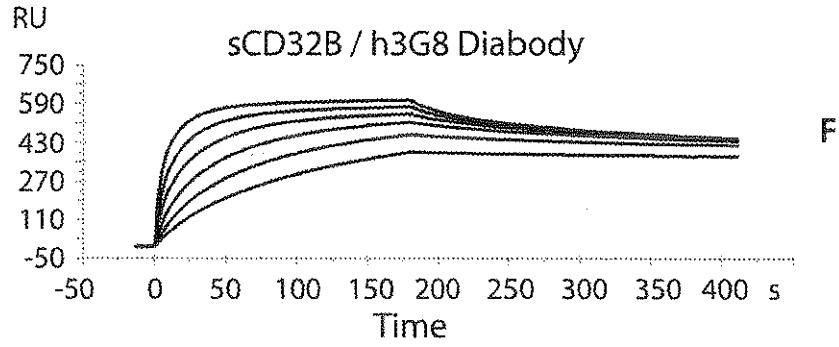


FIG. 7D

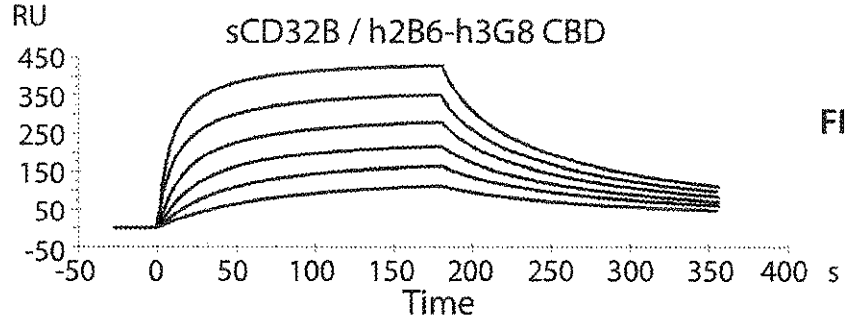


FIG. 7E

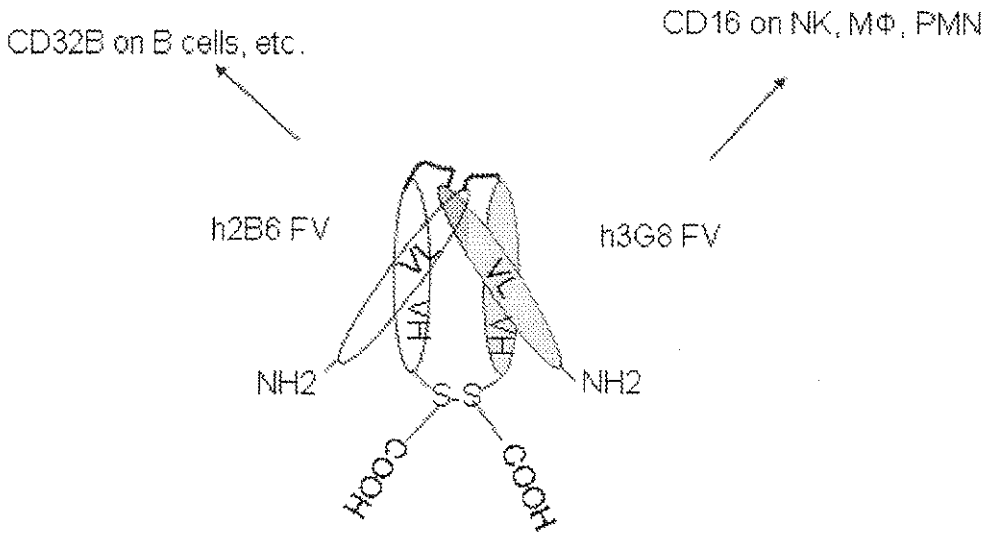

CD32B on B cells, etc.
CD16 on NK, MΦ, PMN
h2B6 FV
h3G8 FV
VL
VH
VH
VL
NH2
NH2
S-S
COOH
COOH

FIG. 8

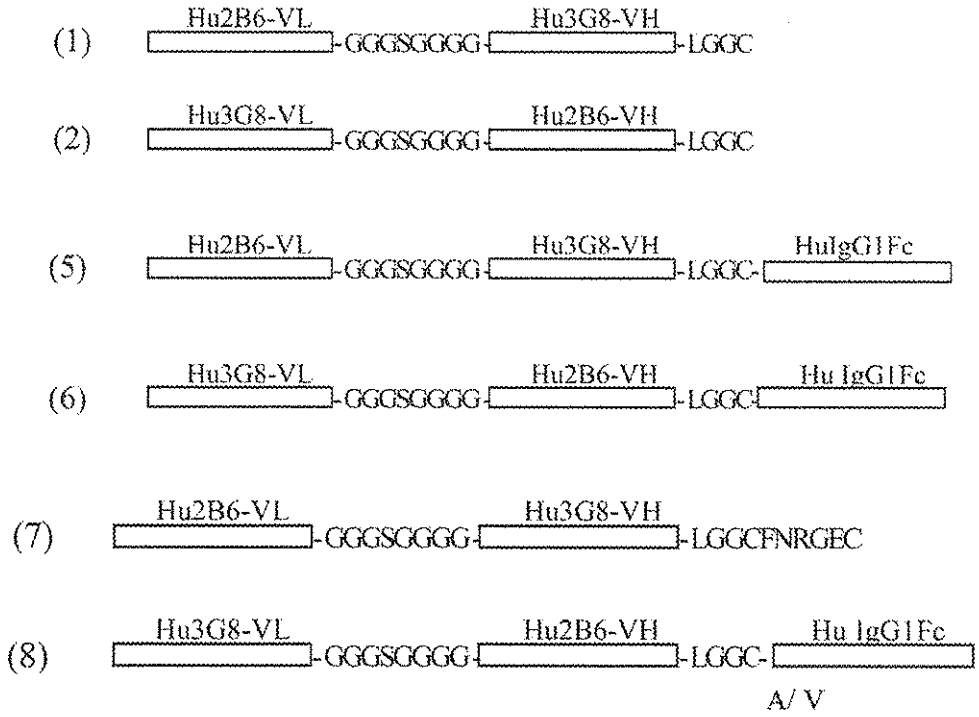

(1)
Hu2B6-VL
-GGGSGGGG-
Hu3G8-VH
-LGGC
(2)
Hu3G8-VL
-GGGSGGGG-
Hu2B6-VH
-LGGC
(5)
Hu2B6-VL
-GGGSGGGG-
Hu3G8-VH
-LGGC-
HuIgG1Fc
(6)
Hu3G8-VL
-GGGSGGGG-
Hu2B6-VH
-LGGC
Hu IgG1Fc
(7)
Hu2B6-VL
-GGGSGGGG-
Hu3G8-VH
-LGGCFNRGEC
(8)
Hu3G8-VL
-GGGSGGGG-
Hu2B6-VH
-LGGC-
Hu IgG1Fc
A/ V

FIG. 9

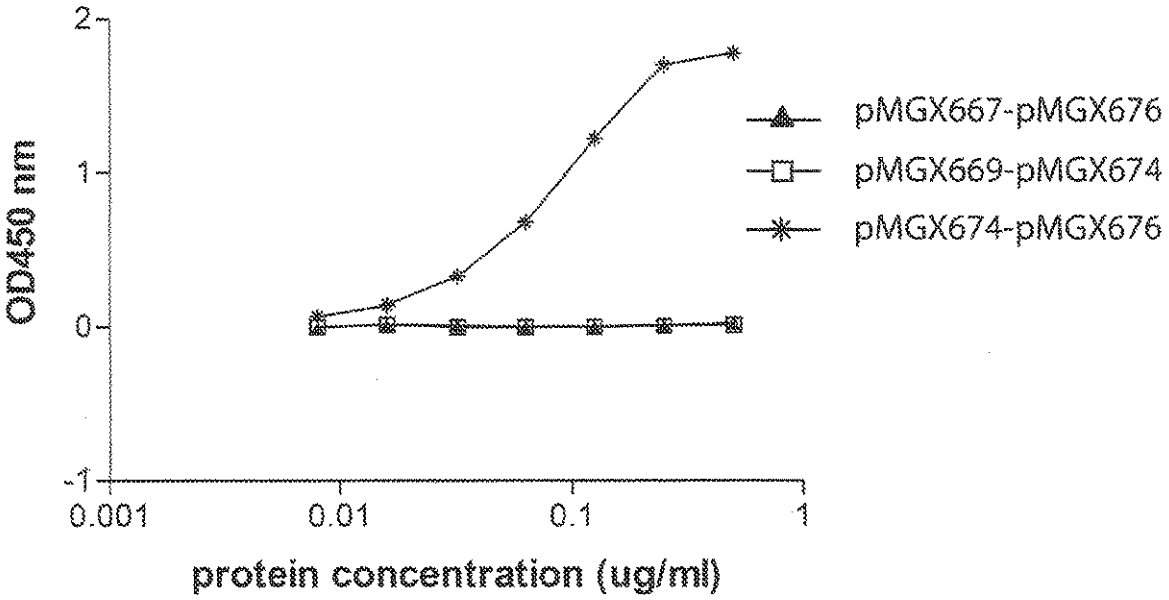

2
1
0
-1
OD450 nm
0.001
0.01
0.1
1
protein concentration (ug/ml)
pMGX667-pMGX676
pMGX669-pMGX674
pMGX674-pMGX676

FIG. 10

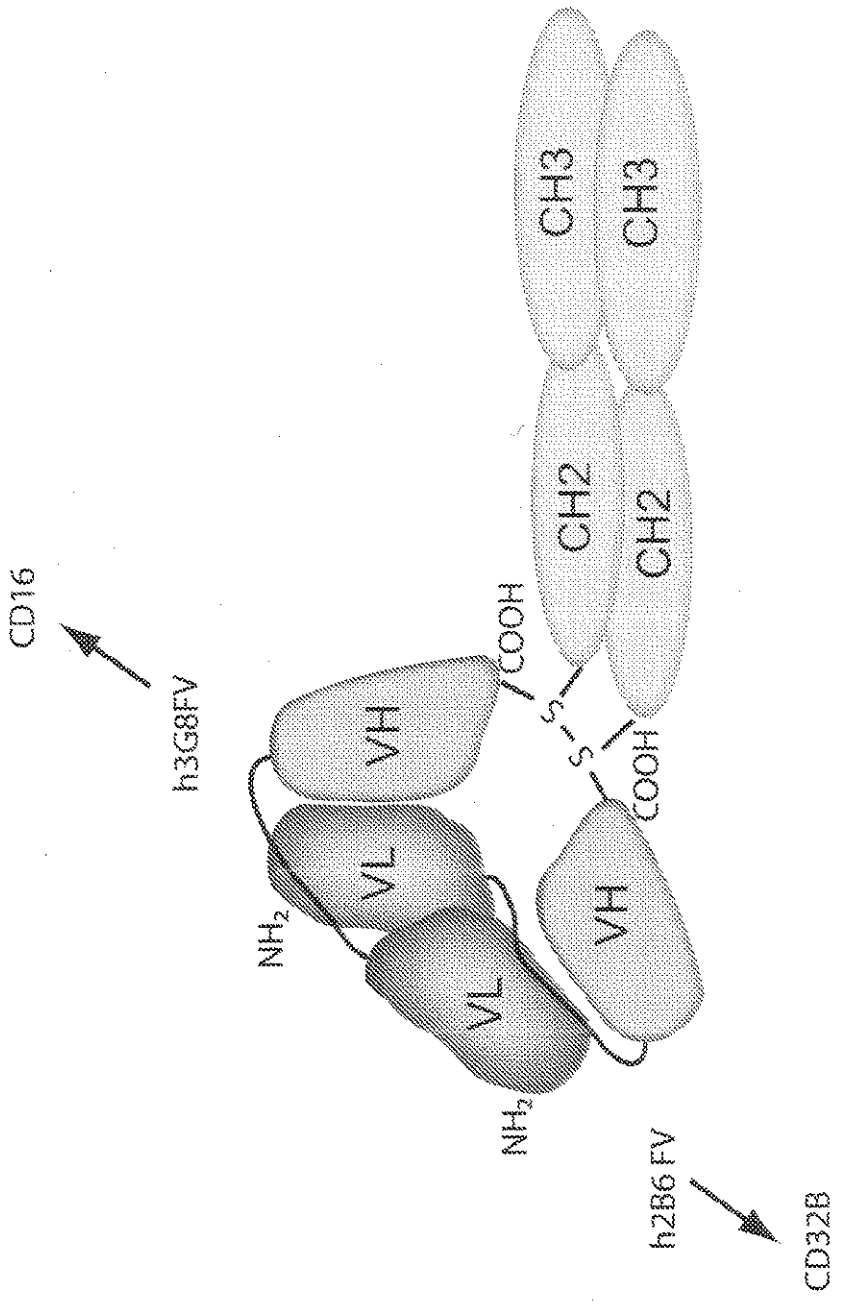

CD16
h3G8FV
VH
COOH
VL
NH2
VL
NH2
CH2
CH2
CH3
CH3
S
S
COOH
VH
h2B6 FV
CD32B

FIG. 11

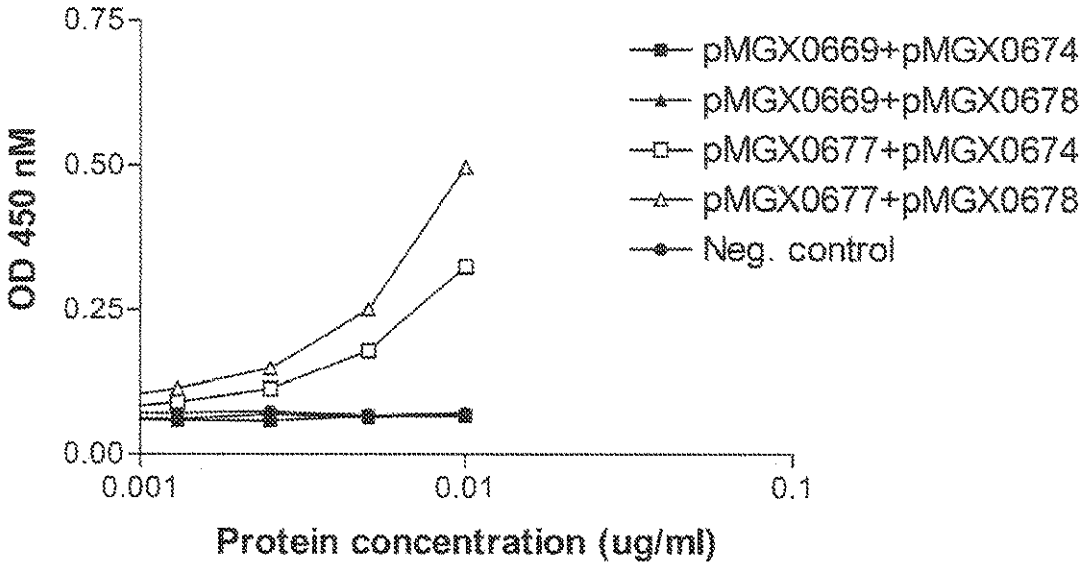

0.75
0.50
0.25
0.00
OD 450 nM
0.001
0.01
0.1
Protein concentration (ug/ml)
pMGX0669+pMGX0674
pMGX0669+pMGX0678
pMGX0677+pMGX0674
pMGX0677+pMGX0678
Neg. control

FIG. 12

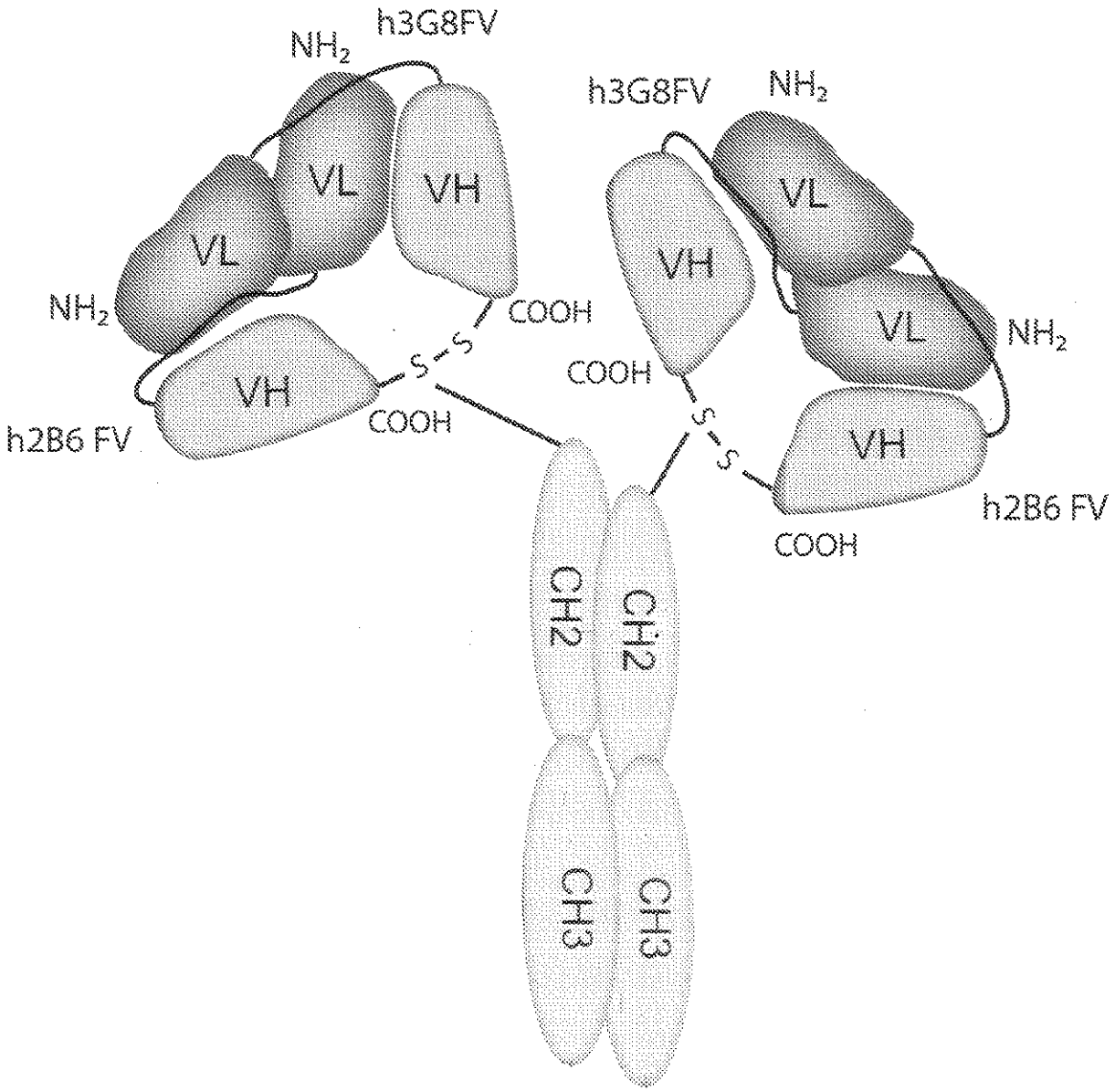

h3G8FV
NH2
VL
VH
VL
NH2
COOH
S-S
VH
COOH
h2B6 FV
h3G8FV
NH2
VL
VH
VL
NH2
COOH
S-S
VH
h2B6 FV
COOH
CH2
CH2
CH3
CH3

FIG. 13

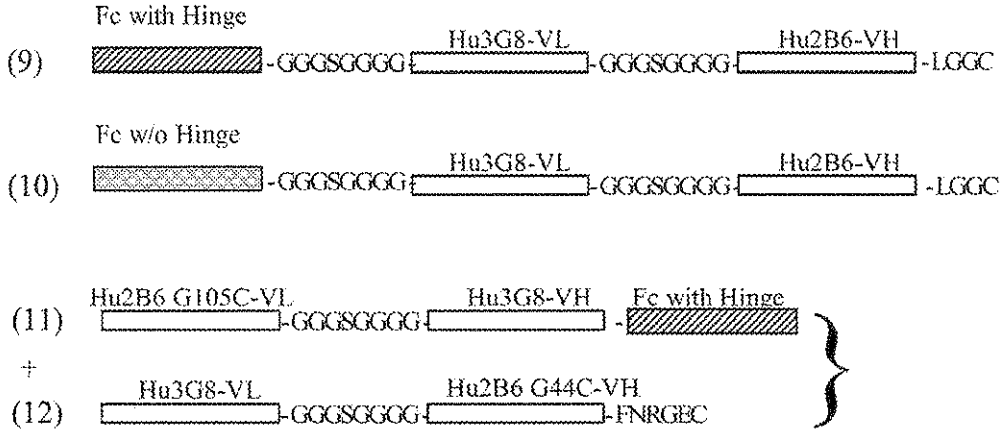

Fc with Hinge
(9)
Hu3G8-VL
Hu2B6-VH
-GGGSGGGG-
-GGGSGGGG-
-LGGC
Fc w/o Hinge
(10)
Hu3G8-VL
Hu2B6-VH
-GGGSGGGG-
-GGGSGGGG-
-LGGC
Hu2B6 G105C-VL
Hu3G8-VH
Fc with Hinge
(11)
-GGGSGGGG-
-
+
Hu3G8-VL
Hu2B6 G44C-VH
(12)
-GGGSGGGG-
-FNRGEC

FIG. 14

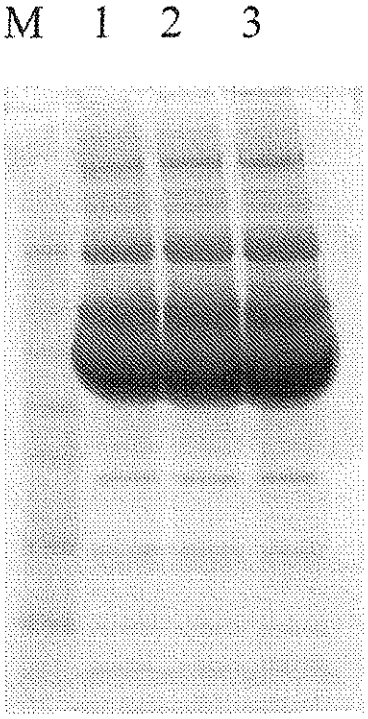


FIG. 15A

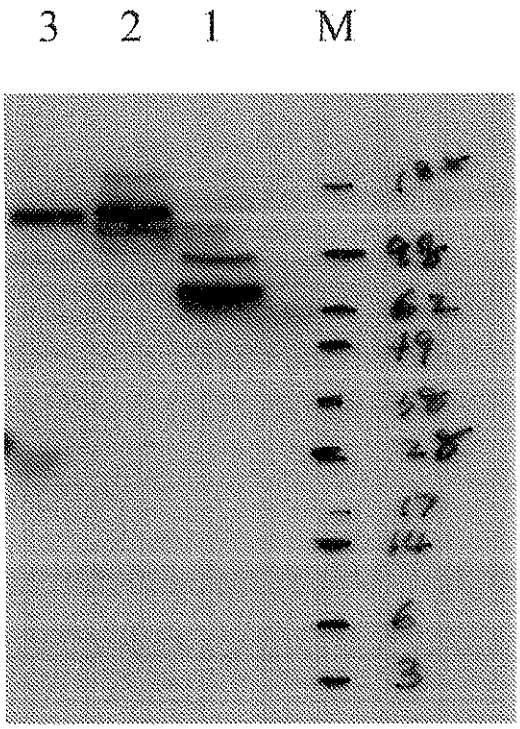


FIG. 15B

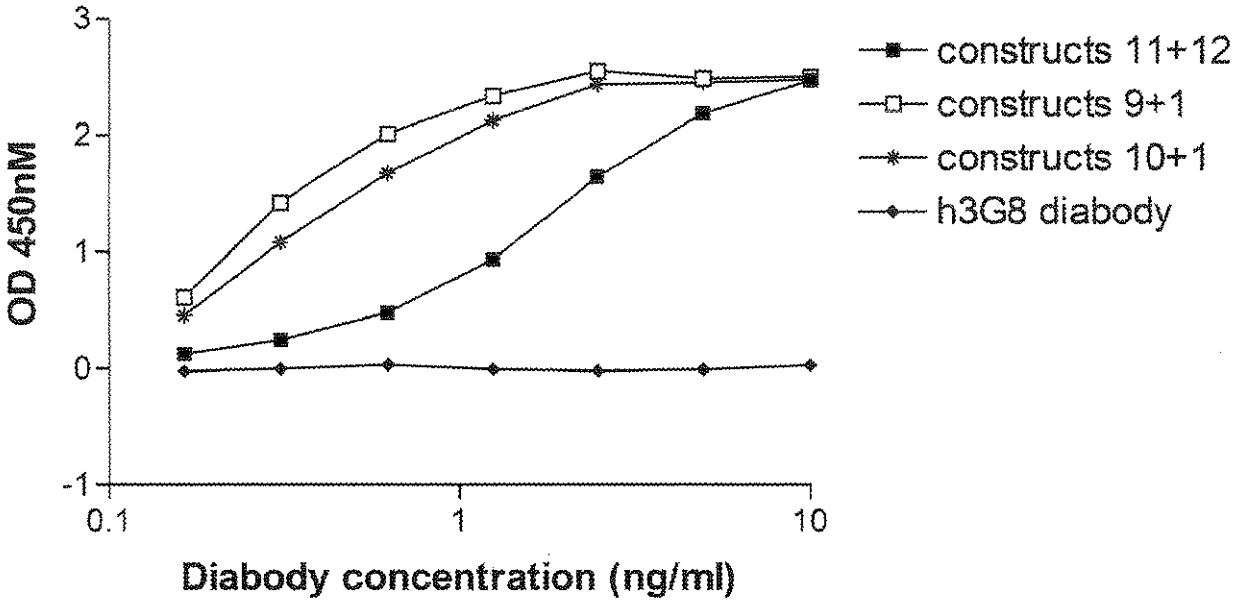

3
2
1
0
-1
OD 450nM
0.1
1
10
Diabody concentration (ng/ml)
constructs 11+12
constructs 9+1
constructs 10+1
h3G8 diabody

FIG. 16

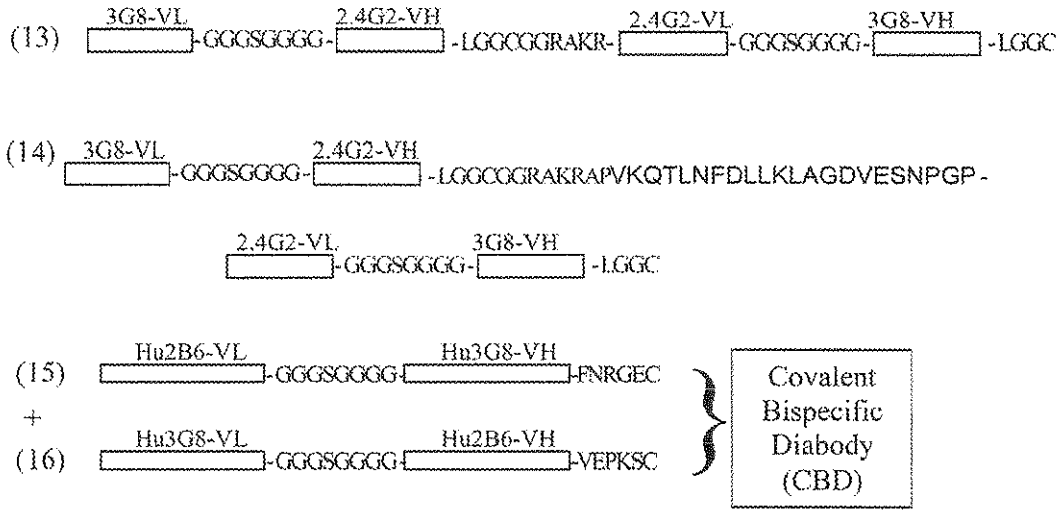

(13) 3G8-VL -GGGSGGGG- 2.4G2-VH -LGGCGGRAKR- 2.4G2-VL -GGGSGGGG- 3G8-VH -LGGC
(14) 3G8-VL -GGGSGGGG- 2.4G2-VH -LGGCGGRAKRAPVKQTLNFDLLKLAGDVESNPGP-
2.4G2-VL -GGGSGGGG- 3G8-VH -LGGC
(15) Hu2B6-VL -GGGSGGGG- Hu3G8-VH -FNRGEC
+
(16) Hu3G8-VL -GGGSGGGG- Hu2B6-VH -VEPKSC
Covalent Bispecific Diabody (CBD)

FIG. 17

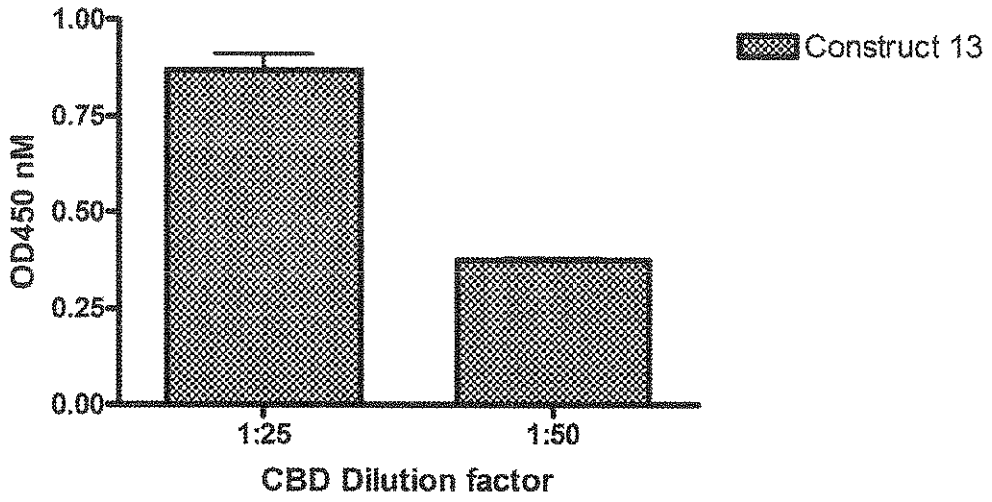

1.00
0.75
0.50
0.25
0.00
OD450 nM
1:25
1:50
CBD Dilution factor
Construct 13

FIG. 18

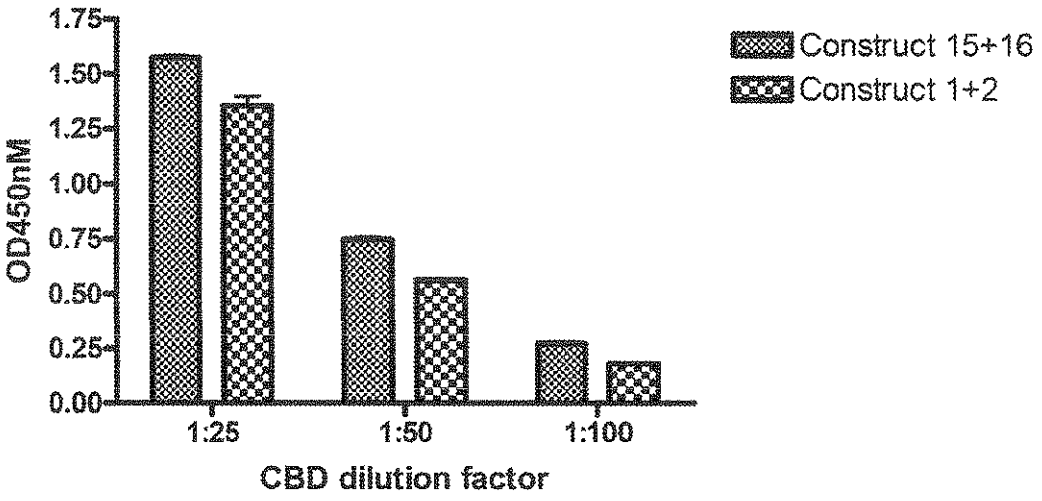

1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
OD450nM
1:25
1:50
1:100
CBD dilution factor
Construct 15+16
Construct 1+2

FIG. 19

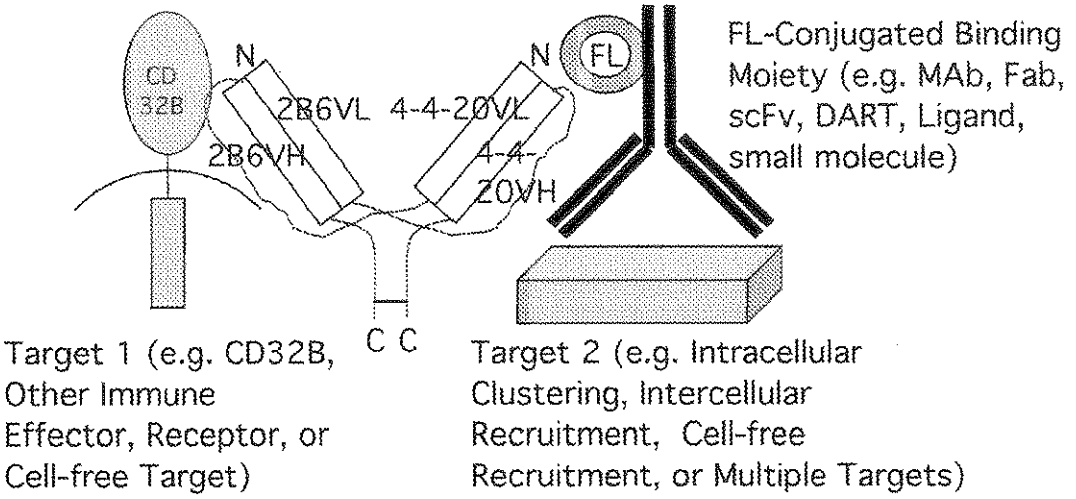

CD 32B
N
2B6VL
2B6VH
4-4-20VL
4-4-
20VH
N
FL
FL-Conjugated Binding Moiety (e.g. MAb, Fab, scFv, DART, Ligand, small molecule)
C C
Target 1 (e.g. CD32B, Other Immune Effector, Receptor, or Cell-free Target)
Target 2 (e.g. Intracellular Clustering, Intercellular Recruitment, Cell-free Recruitment, or Multiple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

FIG. 20

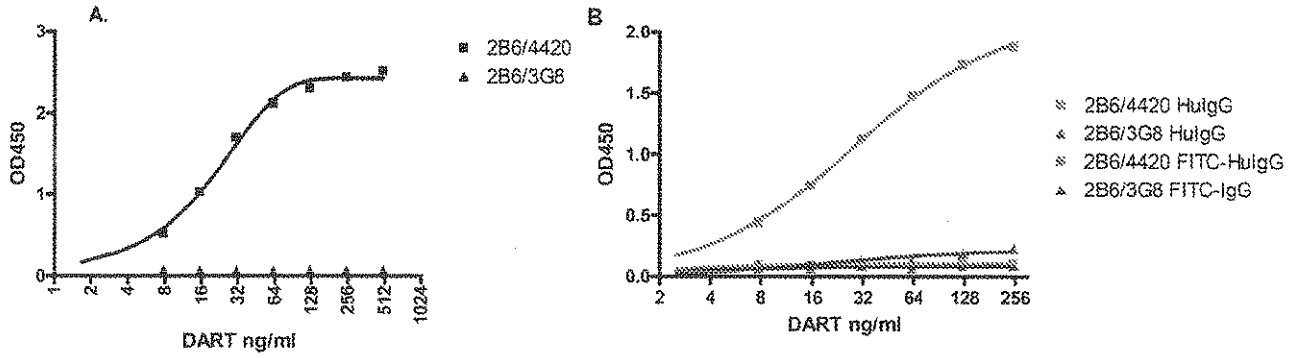


FIG. 21

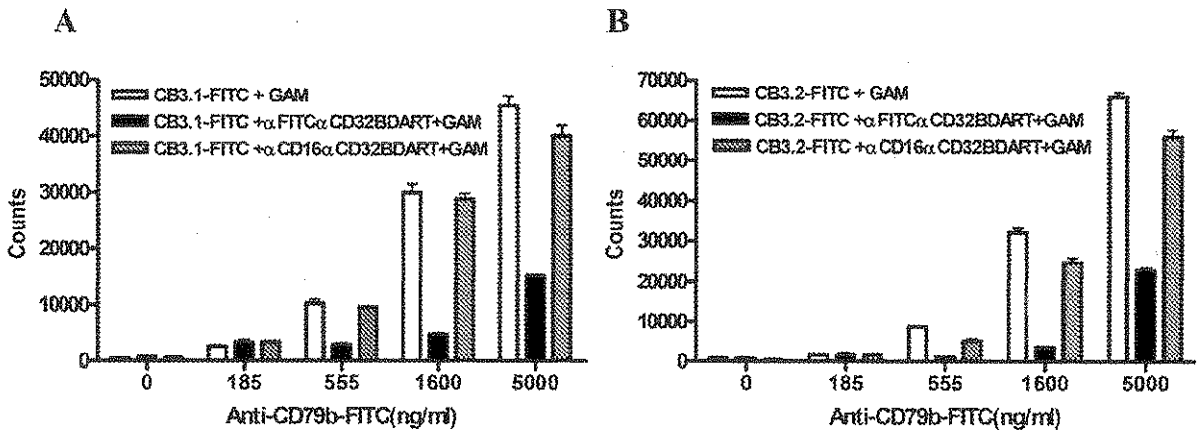


FIG. 22

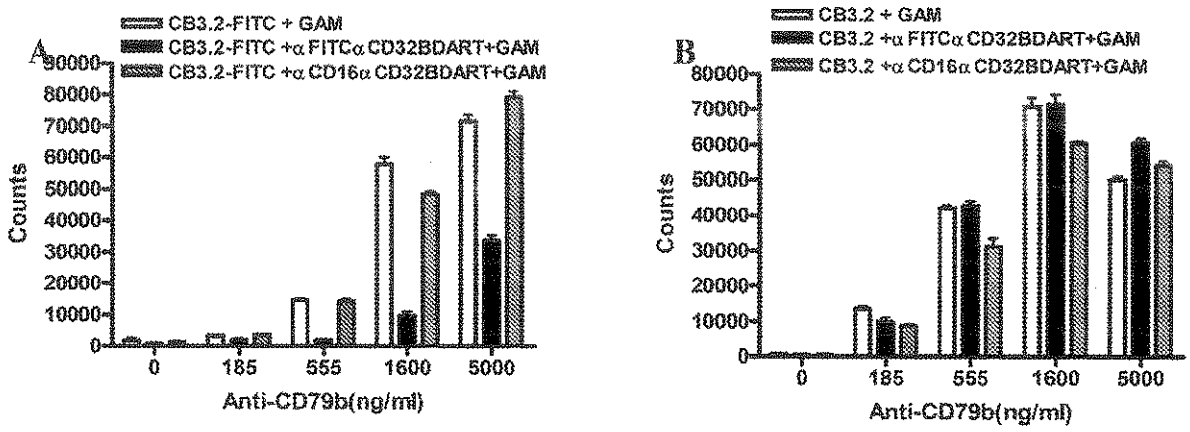
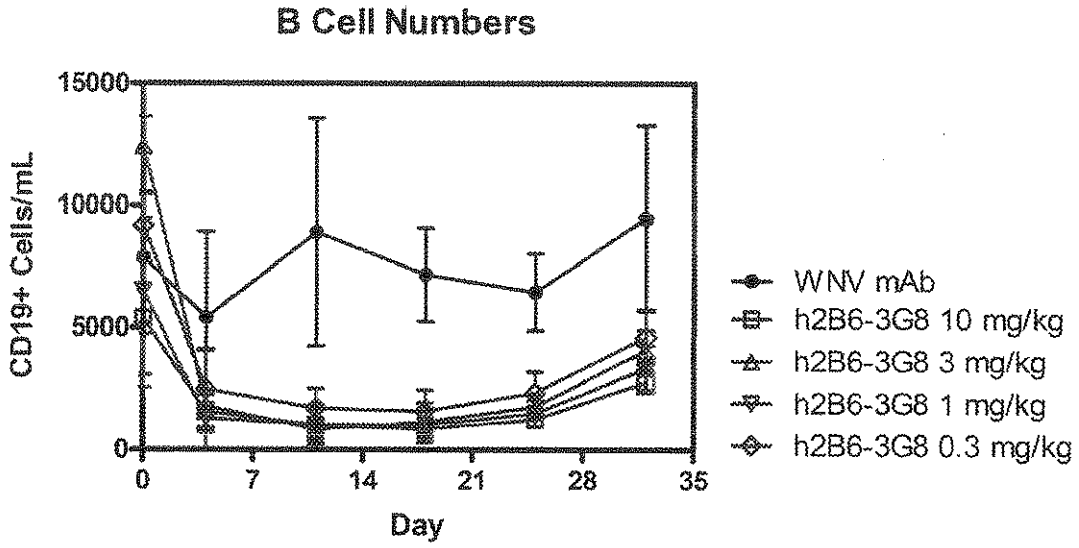


FIG. 23


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

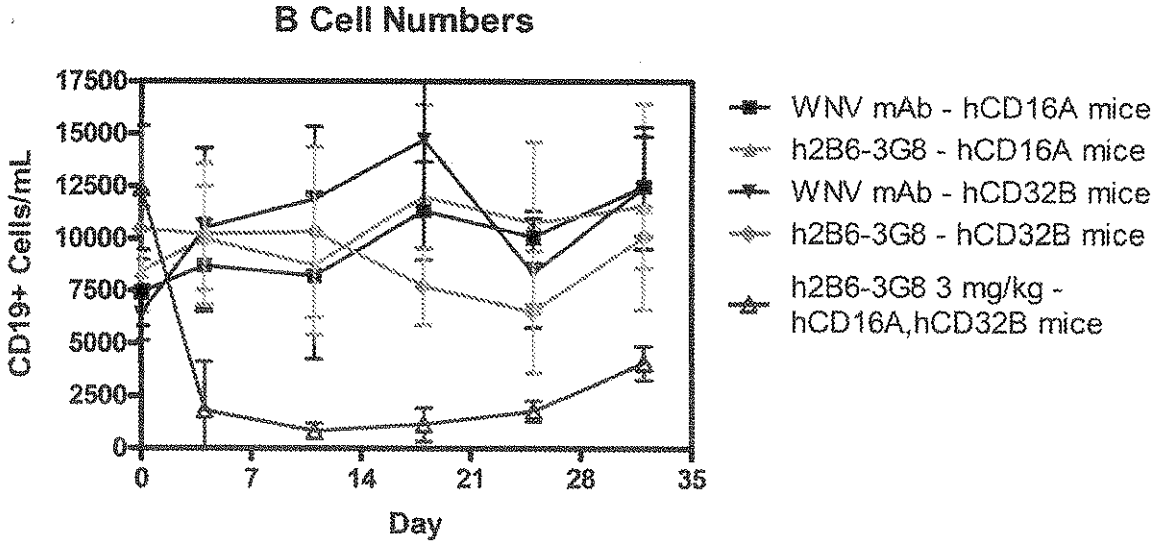

B Cell Numbers
CD19+ Cells/mL
17500
15000
12500
10000
7500
5000
2500
0
0
7
14
21
28
35
Day
WNV mAb - hCD16A mice
h2B6-3G8 - hCD16A mice
WNV mAb - hCD32B mice
h2B6-3G8 - hCD32B mice
h2B6-3G8 3 mg/kg - hCD16A,hCD32B mice

FIG. 24

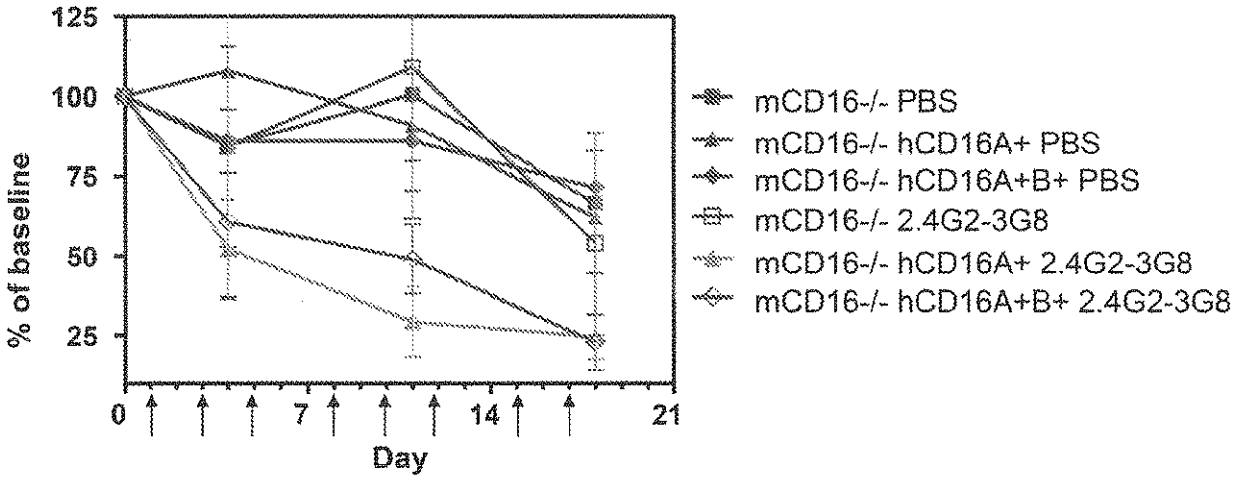

125
100
75
50
25
% of baseline
0
7
14
21
Day
mCD16-/- PBS
mCD16-/- hCD16A+ PBS
mCD16-/- hCD16A+B+ PBS
mCD16-/- 2.4G2-3G8
mCD16-/- hCD16A+ 2.4G2-3G8
mCD16-/- hCD16A+B+ 2.4G2-3G8

FIG. 25

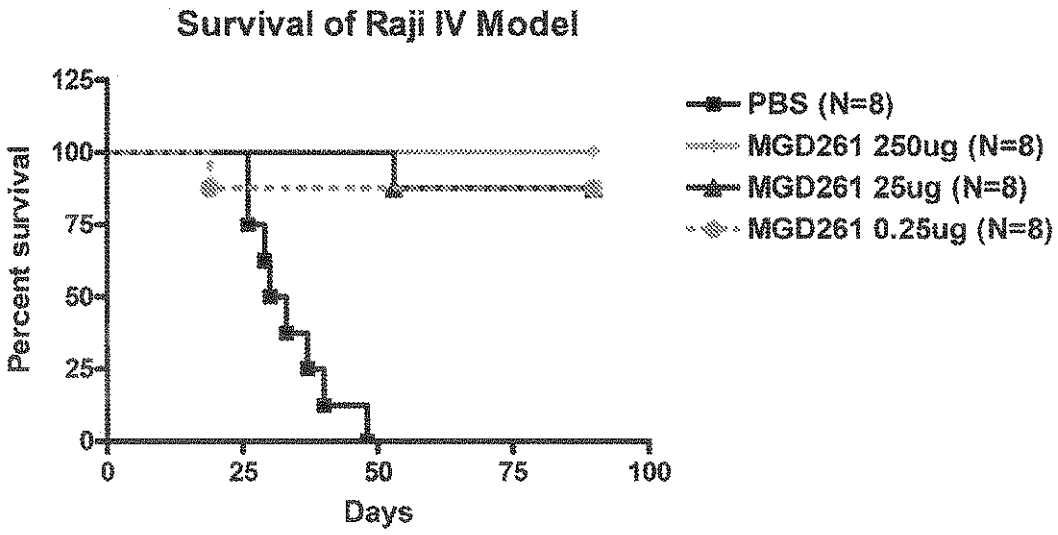

Survival of Raji IV Model
125
100
75
50
25
0
Percent survival
0
25
50
75
100
Days
PBS (N=8)
MGD261 250ug (N=8)
MGD261 25ug (N=8)
MGD261 0.25ug (N=8)

FIG. 26

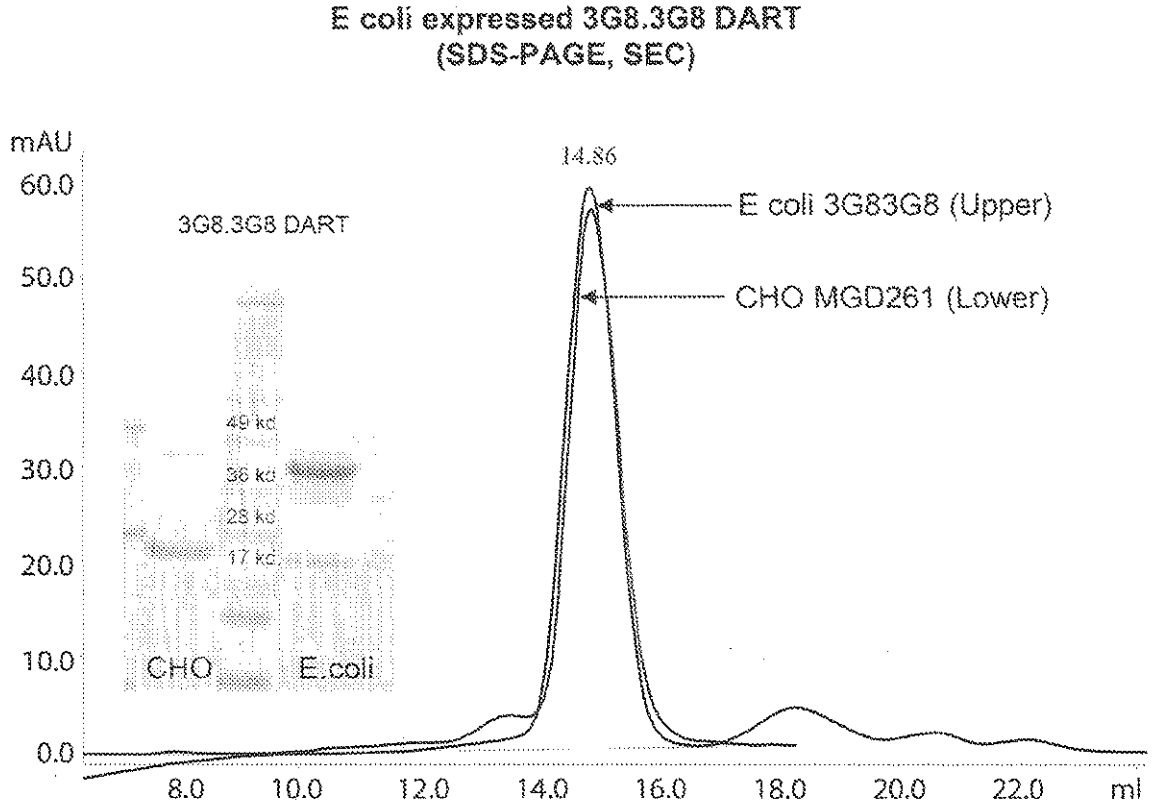

E coli expressed 3G8.3G8 DART
(SDS-PAGE, SEC)
mAU
60.0
50.0
40.0
30.0
20.0
10.0
0.0
14.86
E coli 3G83G8 (Upper)
CHO MGD261 (Lower)
3G8.3G8 DART
49 kd
36 kd
28 kd
17 kd
CHO
E.coli
8.0
10.0
12.0
14.0
16.0
18.0
20.0
22.0
ml

FIG. 27

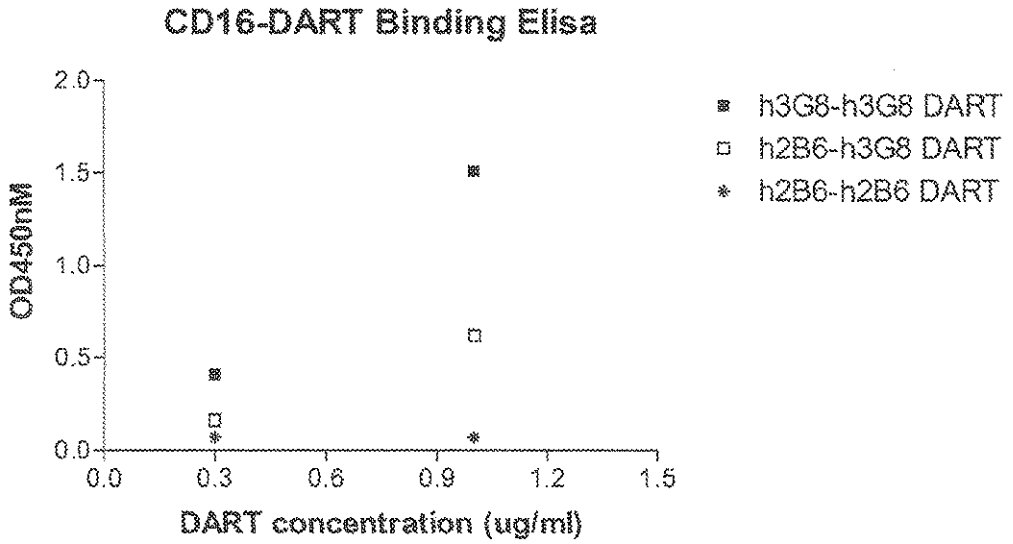

CD16-DART Binding Elisa
OD450nM
2.0
1.5
1.0
0.5
0.0
0.0
0.3
0.6
0.9
1.2
1.5
DART concentration (ug/ml)
h3G8-h3G8 DART
h2B6-h3G8 DART
h2B6-h2B6 DART

FIG. 28

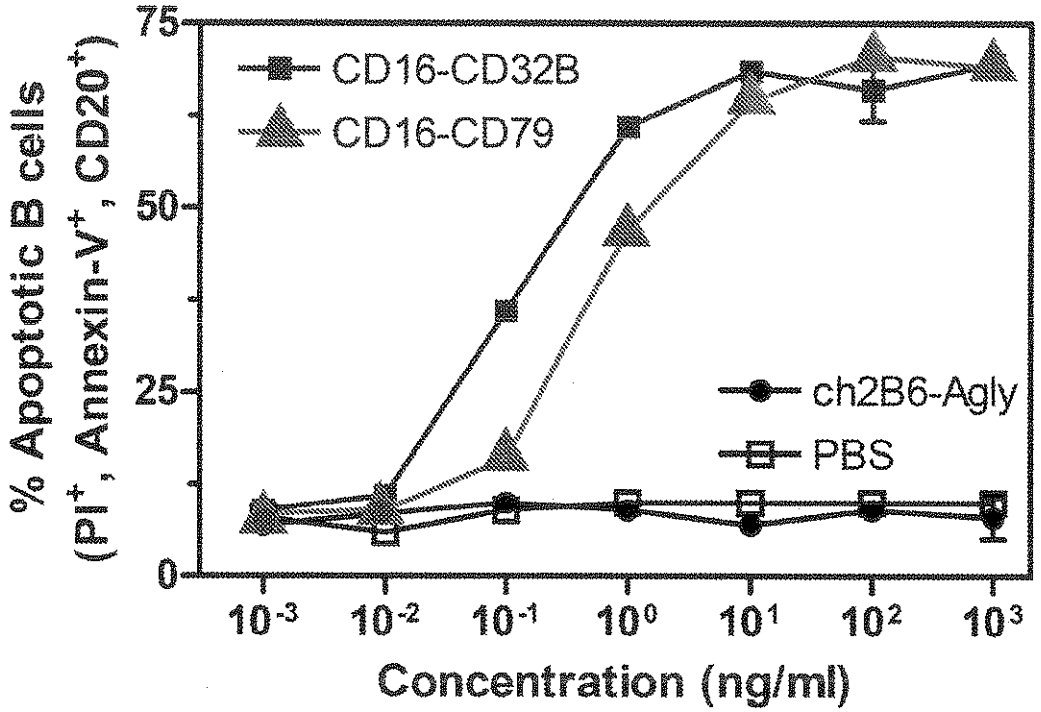

% Apoptotic B cells
(PI+, Annexin-V+, CD20+)
75
50
25
0
CD16-CD32B
CD16-CD79
ch2B6-Agly
PBS
10-3
10-2
10-1
100
101
102
103
Concentration (ng/ml)

FIG. 29

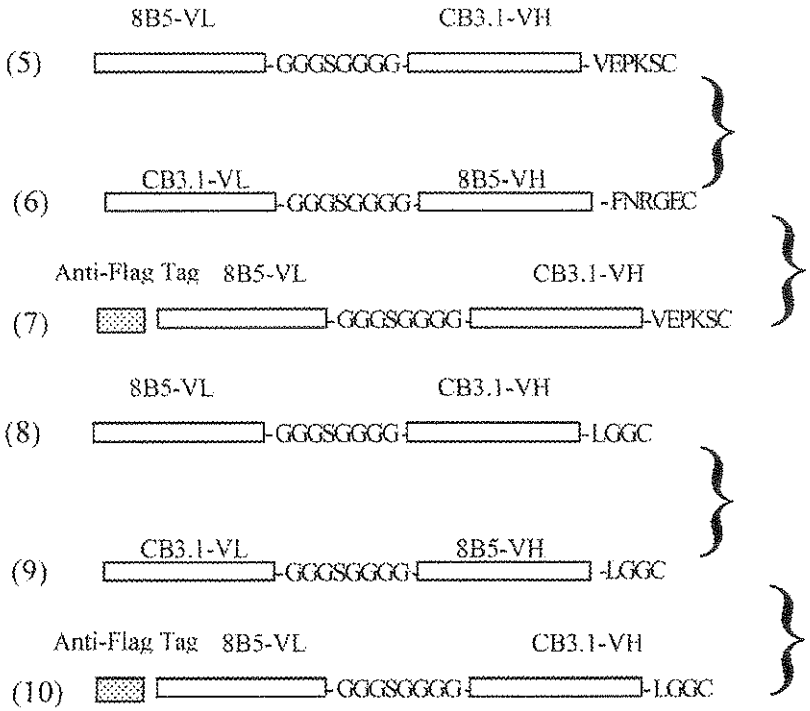

8B5-VL
CB3.1-VH
(5)
GGGSGGGG
VEPKSC
CB3.1-VL
8B5-VH
(6)
GGGSGGGG
FNRGEC
Anti-Flag Tag
8B5-VL
CB3.1-VH
(7)
GGGSGGGG
VEPKSC
8B5-VL
CB3.1-VH
(8)
GGGSGGGG
LGGC
CB3.1-VL
8B5-VH
(9)
GGGSGGGG
LGGC
Anti-Flag Tag
8B5-VL
CB3.1-VH
(10)
GGGSGGGG
LGGC

FIG. 30

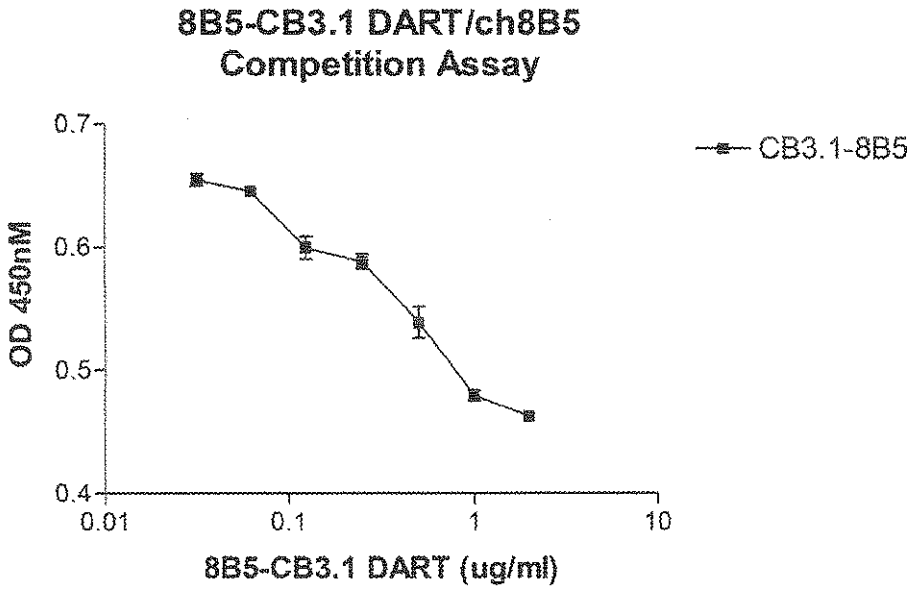

8B5-CB3.1 DART/ch8B5
Competition Assay
CB3.1-8B5
OD 450nM
0.7
0.6
0.5
0.4
0.01
0.1
1
10
8B5-CB3.1 DART (ug/ml)

FIG. 31

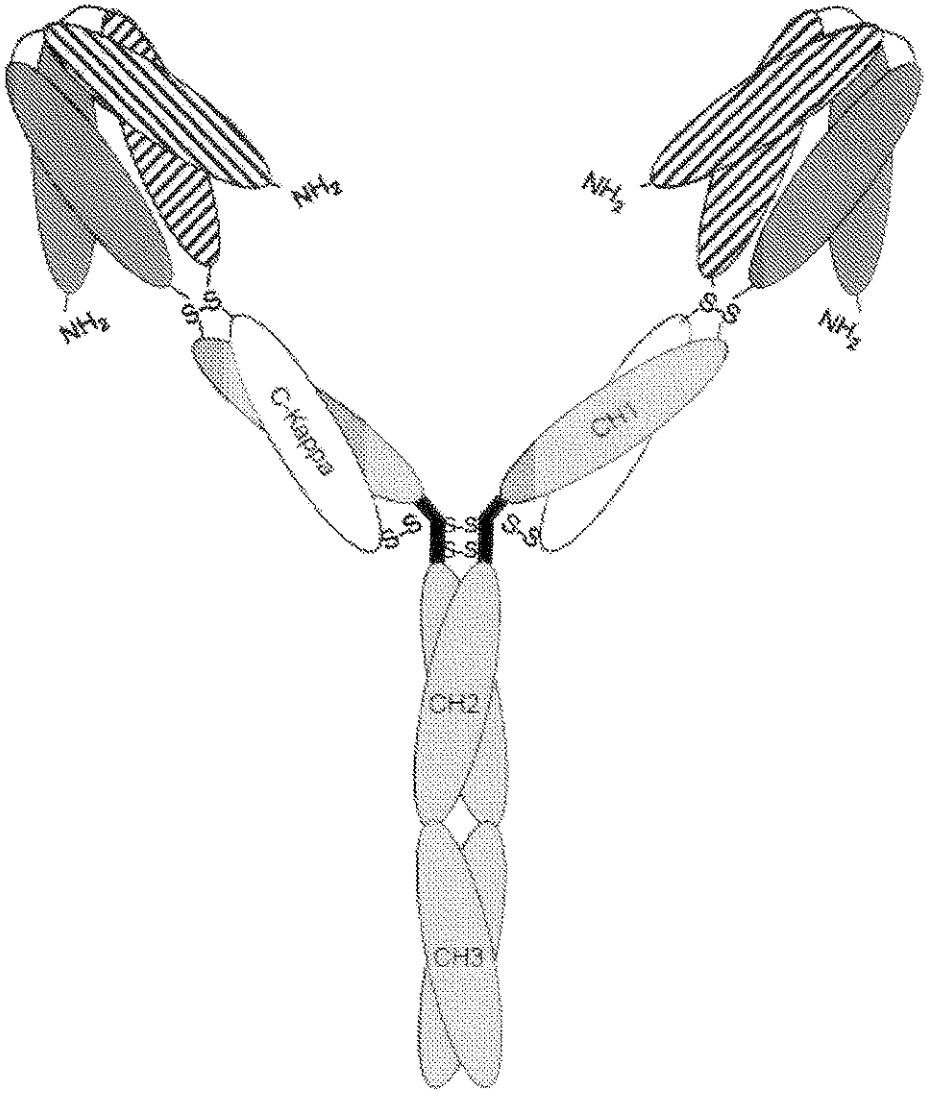
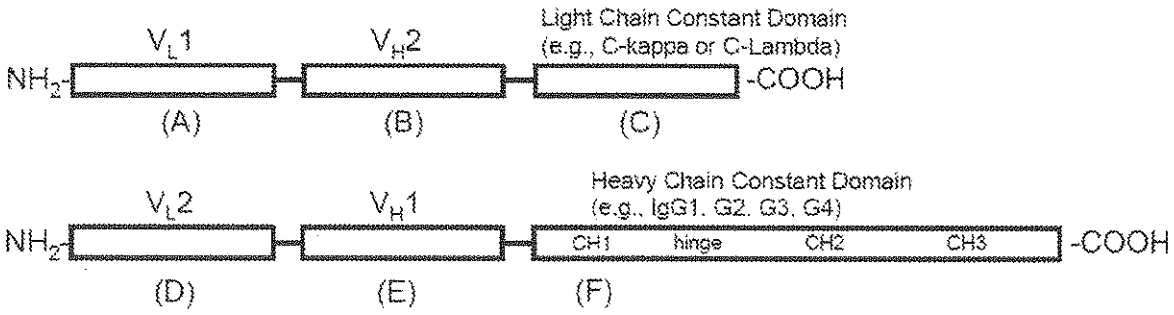


FIG. 32

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

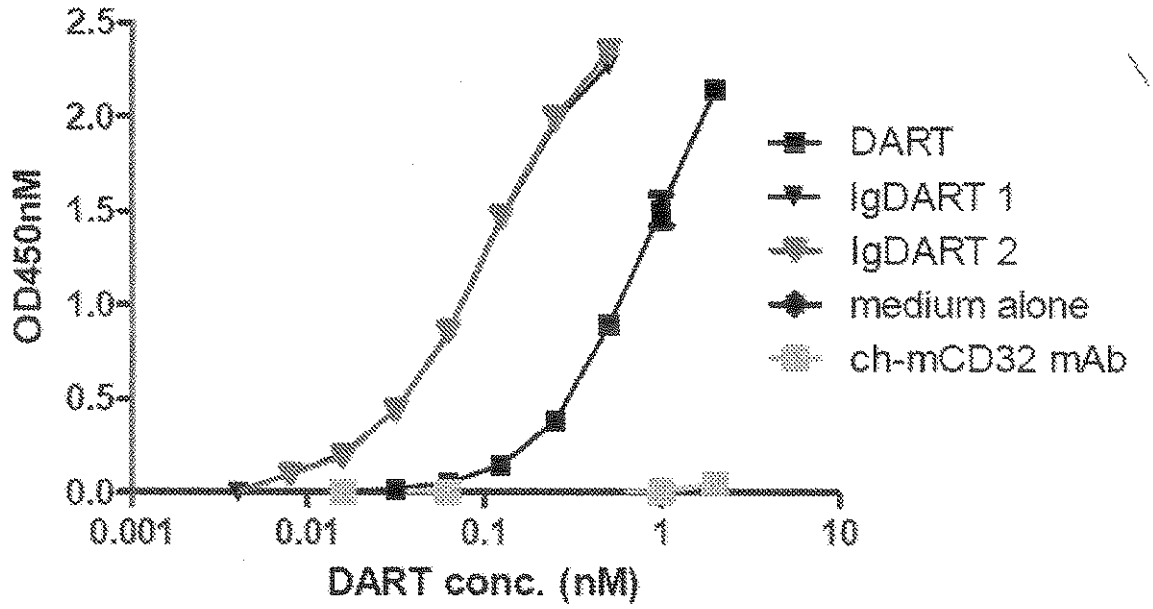


FIG. 34

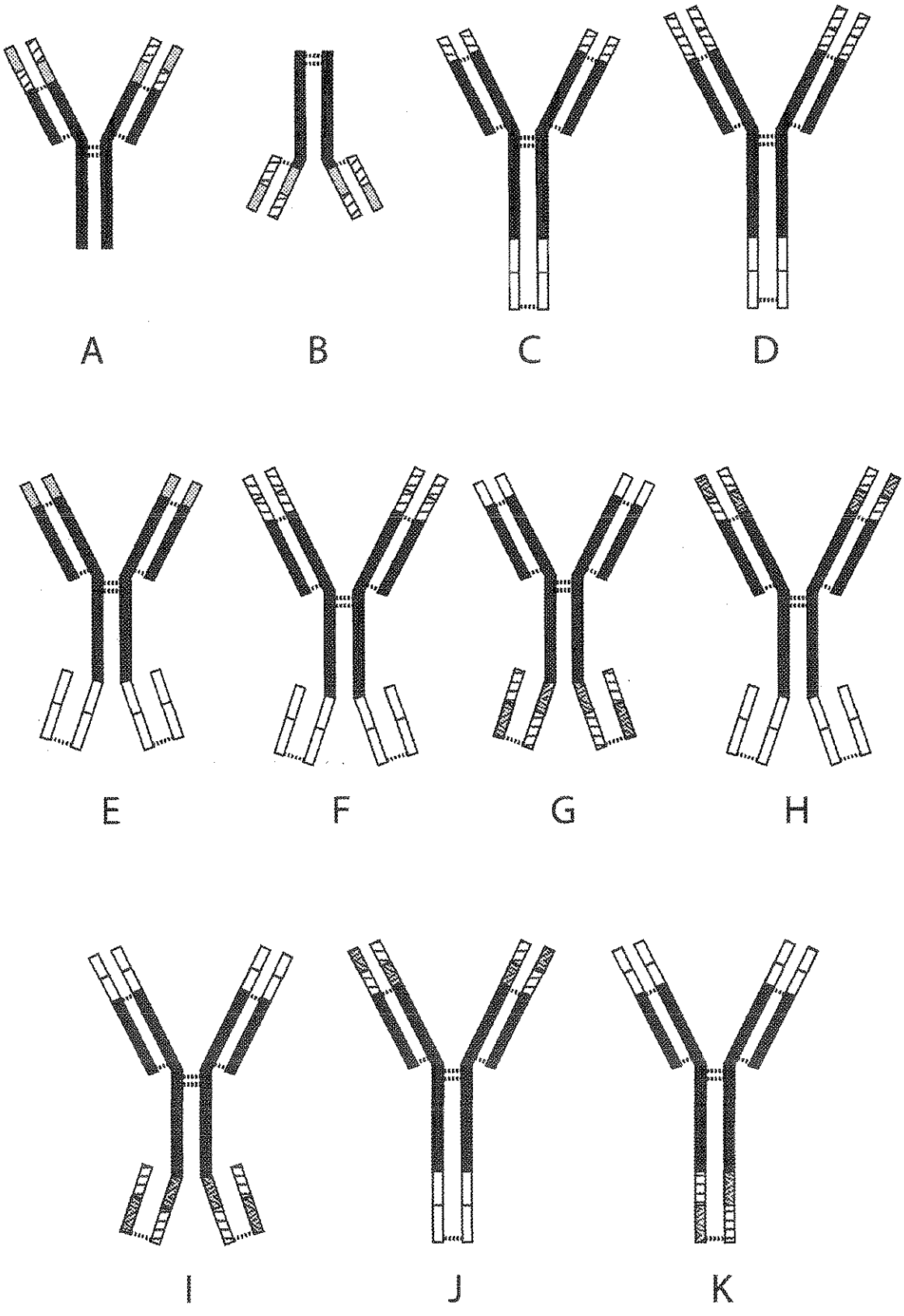


FIG. 35

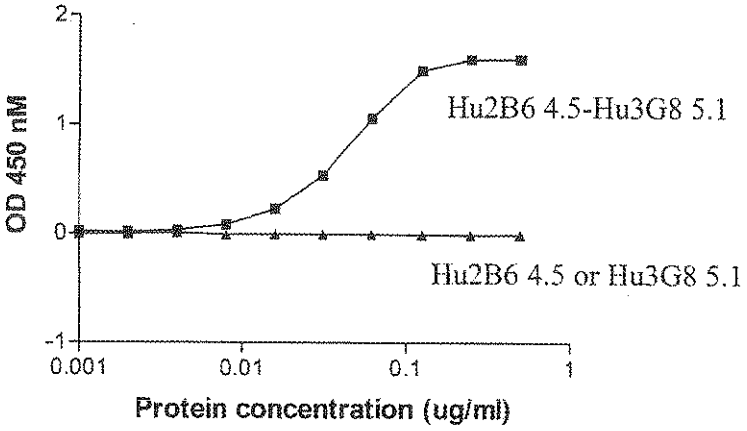

2
1
0
-1
OD 450 nM
Hu2B6 4.5-Hu3G8 5.1
Hu2B6 4.5 or Hu3G8 5.1
0.001
0.01
0.1
1
Protein concentration (ug/ml)

FIG. 36

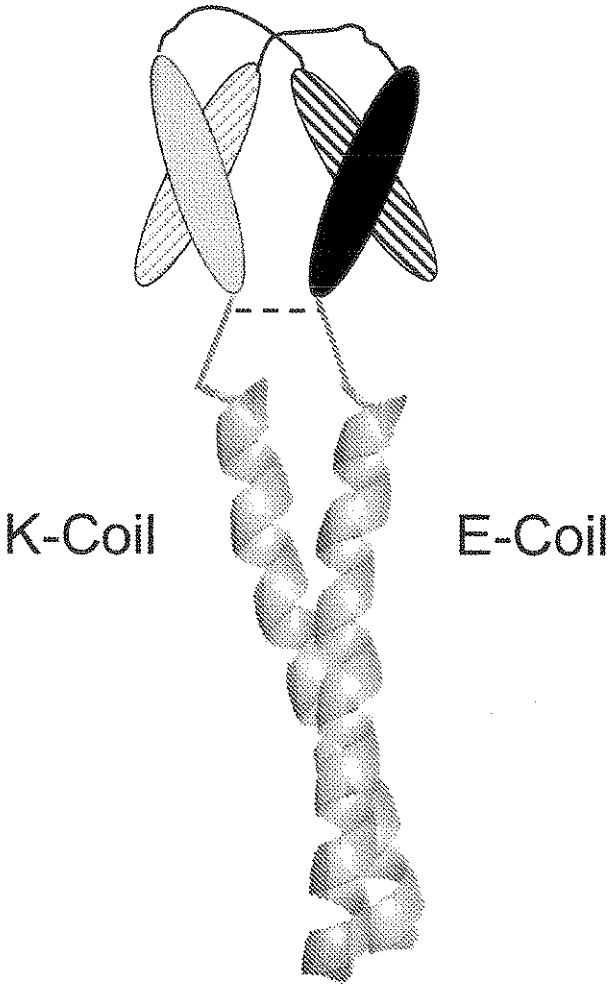


FIG. 37

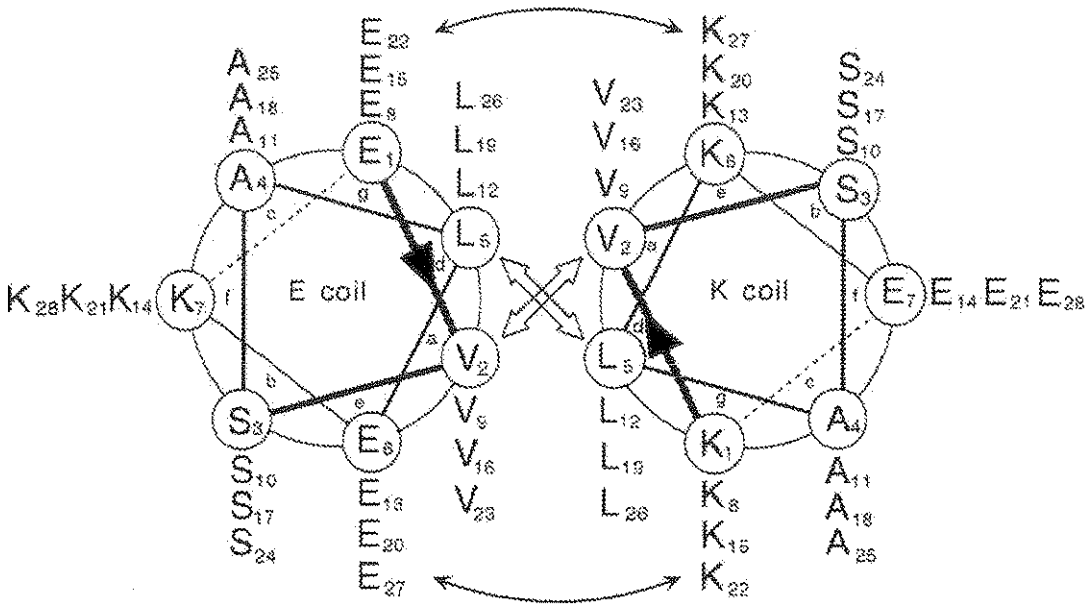

E coil
K coil
A25 A18 A11 A4
E22 E15 E8 E1
L26 L19 L12 L5
V23 V16 V9 V2
K27 K20 K13 K6
S24 S17 S10 S3
K28 K21 K14 K7
E7 E14 E21 E28
S3 S10 S17 S24
E1 E8 E15 E22
V2 V9 V16 V23
L5 L12 L19 L26
K1 K8 K15 K22
A4 A11 A18 A25

FIG. 38

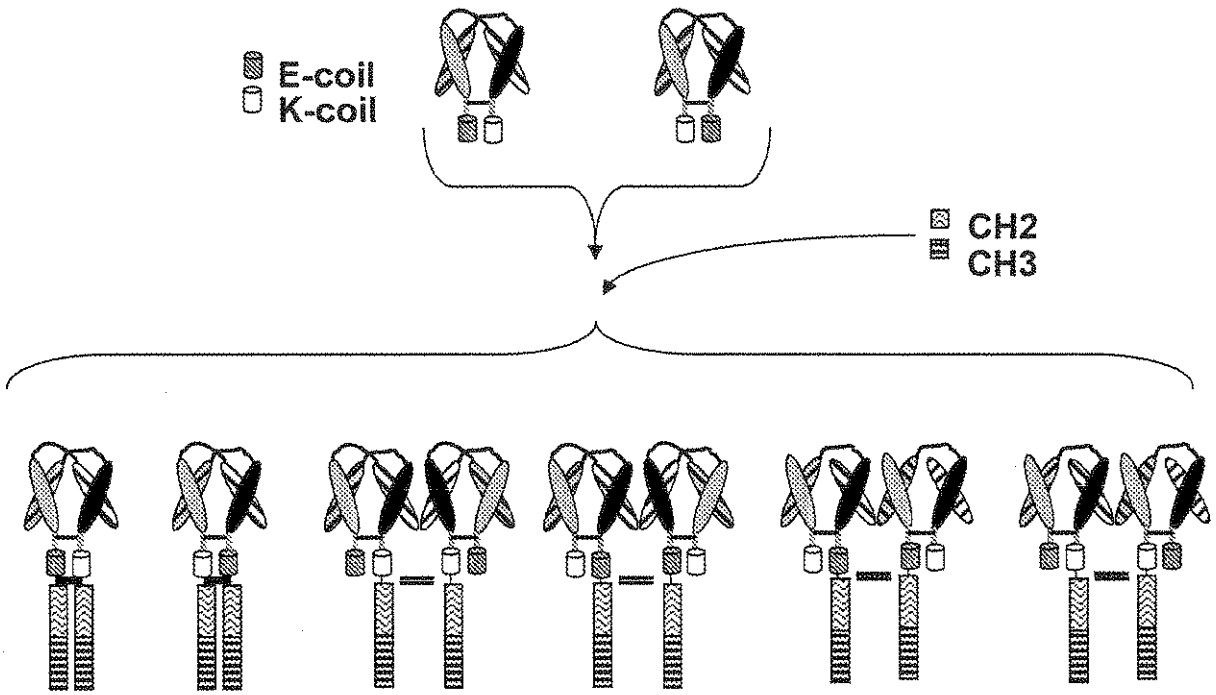


Figure 39

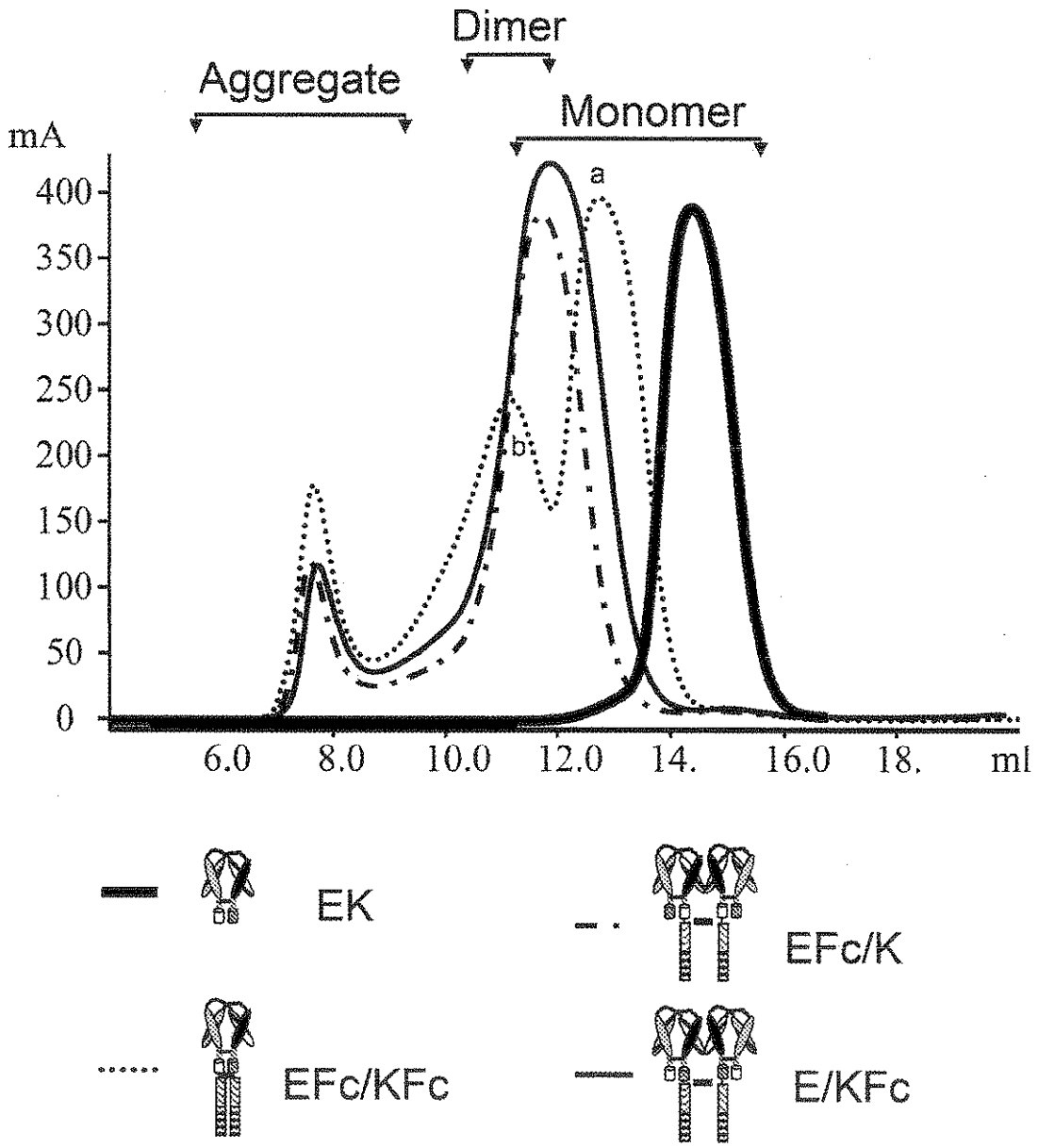

Dimer
Aggregate
Monomer
mA
400
350
300
250
200
150
100
50
0
a
b
6.0
8.0
10.0
12.0
14.
16.0
18.
ml
EK
EFc/K
EFc/KFc
E/KFc

Figure 40

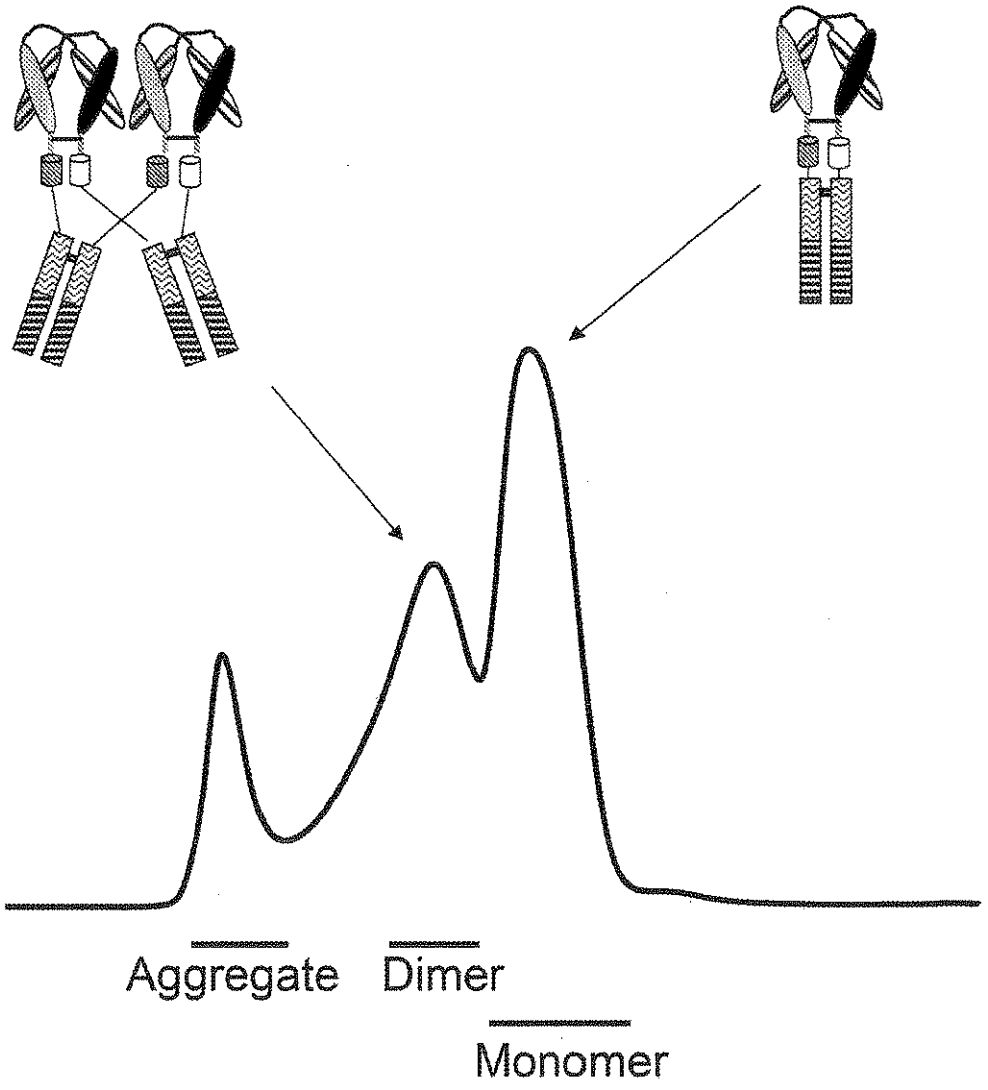


Figure 41

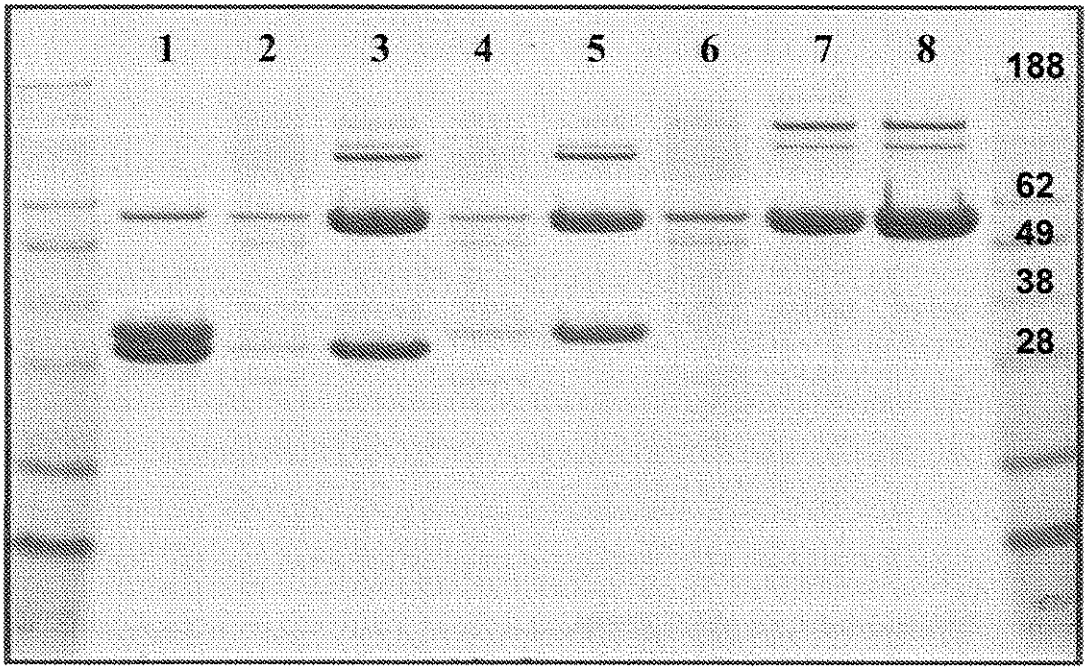


Figure 42

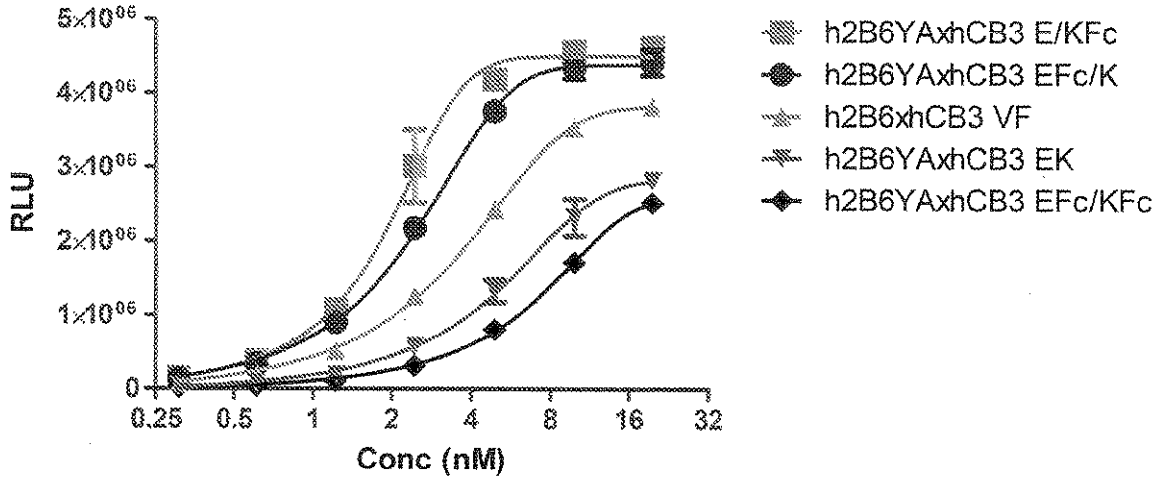

5x10^06
4x10^06
3x10^06
2x10^06
1x10^06
0
RLU
0.25
0.5
1
2
4
8
16
32
Conc (nM)
h2B6YAxhCB3 E/KFc
h2B6YAxhCB3 EFc/K
h2B6xhCB3 VF
h2B6YAxhCB3 EK
h2B6YAxhCB3 EFc/KFc

Figure 43

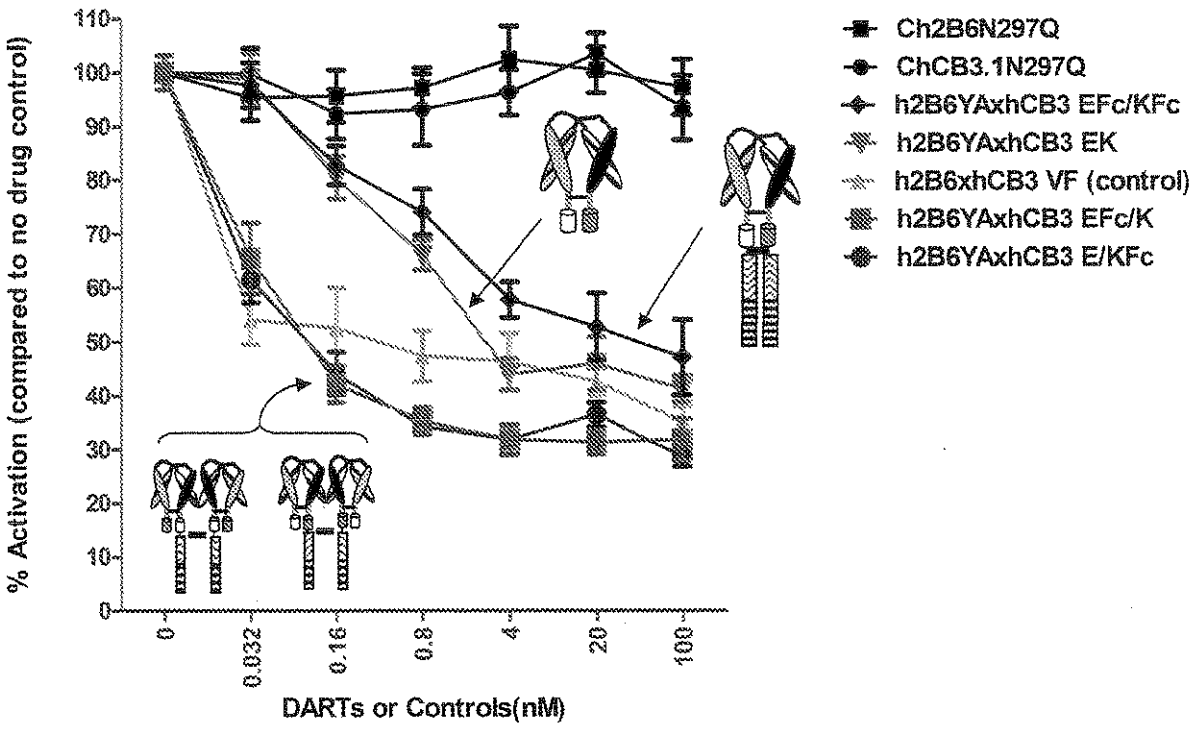

% Activation (compared to no drug control)
110
100
90
80
70
60
50
40
30
20
10
0
0
0.032
0.16
0.8
4
20
100
DARTs or Controls(nM)
Ch2B6N297Q
ChCB3.1N297Q
h2B6YAxhCB3 EFc/KFc
h2B6YAxhCB3 EK
h2B6xhCB3 VF (control)
h2B6YAxhCB3 EFc/K
h2B6YAxhCB3 E/KFc

Figure 44

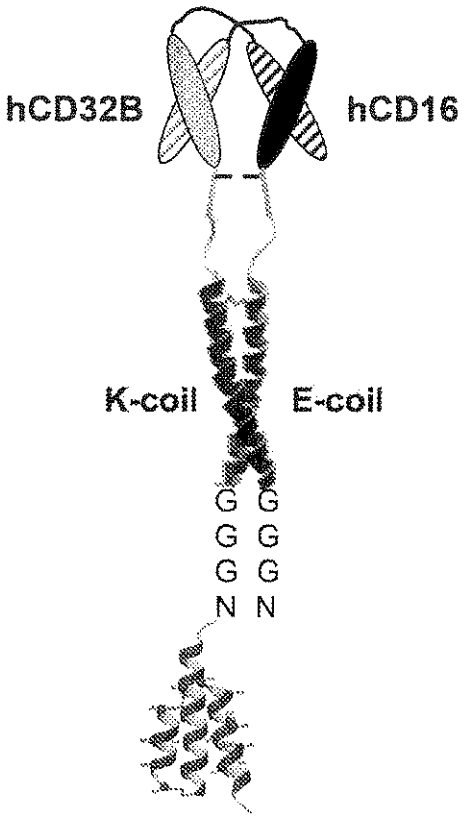


FIG. 45

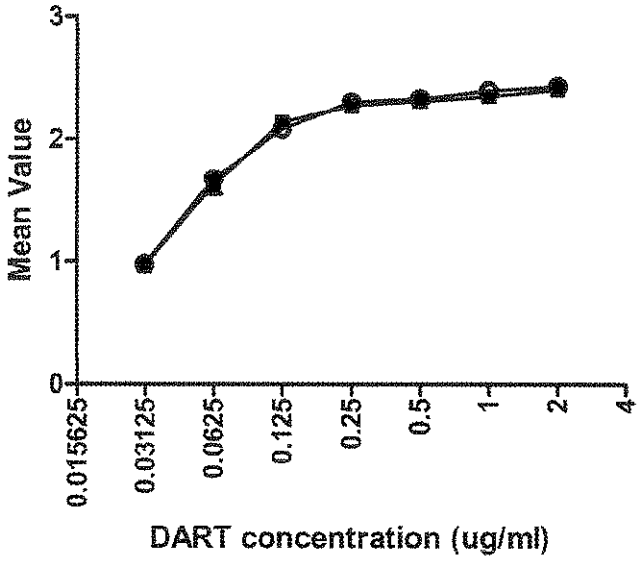


FIG. 46A

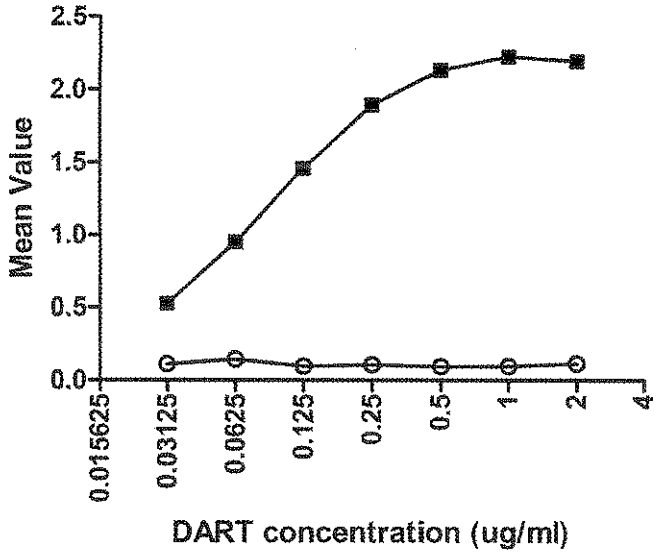


FIG. 46B

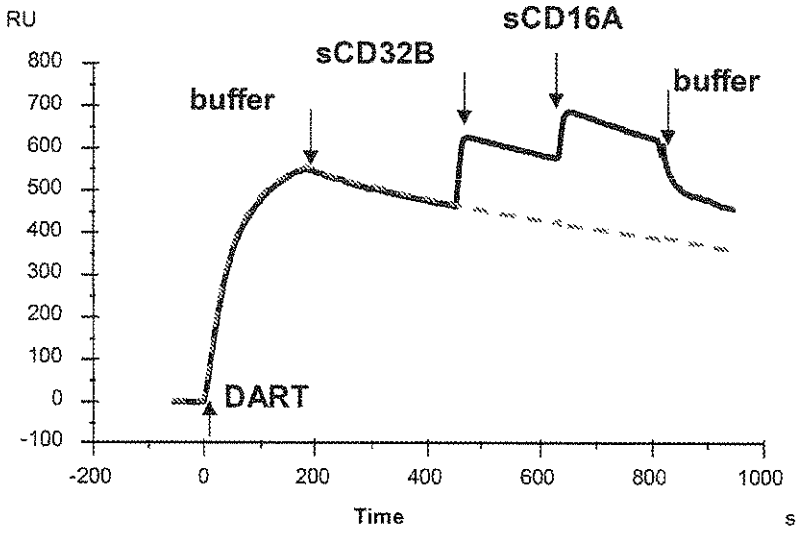
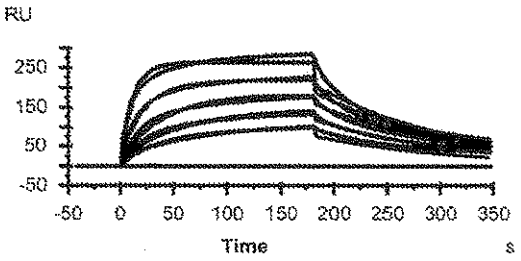

RU
800
700
600
500
400
300
200
100
0
-100
buffer
sCD32B
sCD16A
buffer
DART
-200
0
200
400
600
800
1000
Time
s

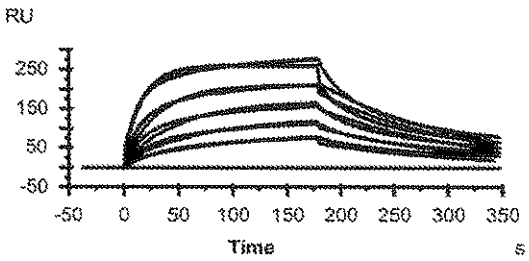
FIG. 46C

FIG. 46D
DART



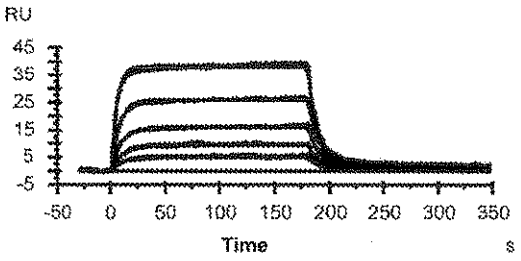
sCD32B
ka1/Ms 3.7e5 kd,1/s 7.4e-3
Kd,nM=20

FIG. 46E
ABD-DART



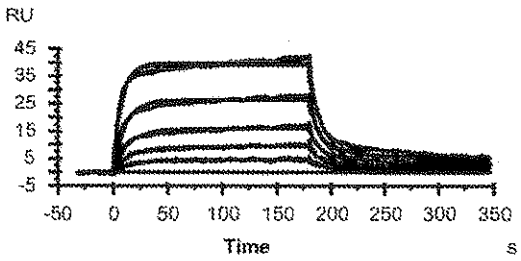
sCD32B
ka1/Ms 2.4e5 kd,1/s 7.2e-3
Kd,nM=30

FIG. 46F
DART



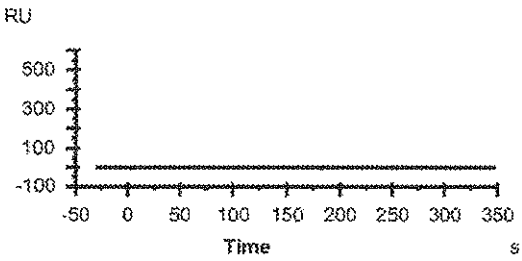
sCD16A
ka1/Ms5.7e5 kd,1/s0.090
Kd,nM=158

FIG. 46G
ABD-DART



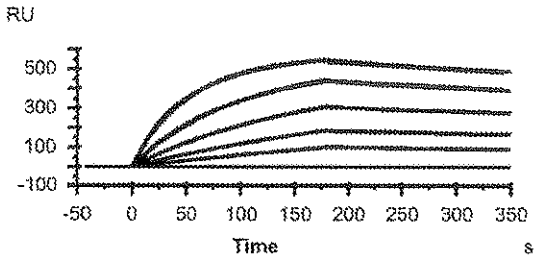
sCD16A
ka1/Ms 3.8e5 kd,1/s 0.052
Kd,nM=137

FIG. 46H
DART



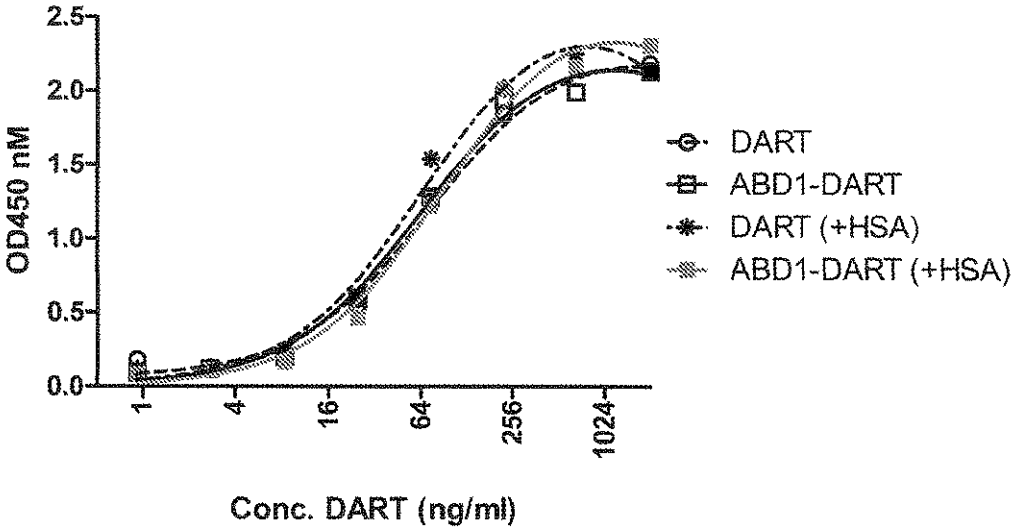
HSA

FIG. 46I
ABD-DART



HSA
ka1/Ms 0.9e5 kd,1/s 6.4e-4
Kd,nM=7.1

FIG. 46J


2.5
2.0
1.5
1.0
0.5
0.0
OD450 nM
1
4
16
64
256
1024
Conc. DART (ng/ml)
DART
ABD1-DART
DART (+HSA)
ABD1-DART (+HSA)

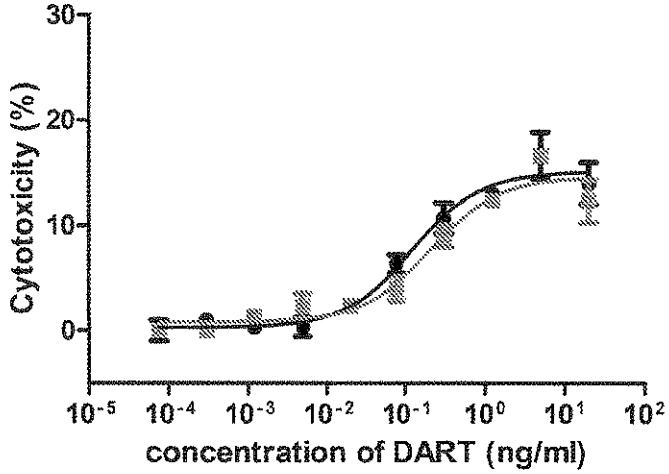


FIG. 47A

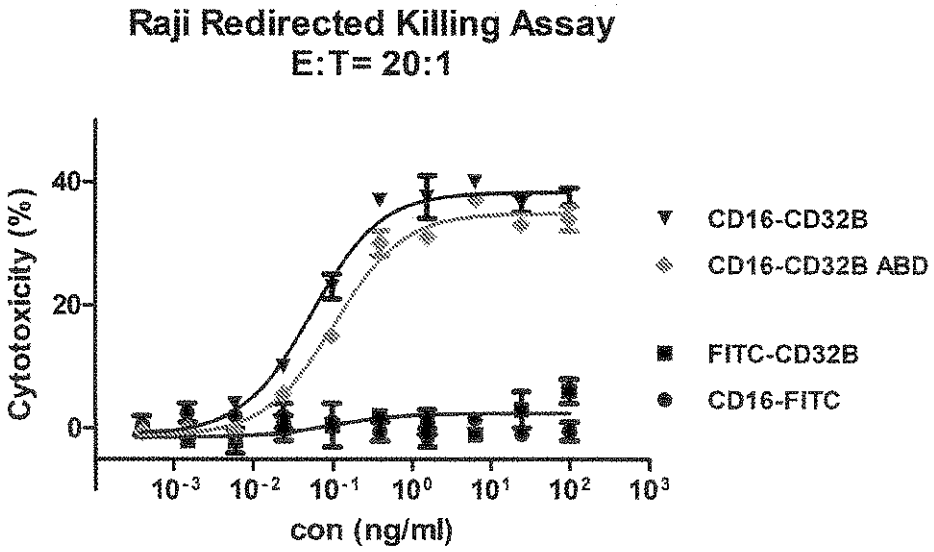


FIG. 47B

FIG. 48

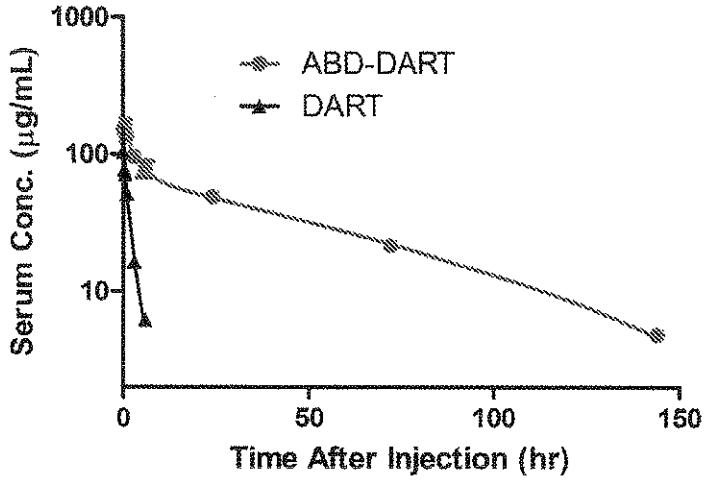


FIG. 49A

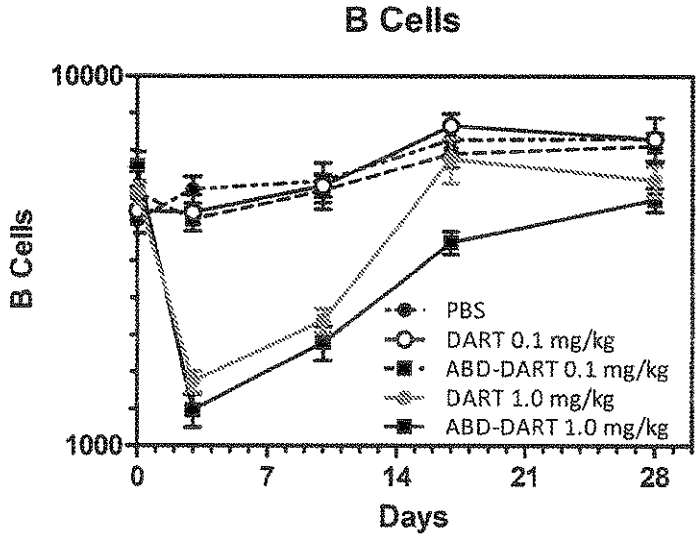


FIG. 49B

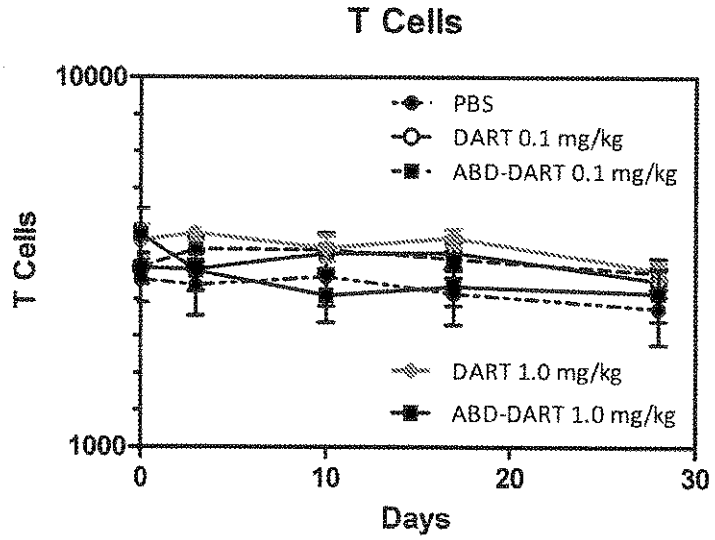


FIG. 49C

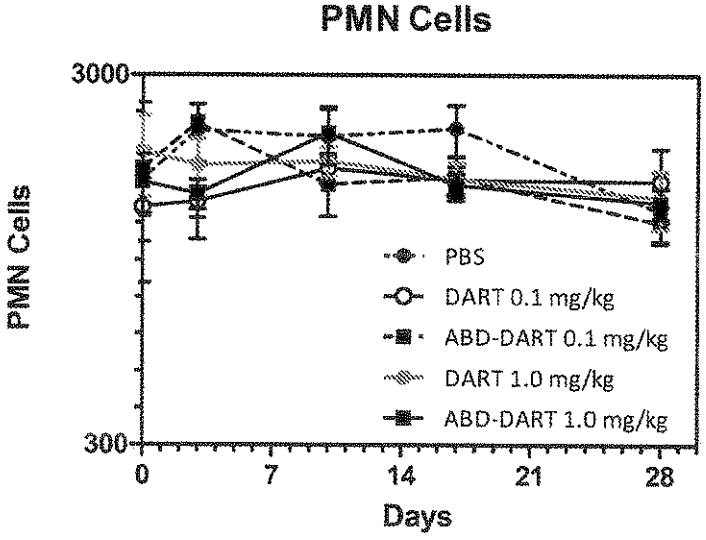


FIG. 50A

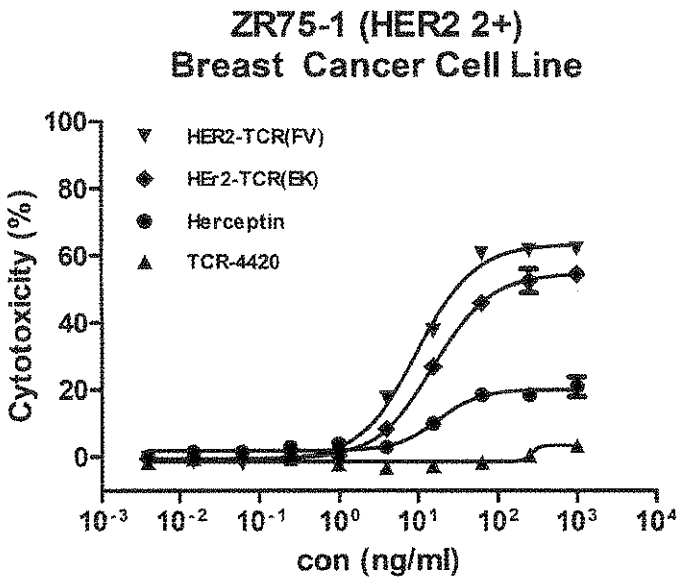


FIG. 50B

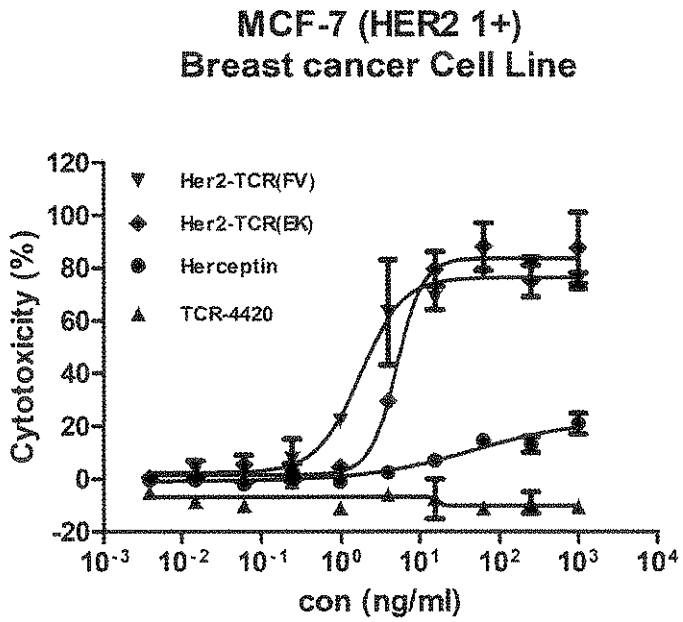


FIG. 50C

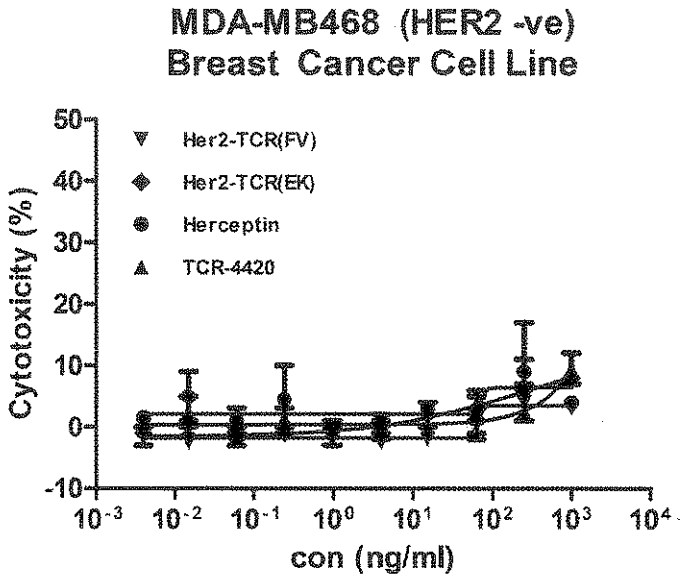


FIG. 50D

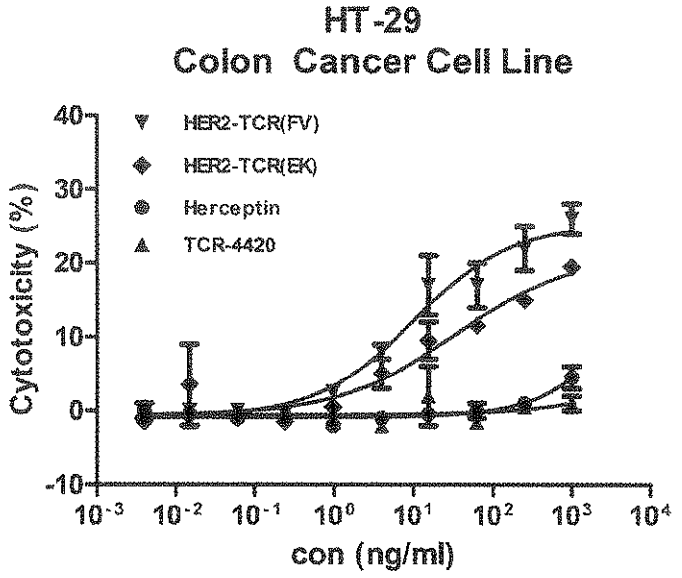


FIG. 50E

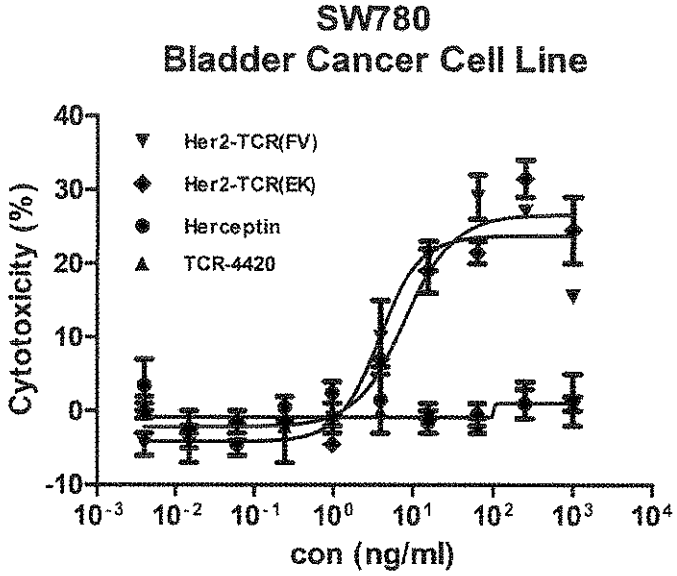


FIG. 51A

Affinity of purified KYK2 4420 captured with sFITC and detection with polyclonal anti EK Ab 030210

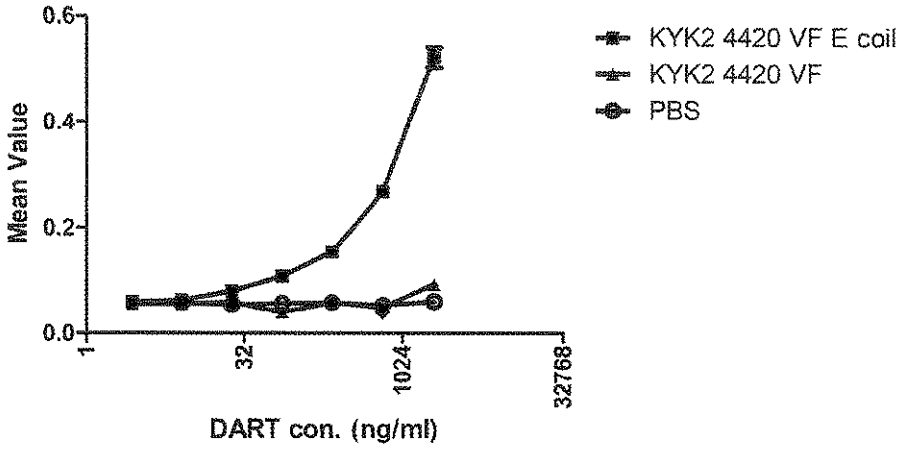


FIG 51B

KYK2-4420 VF E coil P312-009, CHO Trans.

0.45mg/L MSA Purified

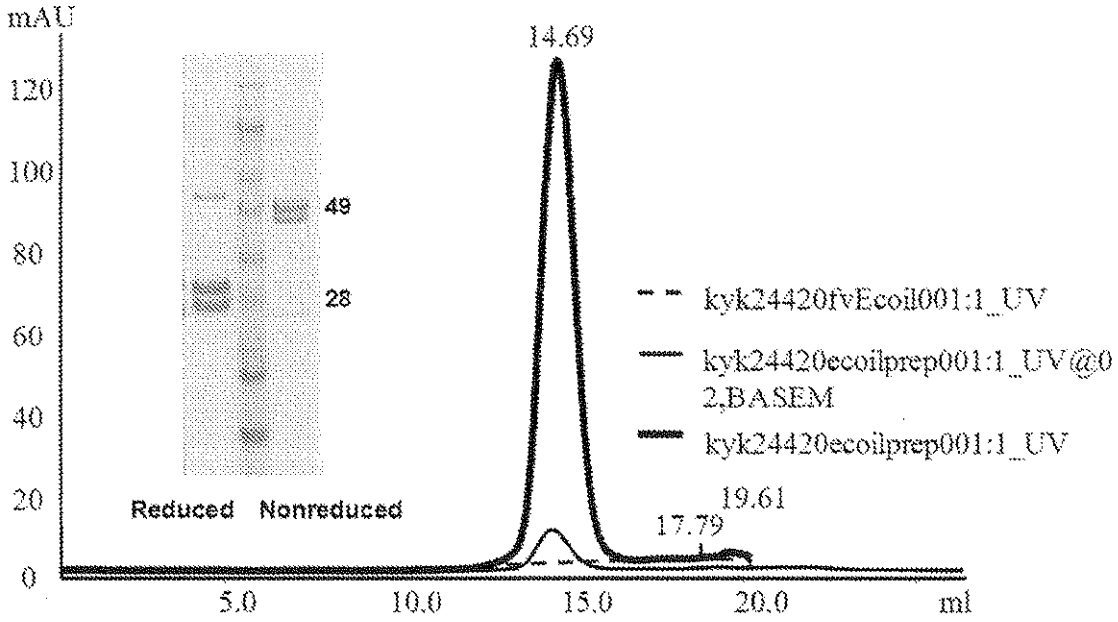


FIG. 52

Affinity of KYK2 4420 VF Ecoil DART captured with FITC antigen and detected with NKG2D Fc 012210

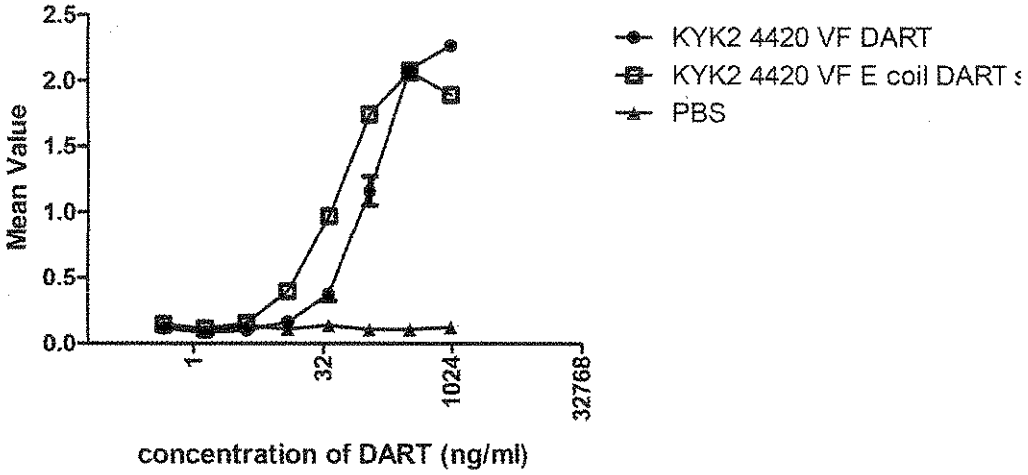
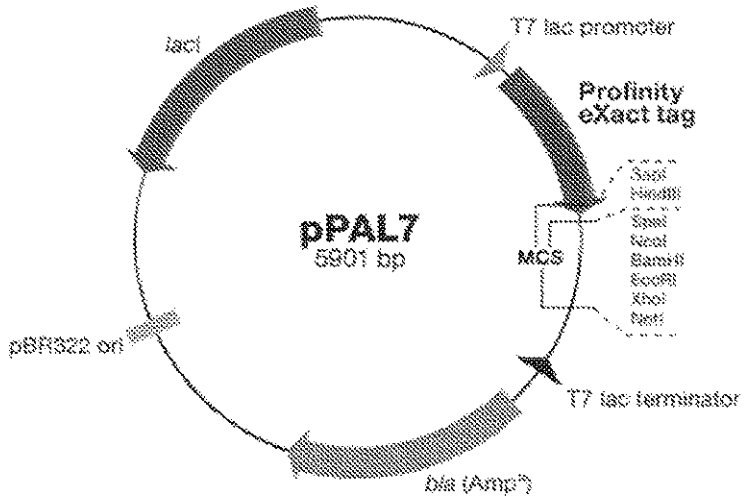
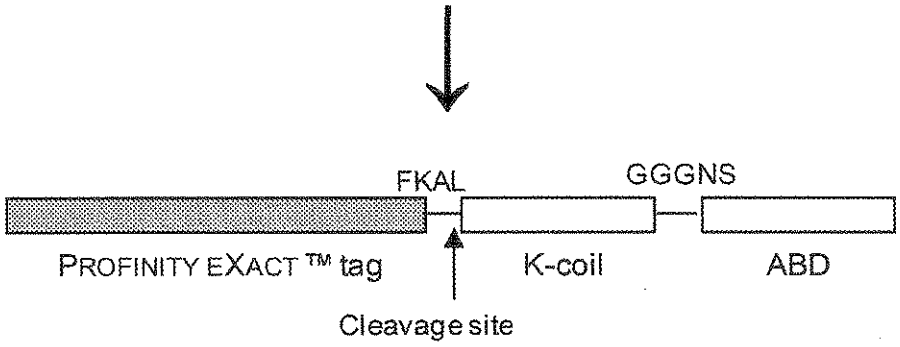


FIG. 53



+





pPAL7 Kcoil-ABD

FIG. 54

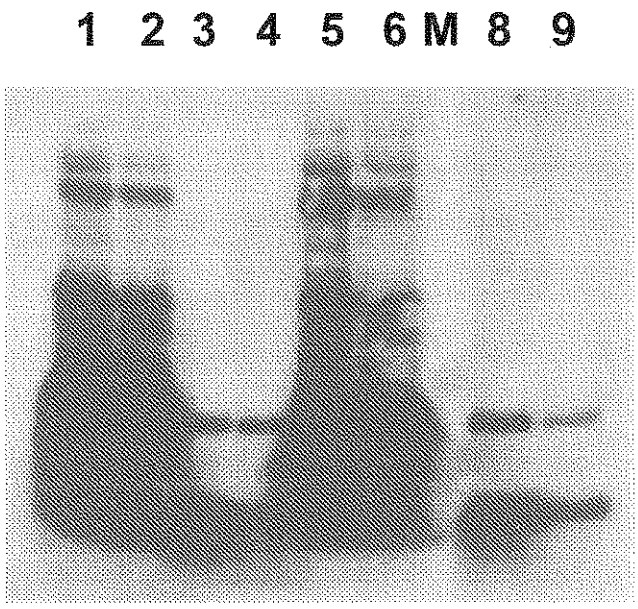

1 2 3 4 5 6 M 8 9

FIG. 55

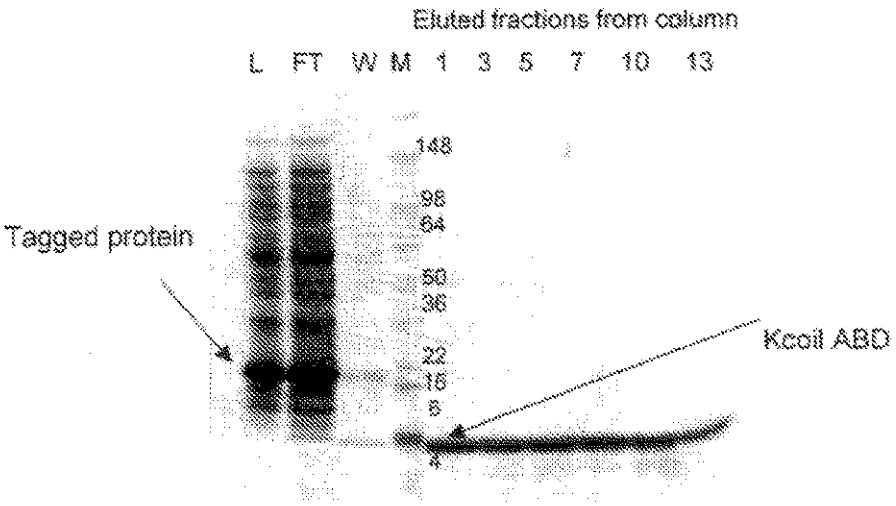

Eluted fractions from column
L FT W M 1 3 5 7 10 13
148
98
64
50
36
22
16
6
4
Tagged protein
Kcoil ABD

FIG. 56A

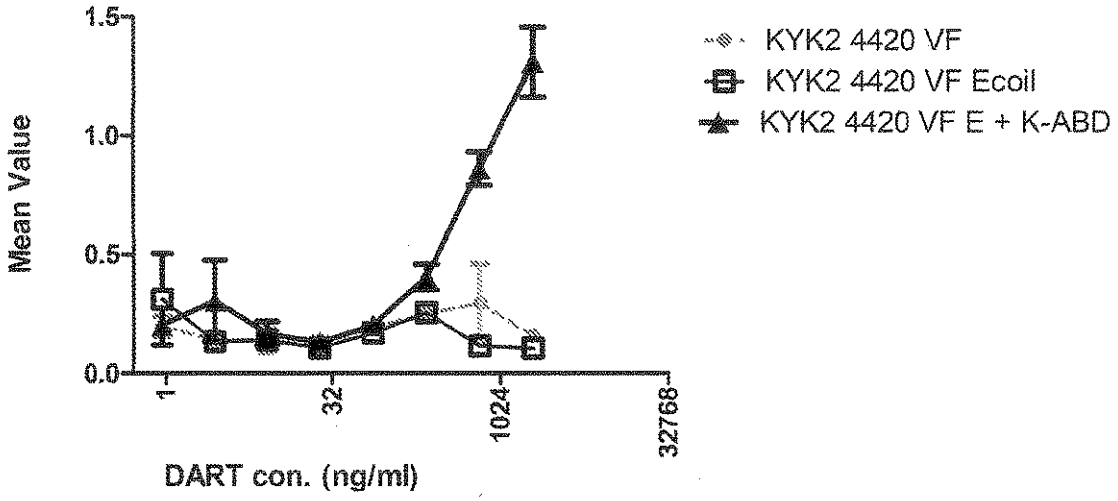


FIG. 56B

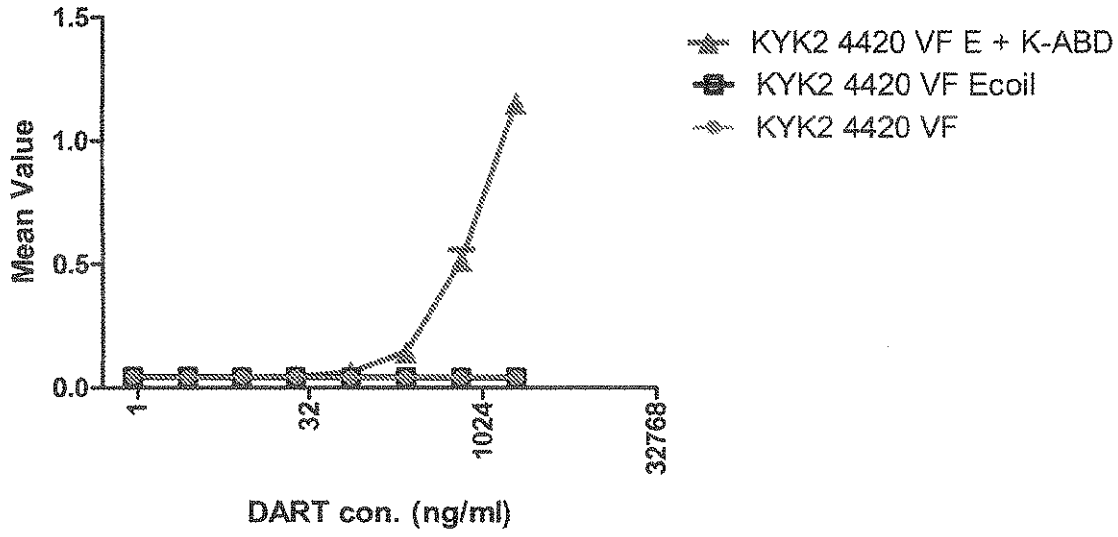


FIG. 56C

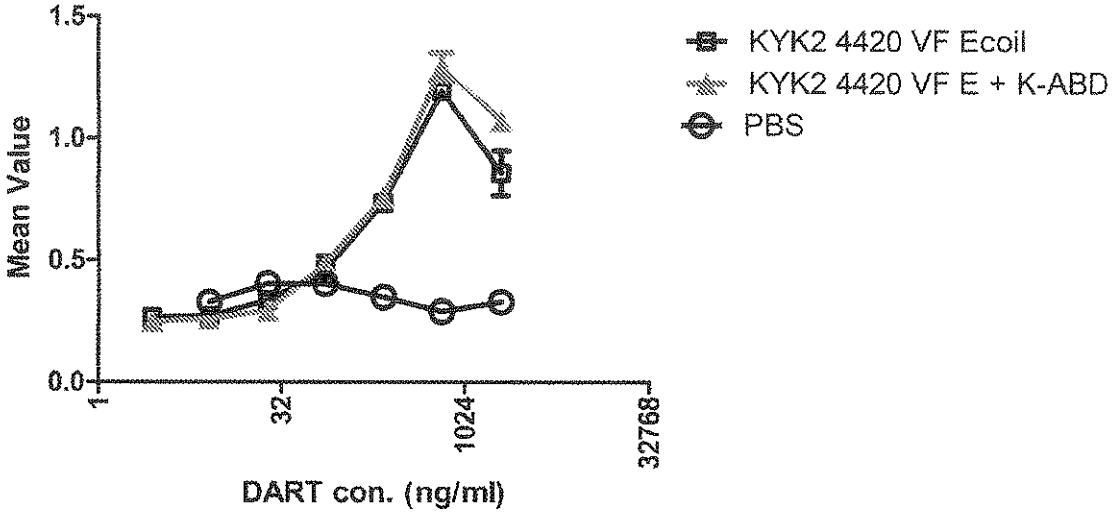


FIG. 57

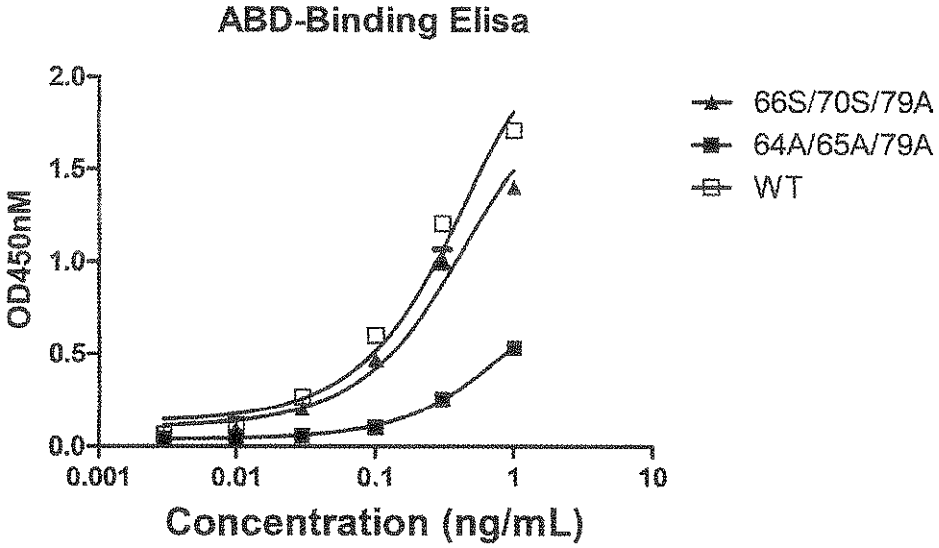


FIG. 58

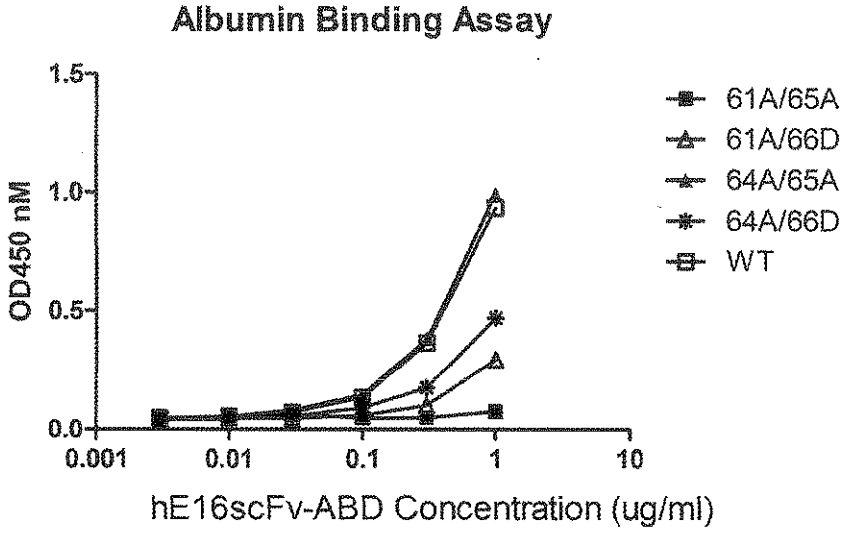

Albumin Binding Assay
OD450 nM
1.5
1.0
0.5
0.0
0.001
0.01
0.1
1
10
hE16scFv-ABD Concentration (ug/ml)
61A/65A
61A/66D
64A/65A
64A/66D
WT

FIG. 59

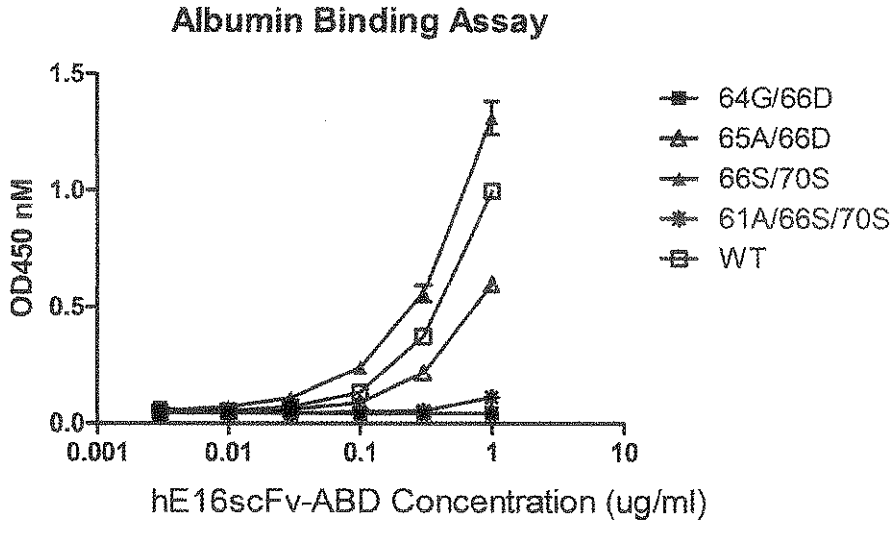

Albumin Binding Assay
OD450 nM
1.5
1.0
0.5
0.0
0.001
0.01
0.1
1
10
hE16scFv-ABD Concentration (ug/ml)
64G/66D
65A/66D
66S/70S
61A/66S/70S
WT

FIG. 60

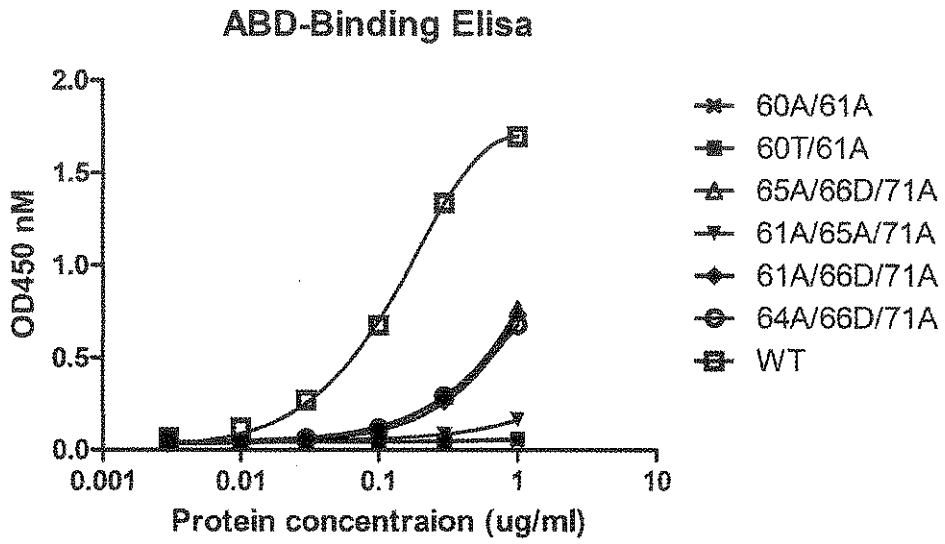

ABD-Binding Elisa
OD450 nM
2.0
1.5
1.0
0.5
0.0
0.001
0.01
0.1
1
10
Protein concentraion (ug/ml)
60A/61A
60T/61A
65A/66D/71A
61A/65A/71A
61A/66D/71A
64A/66D/71A
WT

FIG. 61

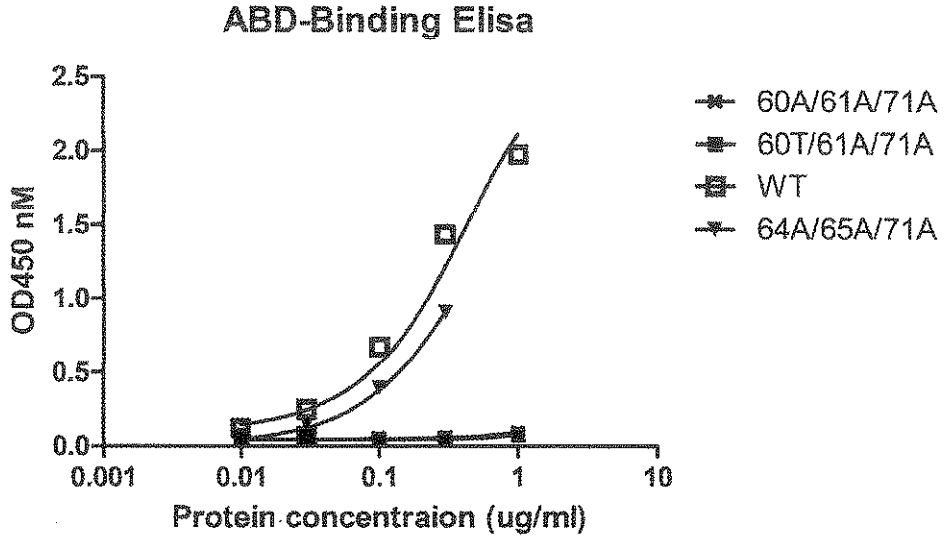

ABD-Binding Elisa
OD450 nM
2.5
2.0
1.5
1.0
0.5
0.0
0.001
0.01
0.1
1
10
Protein concentraion (ug/ml)
60A/61A/71A
60T/61A/71A
WT
64A/65A/71A

FIG. 62

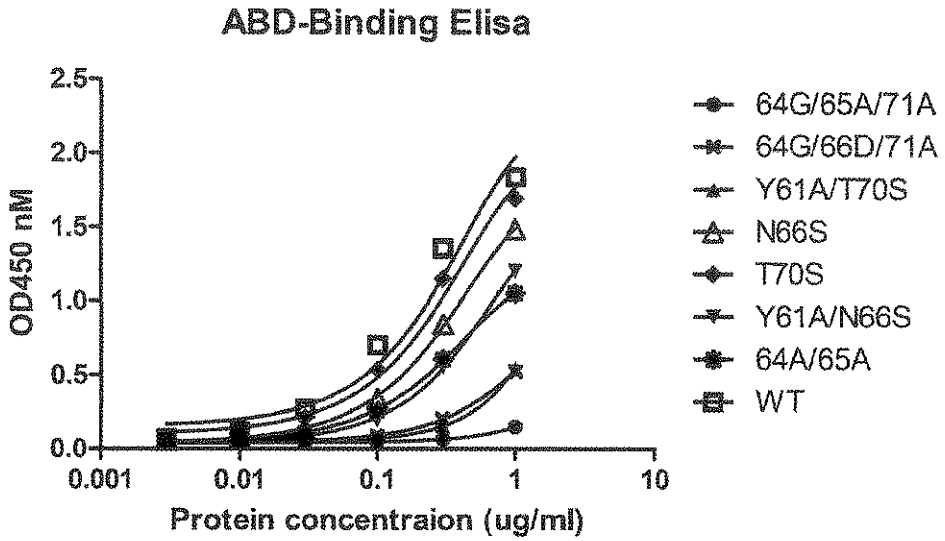

ABD-Binding Elisa
OD450 nM
2.5
2.0
1.5
1.0
0.5
0.0
0.001
0.01
0.1
1
10
Protein concentraion (ug/ml)
64G/65A/71A
64G/66D/71A
Y61A/T70S
N66S
T70S
Y61A/N66S
64A/65A
WT

FIG. 63

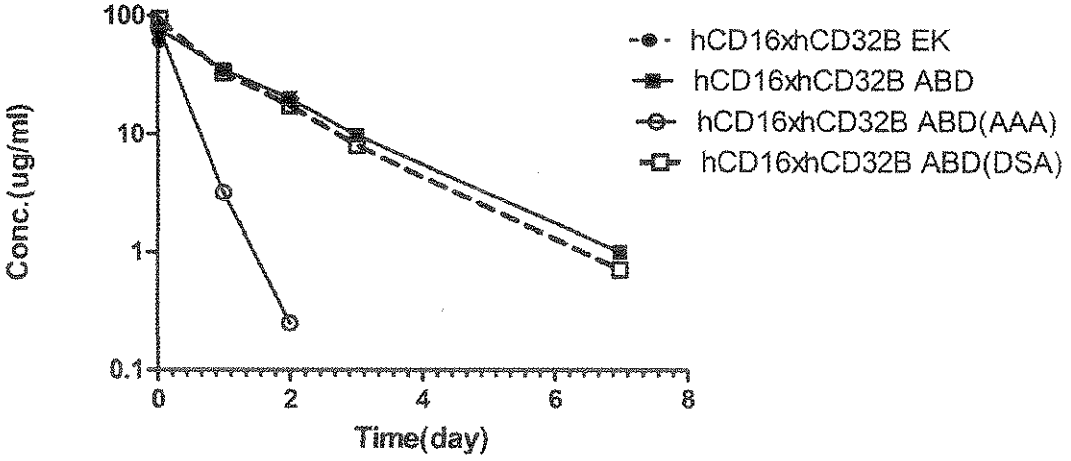

Conc.(ug/ml)
100
10
1
0.1
0
2
4
6
8
Time(day)
hCD16xhCD32B EK
hCD16xhCD32B ABD
hCD16xhCD32B ABD(AAA)
hCD16xhCD32B ABD(DSA)

FIG.64A

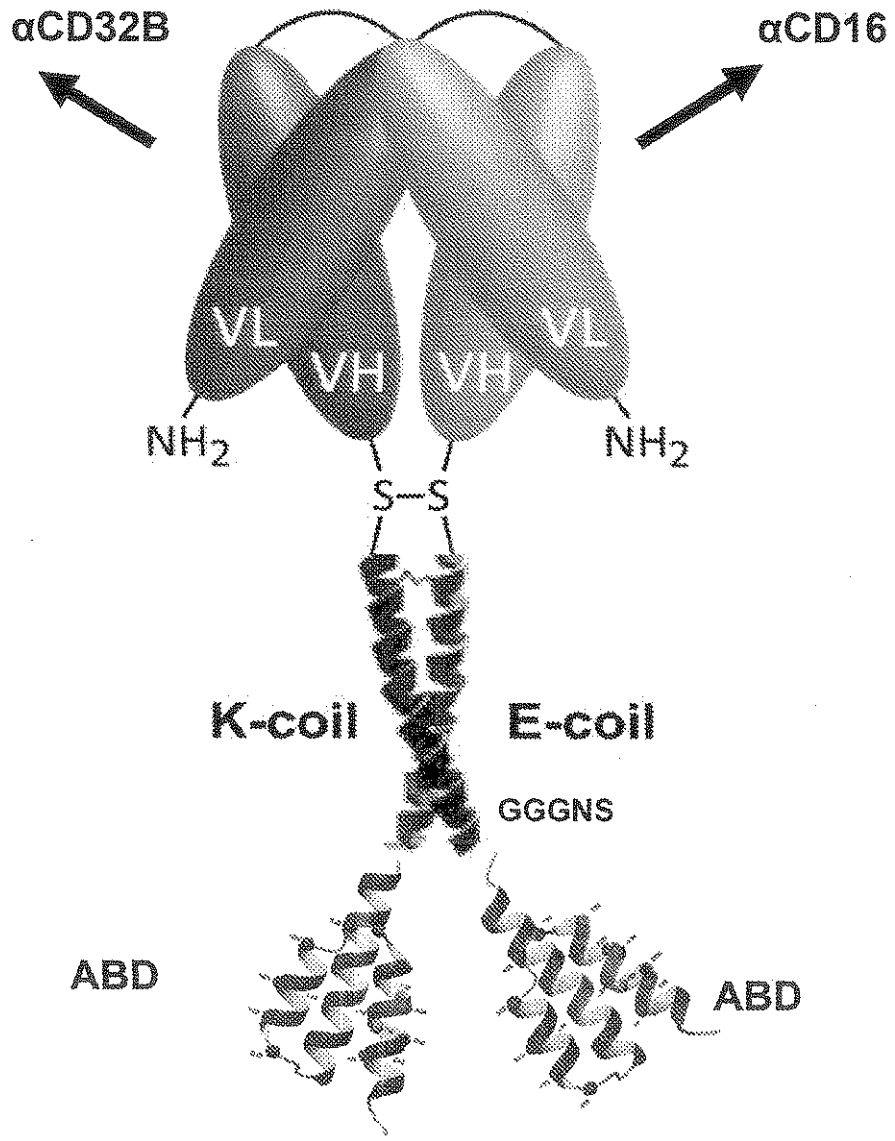


FIG.64B

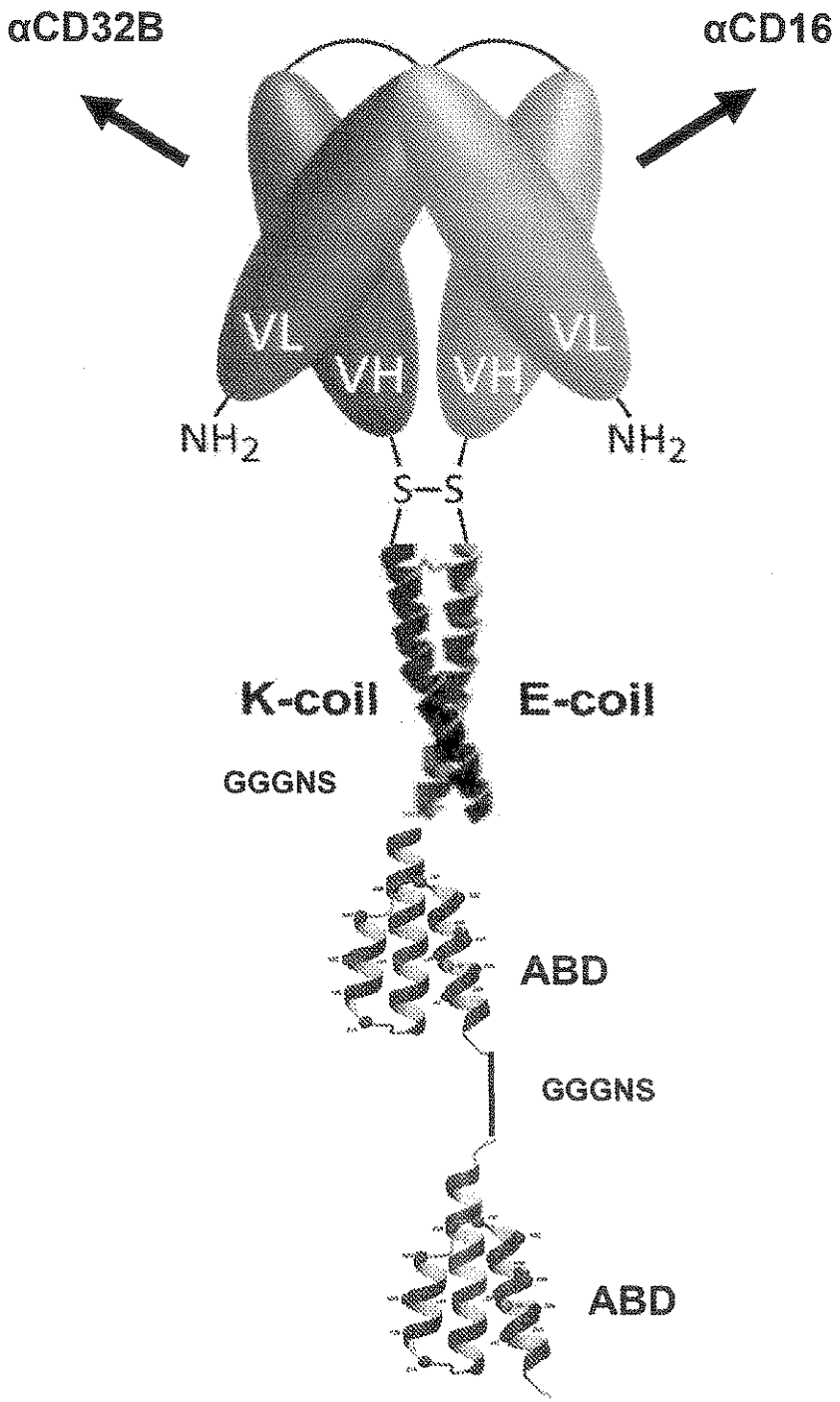

αCD32B
αCD16
VL
VH
VH
VL
NH2
NH2
S—S
K-coil
E-coil
GGGNS
ABD
GGGNS
ABD

FIG.65A

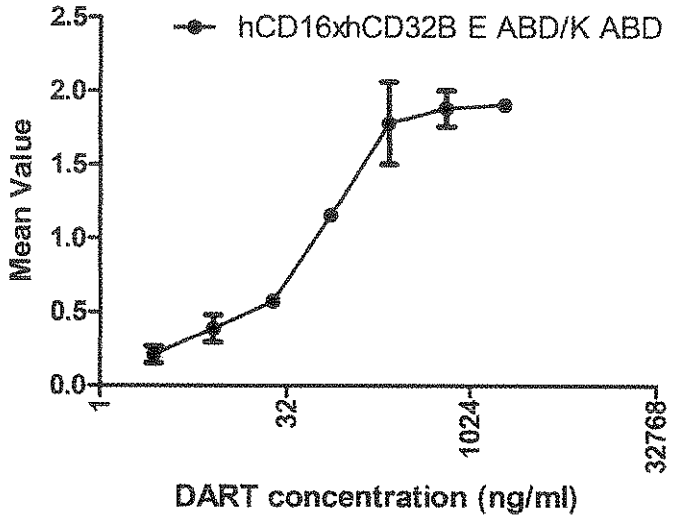


FIG.65B

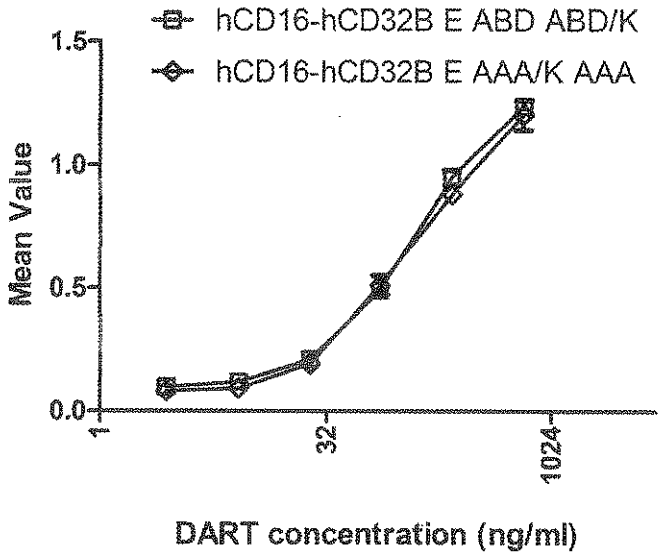


FIG.65C

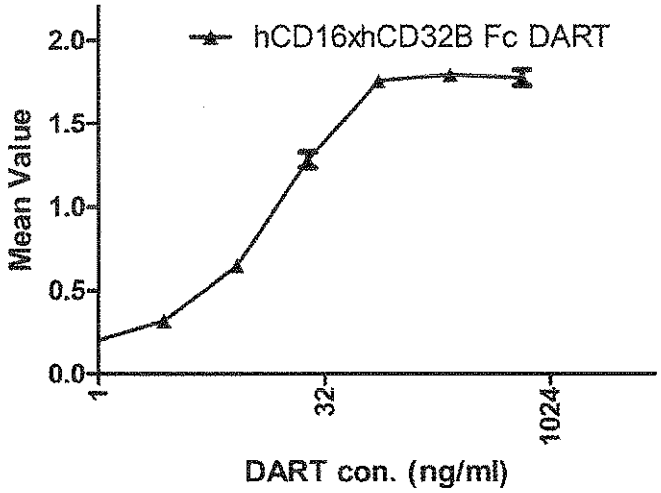
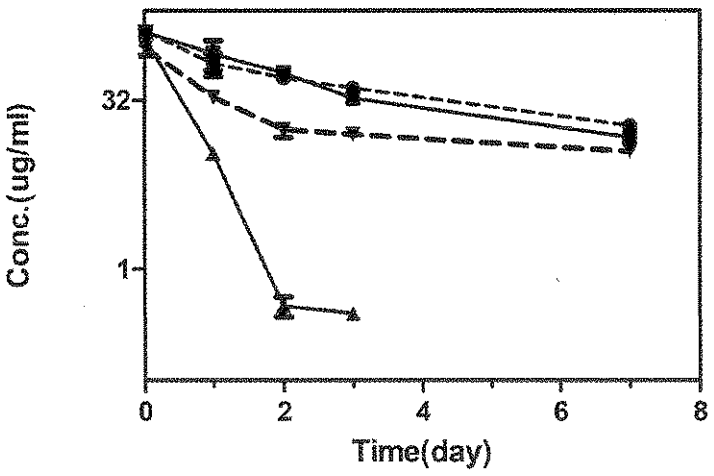


FIG.66



hCD16xhCD32B E ABD / K ABD
hCD16xhCD32B E ABD-ABD / K
h2B6-h3G8 E ABD(AAA) / K ABD(AAA)
hCD16xhCD32B E Fc / K