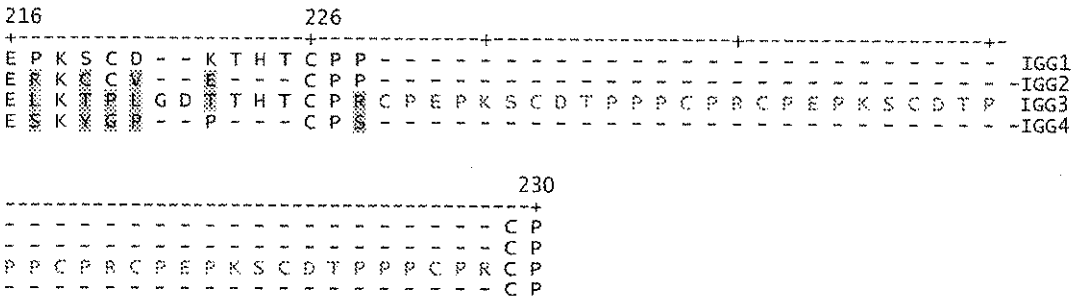


FIG. 1A

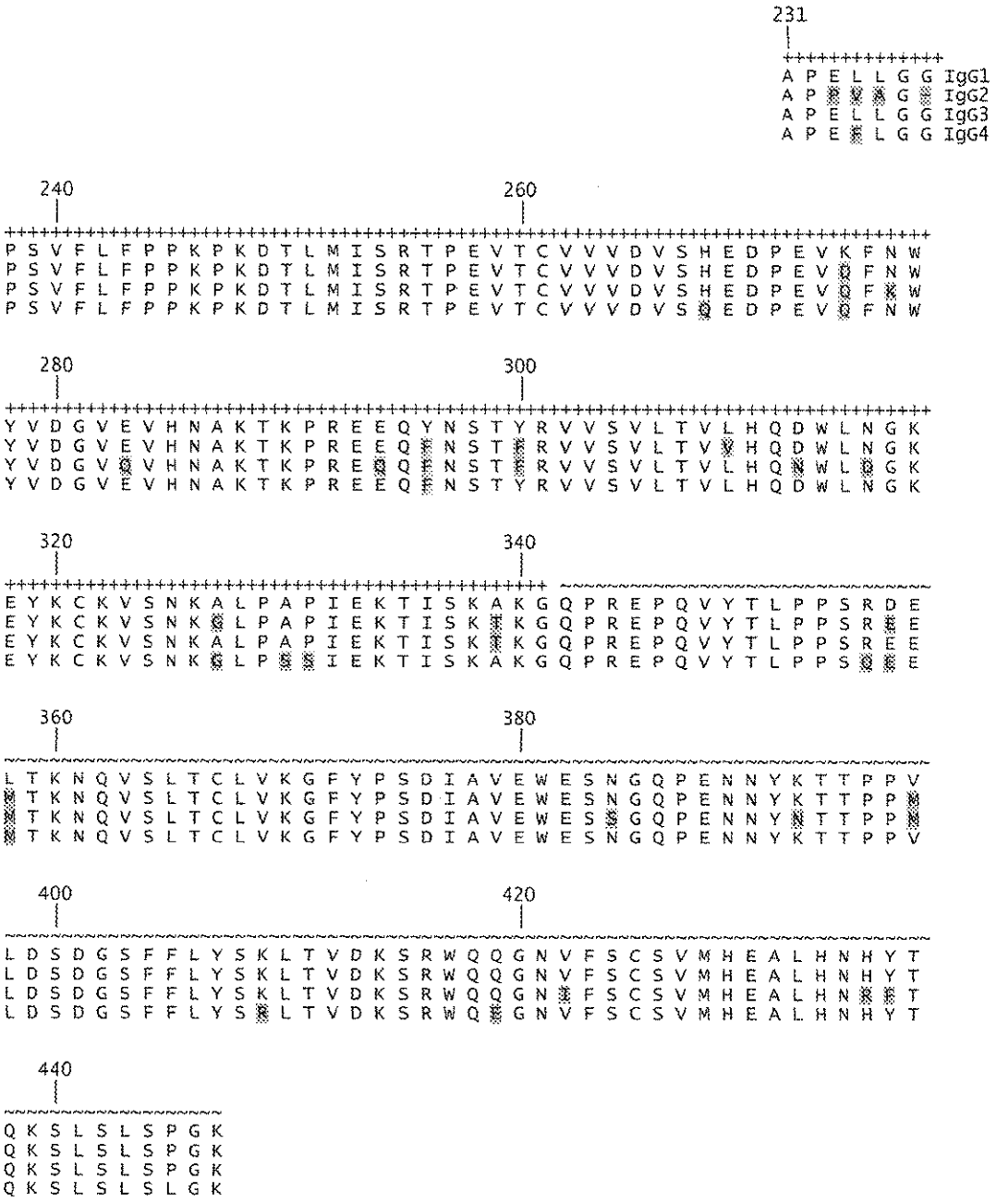

231
A P E L L G G IgG1
A P X X X G X IgG2
A P E L L G G IgG3
A P E X L G G IgG4
240
260
P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S H E D P E V K F N W
P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S H E D P E V X F N W
P S V F L F P P K P K D T L M I S R T P E V T C V V V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

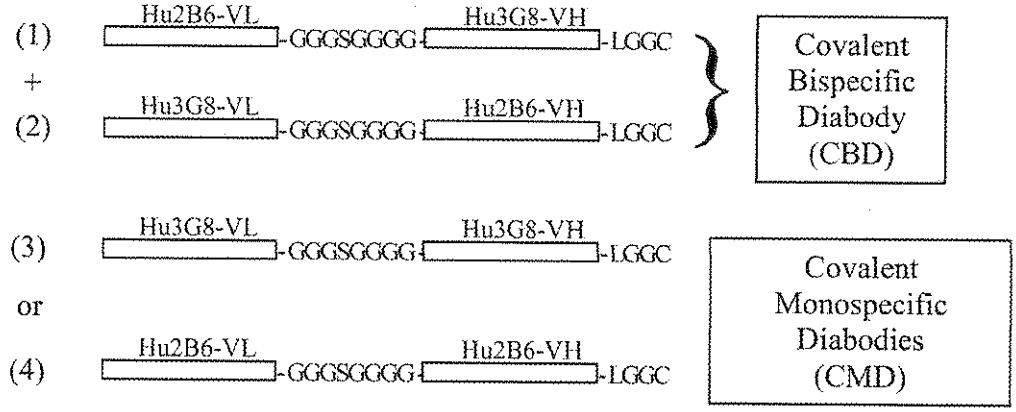

(1)
Hu2B6-VL
-GGGSGGGG-
Hu3G8-VH
-LGGC
+
(2)
Hu3G8-VL
-GGGSGGGG-
Hu2B6-VH
-LGGC
Covalent Bispecific Diabody (CBD)
(3)
Hu3G8-VL
-GGGSGGGG-
Hu3G8-VH
-LGGC
or
(4)
Hu2B6-VL
-GGGSGGGG-
Hu2B6-VH
-LGGC
Covalent Monospecific Diabodies (CMD)

FIG. 2

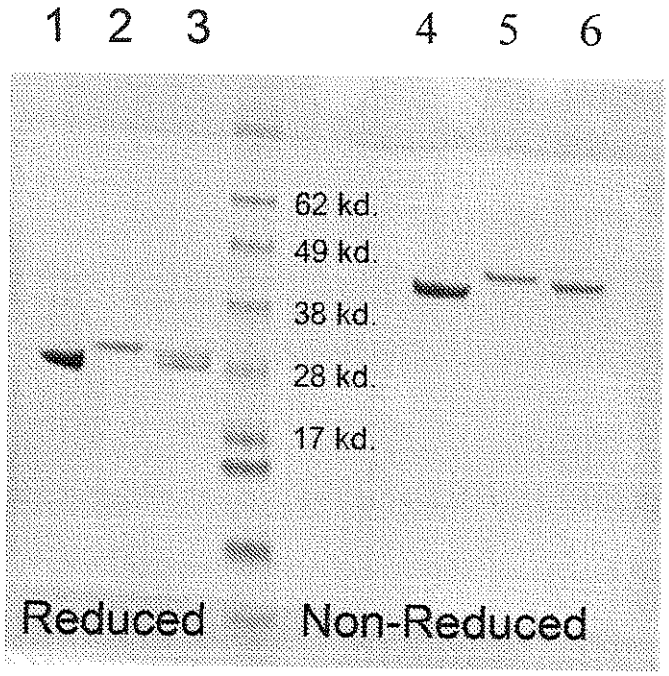


FIG. 3

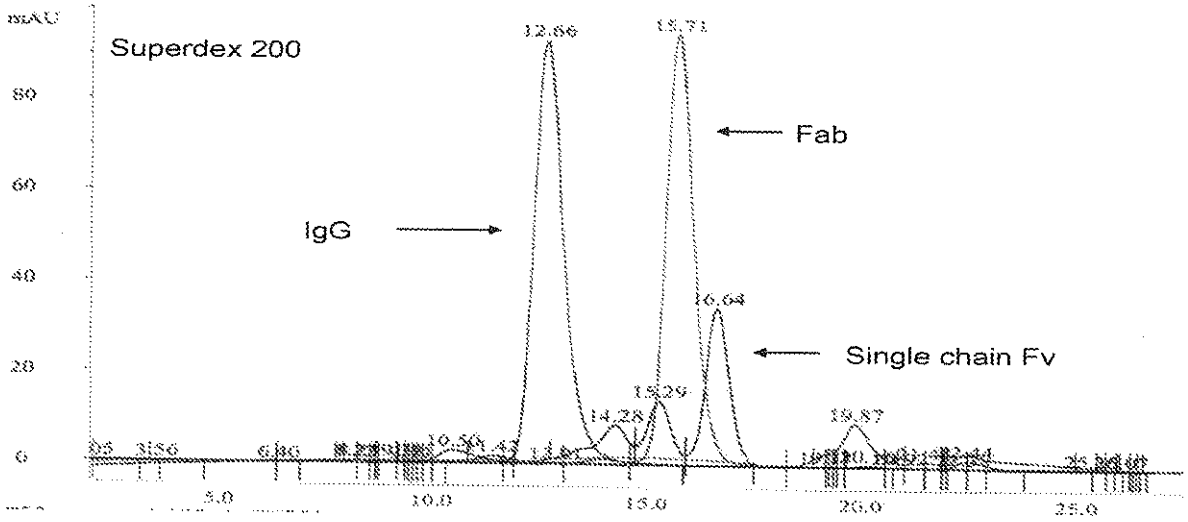

mAU
Superdex 200
80
60
40
20
0
12.66
15.71
Fab
IgG
16.64
Single chain Fv
15.29
14.28
19.87
5.0
10.0
15.0
20.0
25.0

FIG. 4A

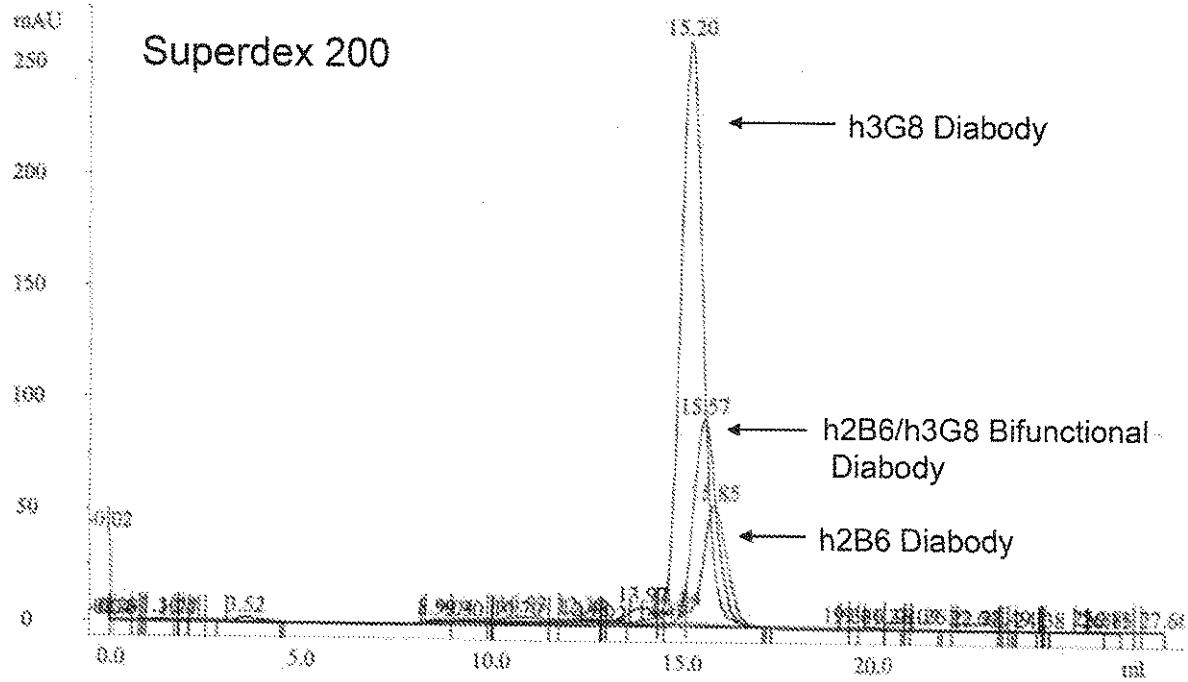

mAU
250
200
150
100
50
0
Superdex 200
15.20
h3G8 Diabody
15.57
h2B6/h3G8 Bifunctional
Diabody
h2B6 Diabody
0.0
5.0
10.0
15.0
20.0
ml

FIG. 4B

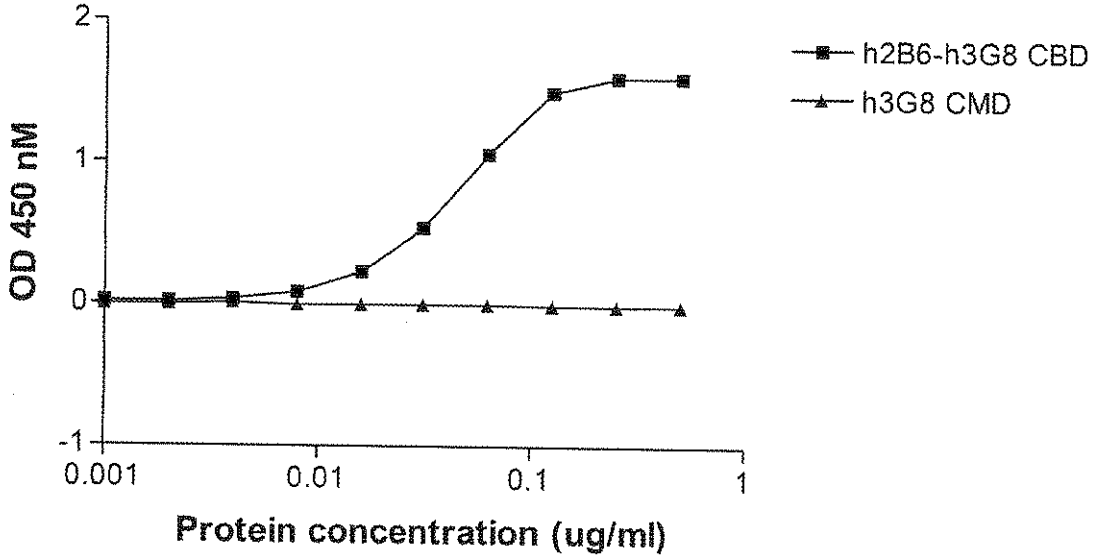

2
1
0
-1
OD 450 nM
0.001
0.01
0.1
1
Protein concentration (ug/ml)
h2B6-h3G8 CBD
h3G8 CMD

FIG. 5

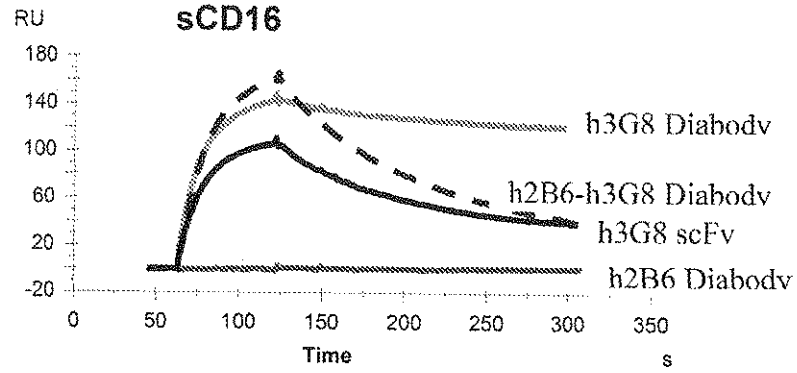


FIG. 6A

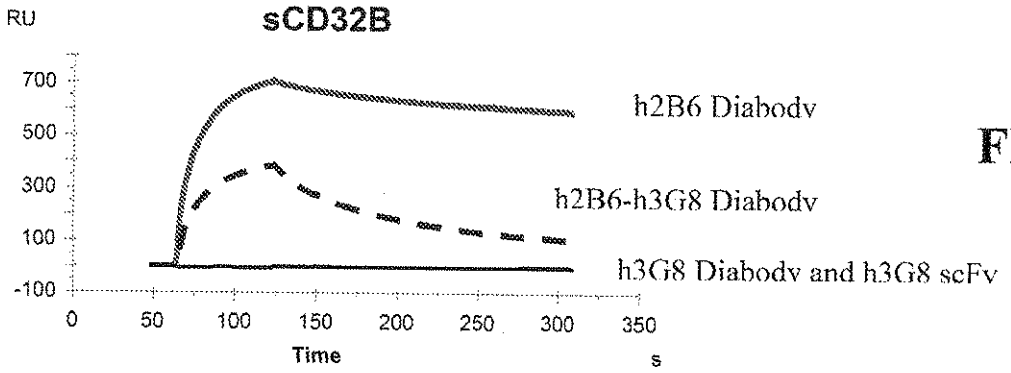


FIG. 6B

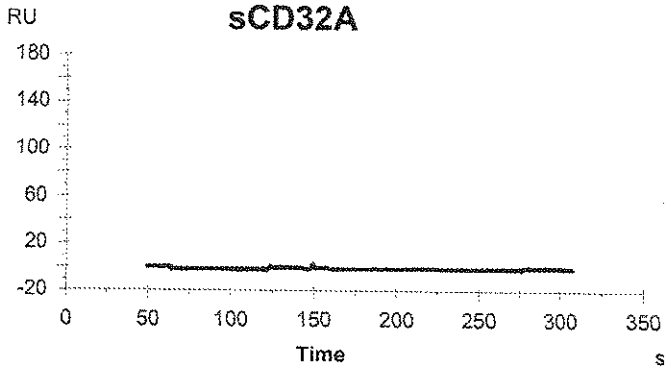


FIG. 6C

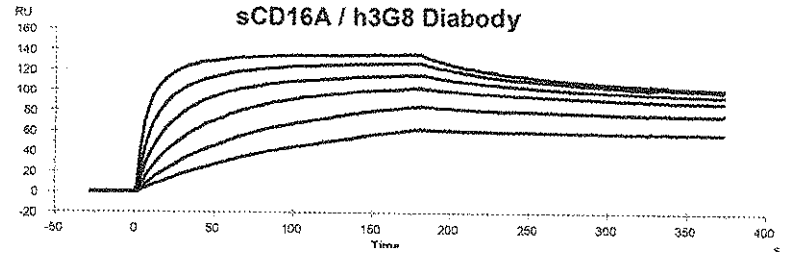


FIG. 7A

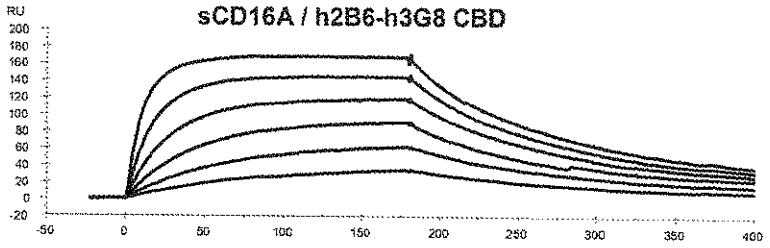


FIG. 7B

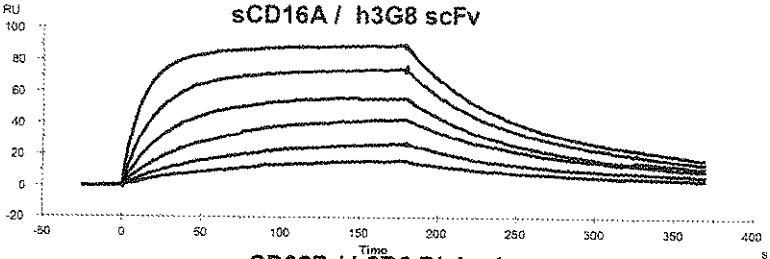


FIG. 7C

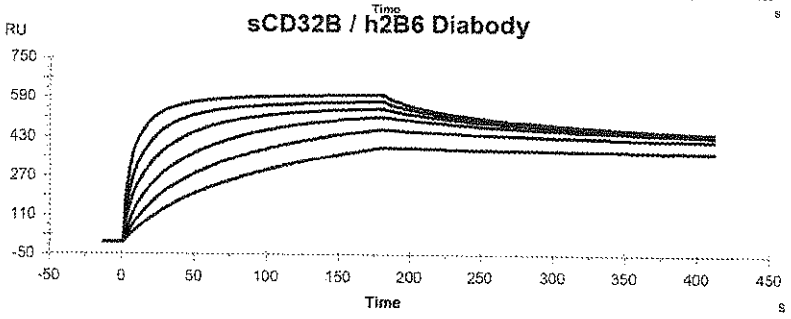


FIG. 7D

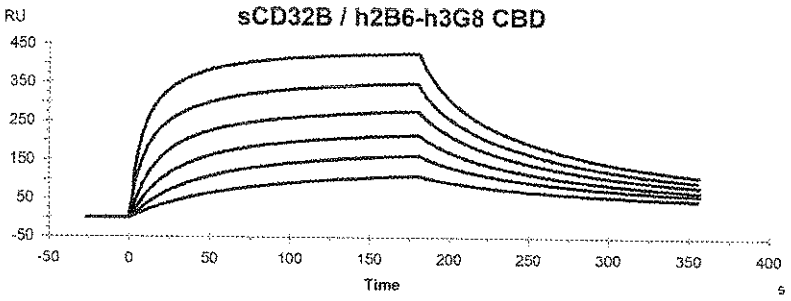


FIG. 7E

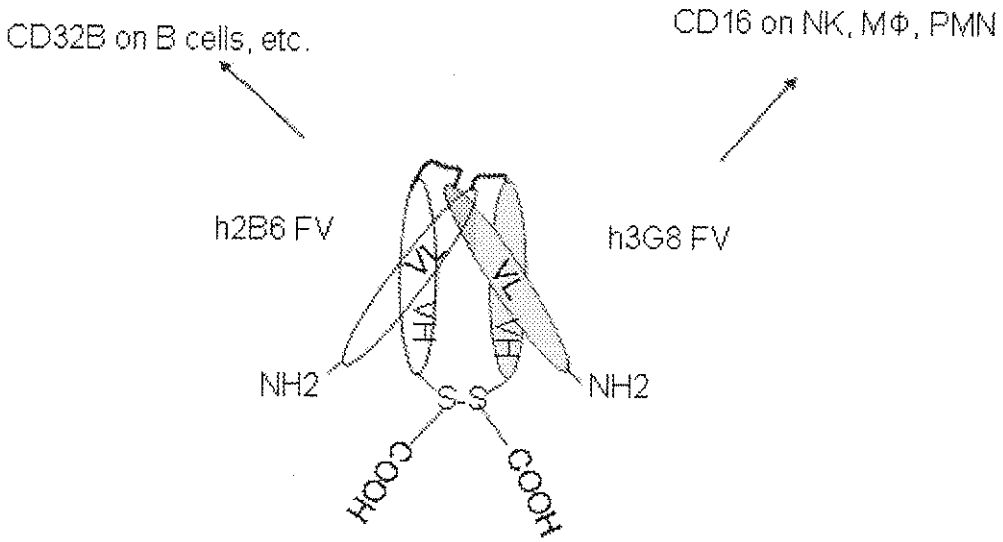

CD32B on B cells, etc.
CD16 on NK, MΦ, PMN
h2B6 FV
h3G8 FV
VL
VH
VL
VH
NH2
NH2
S-S
HOOC
COOH

FIG. 8

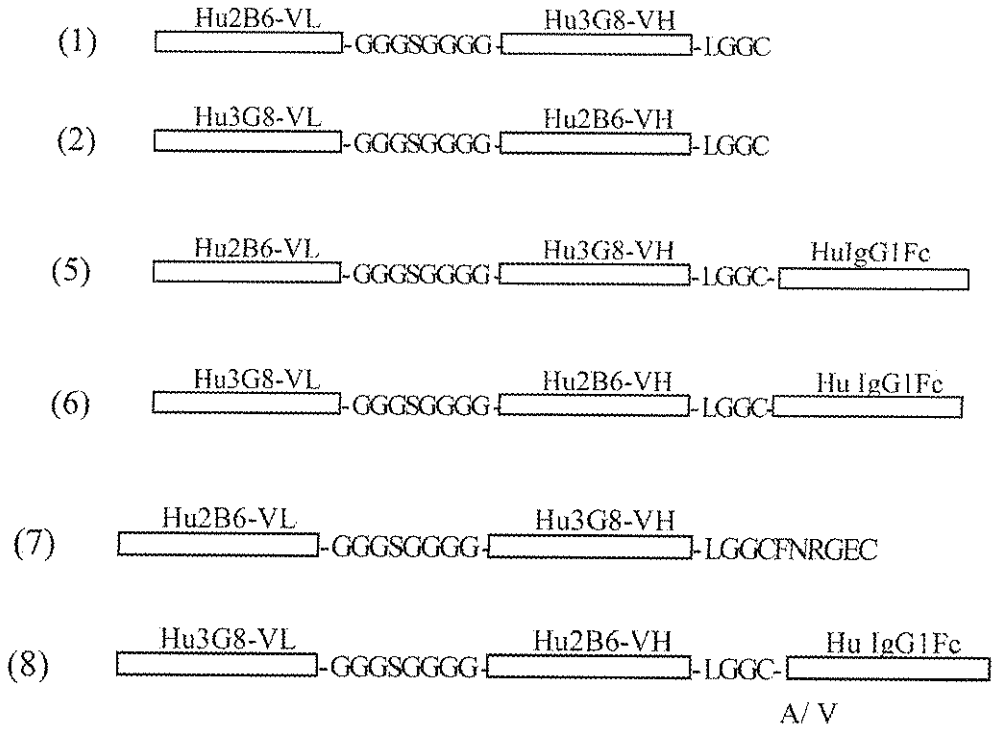

(1)
Hu2B6-VL
-GGGSGGGG-
Hu3G8-VH
-LGGC
(2)
Hu3G8-VL
-GGGSGGGG-
Hu2B6-VH
-LGGC
(5)
Hu2B6-VL
-GGGSGGGG-
Hu3G8-VH
-LGGC-
HuIgG1Fc
(6)
Hu3G8-VL
-GGGSGGGG-
Hu2B6-VH
-LGGC-
Hu IgG1Fc
(7)
Hu2B6-VL
-GGGSGGGG-
Hu3G8-VH
-LGGCFNRGEC
(8)
Hu3G8-VL
-GGGSGGGG-
Hu2B6-VH
-LGGC-
Hu IgG1Fc
A/ V

FIG. 9

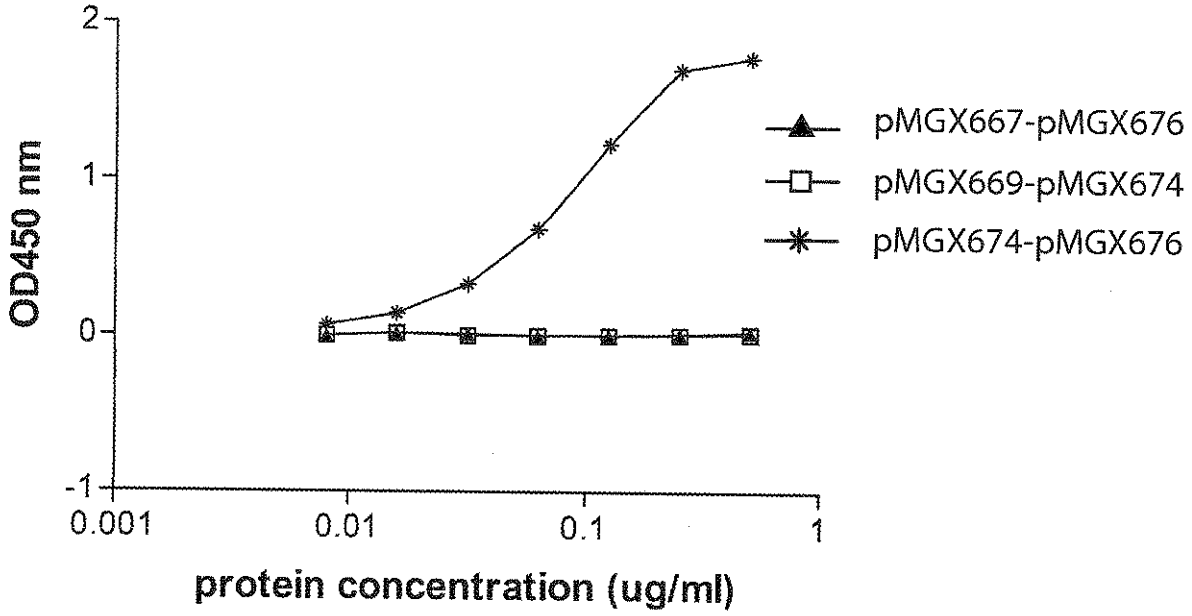

2
1
0
-1
OD450 nm
0.001
0.01
0.1
1
protein concentration (ug/ml)
pMGX667-pMGX676
pMGX669-pMGX674
pMGX674-pMGX676

FIG. 10

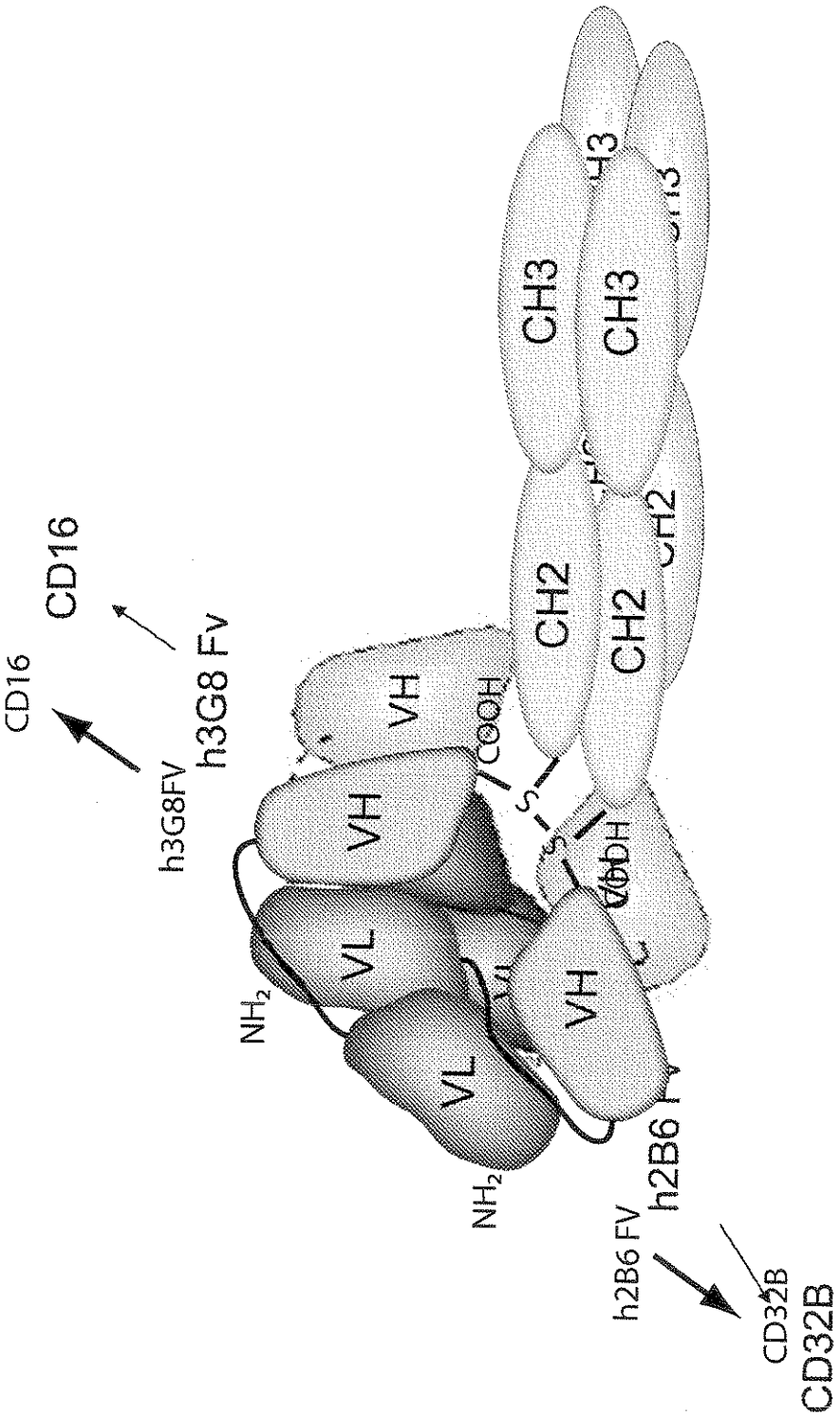

CD16
CD16
h3G8FV
h3G8 Fv
VH
VH
VL
VL
NH2
NH2
CH2
CH2
CH3
CH3
VH
h2B6 FV
h2B6 Fv
CD32B
CD32B

FIG. 11

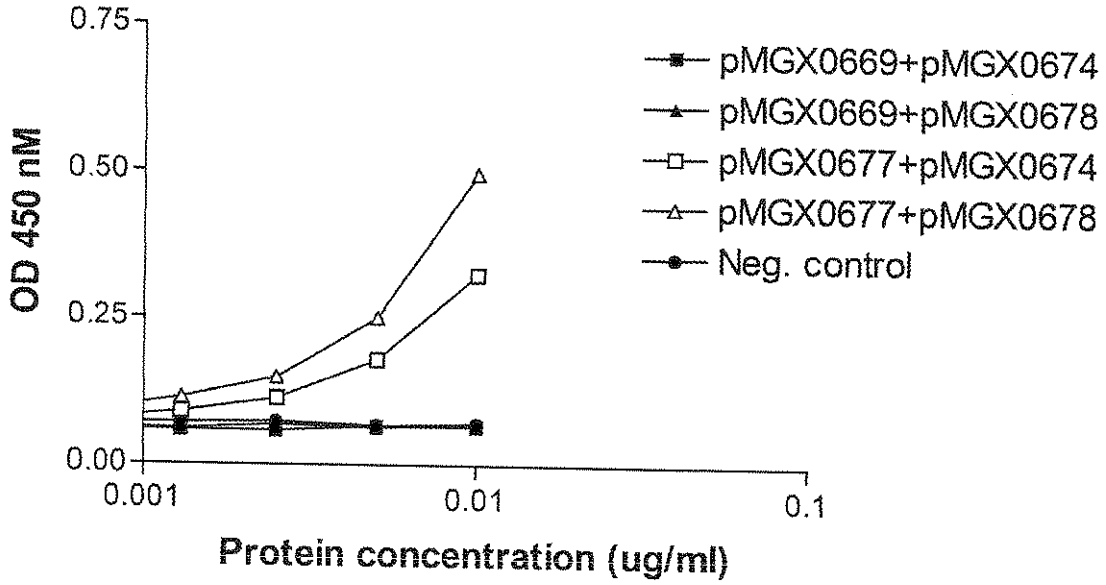

0.75
0.50
0.25
0.00
OD 450 nM
0.001
0.01
0.1
Protein concentration (ug/ml)
pMGX0669+pMGX0674
pMGX0669+pMGX0678
pMGX0677+pMGX0674
pMGX0677+pMGX0678
Neg. control

FIG. 12

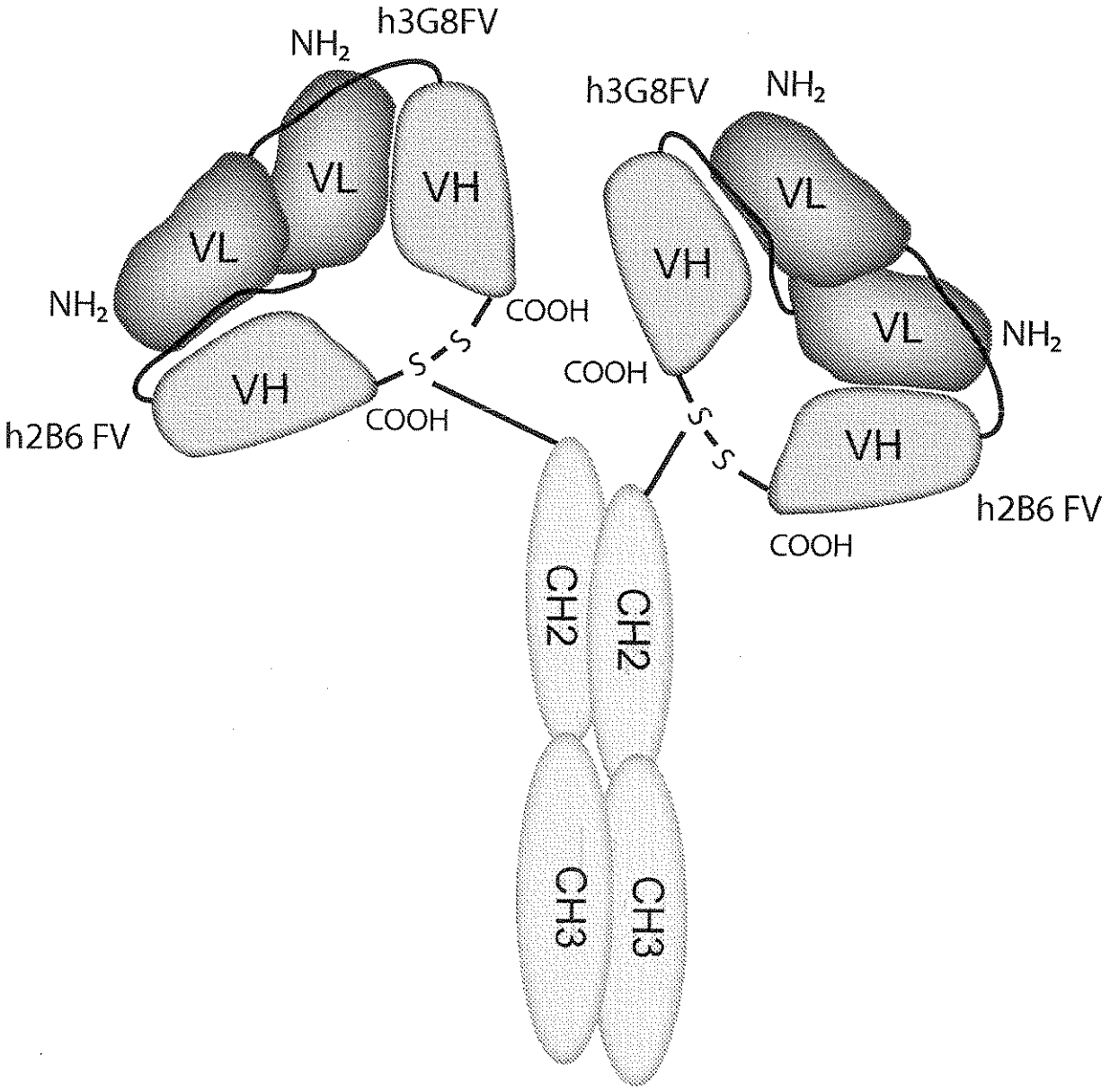

h3G8FV
NH2
VL
VH
VL
NH2
COOH
S
S
VH
COOH
h2B6 FV
h3G8FV
NH2
VL
VH
VL
NH2
COOH
S
S
VH
h2B6 FV
COOH
CH2
CH2
CH3
CH3

FIG. 13

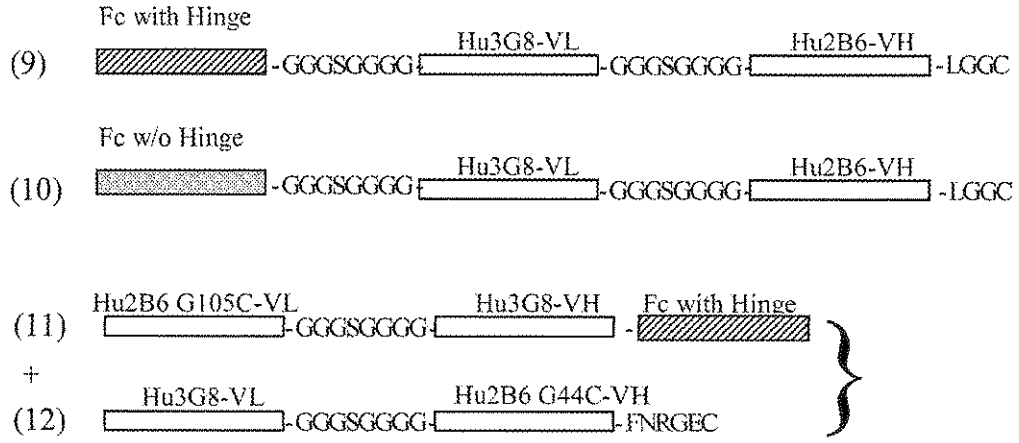

Fc with Hinge
(9)
Hu3G8-VL
Hu2B6-VH
-GGGSGGGG-
-GGGSGGGG-
-LGGC
Fc w/o Hinge
(10)
Hu3G8-VL
Hu2B6-VH
-GGGSGGGG-
-GGGSGGGG-
-LGGC
Hu2B6 G105C-VL
Hu3G8-VH
Fc with Hinge
(11)
-GGGSGGGG-
-
+
Hu3G8-VL
Hu2B6 G44C-VH
(12)
-GGGSGGGG-
-FNRGEC

FIG. 14

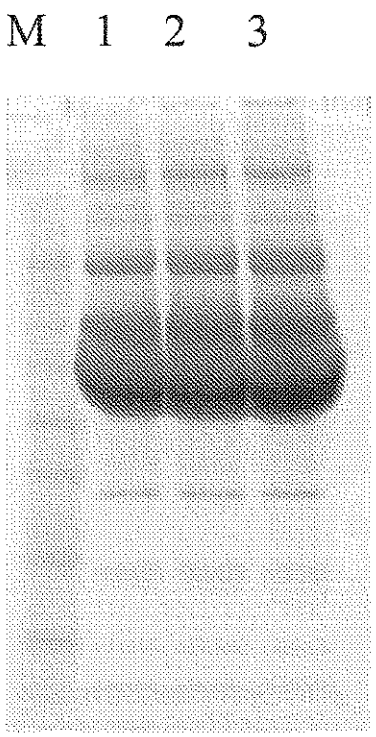


FIG. 15A

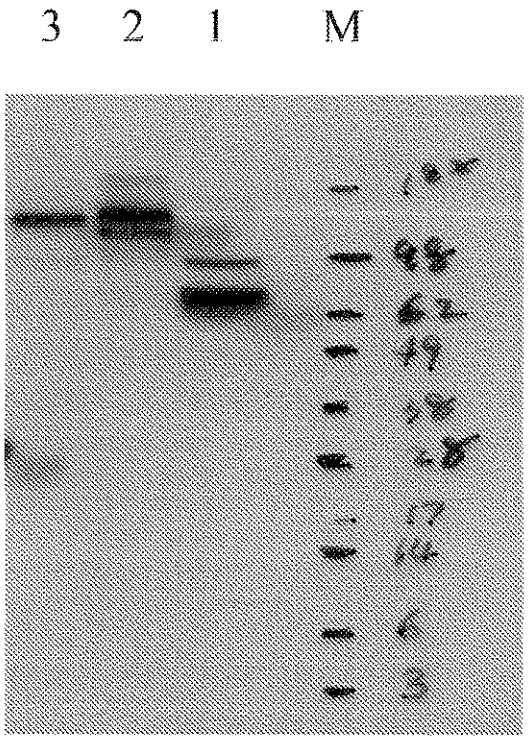


FIG. 15B

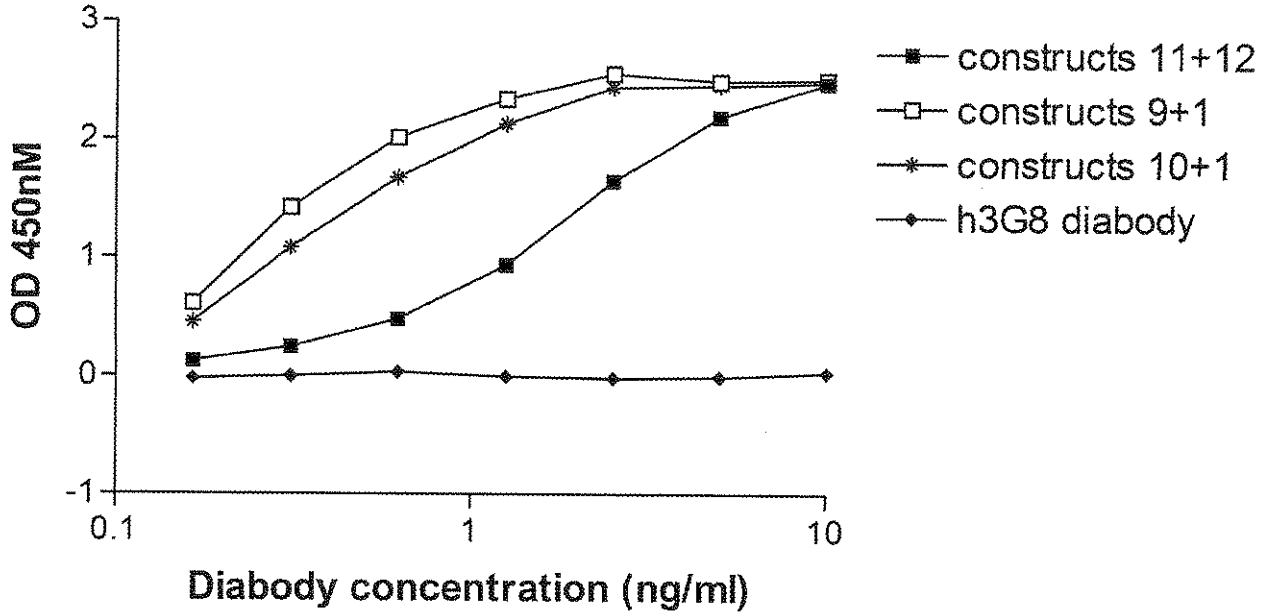

3
2
1
0
-1
OD 450nM
0.1
1
10
Diabody concentration (ng/ml)
constructs 11+12
constructs 9+1
constructs 10+1
h3G8 diabody

FIG. 16

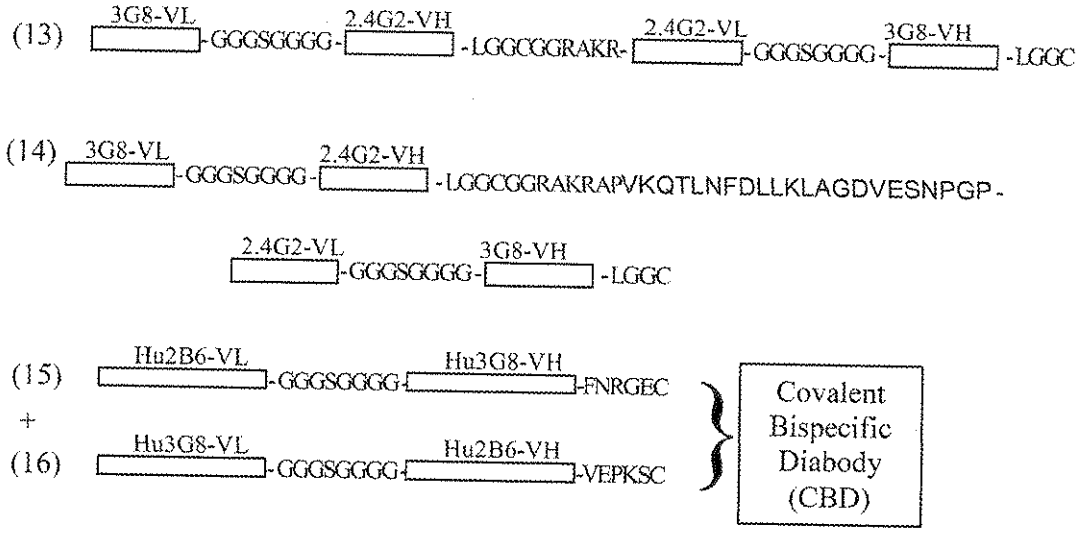

(13) 3G8-VL -GGGSGGGG- 2.4G2-VH -LGGCGGRAKR- 2.4G2-VL -GGGSGGGG- 3G8-VH -LGGC
(14) 3G8-VL -GGGSGGGG- 2.4G2-VH -LGGCGGRAKRAPVKQTLNFDLLKLAGDVESNPGP-
2.4G2-VL -GGGSGGGG- 3G8-VH -LGGC
(15) Hu2B6-VL -GGGSGGGG- Hu3G8-VH -FNRGEC
+
(16) Hu3G8-VL -GGGSGGGG- Hu2B6-VH -VEPKSC
Covalent Bispecific Diabody (CBD)

FIG. 17

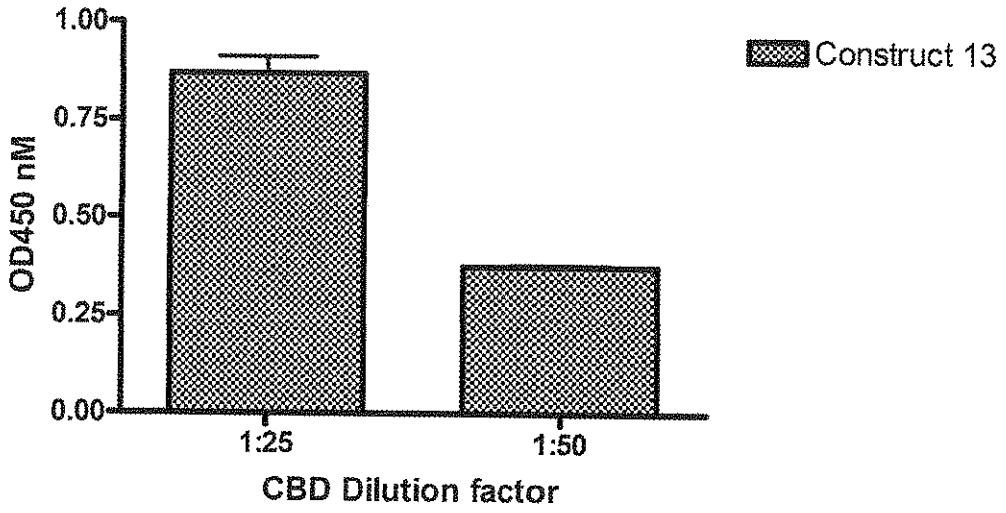

1.00
0.75
0.50
0.25
0.00
OD450 nM
1:25
1:50
CBD Dilution factor
Construct 13

FIG. 18

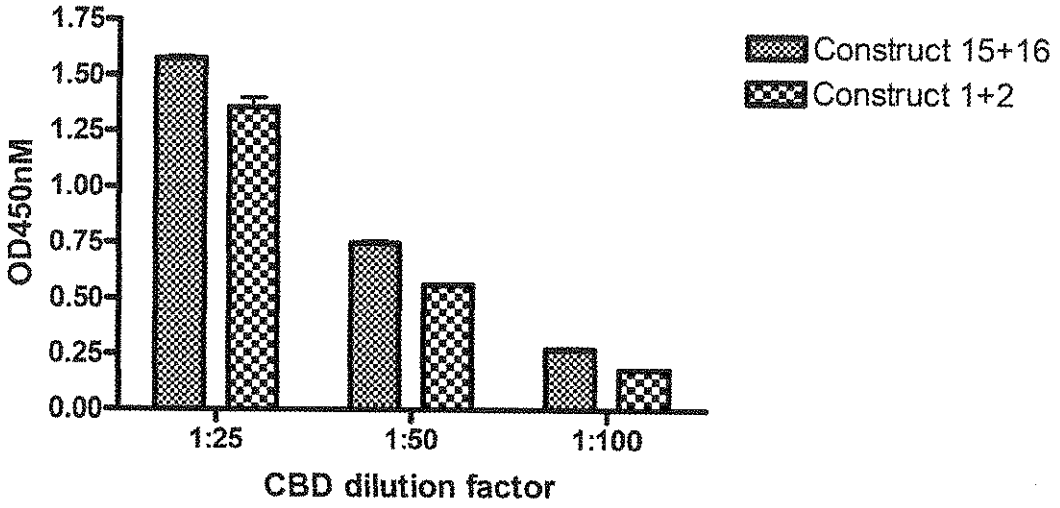

1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
OD450nM
1:25
1:50
1:100
CBD dilution factor
Construct 15+16
Construct 1+2

FIG. 19

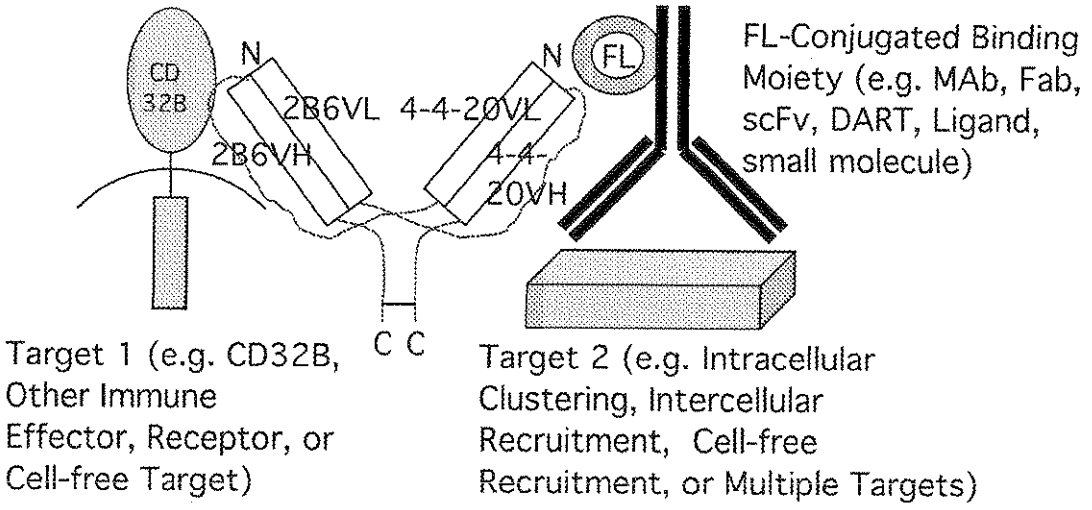

CD 32B
N
2B6VL
2B6VH
4-4-20VL
4-4-20VH
N
FL
FL-Conjugated Binding Moiety (e.g. MAb, Fab, scFv, DART, Ligand, small molecule)
C C
Target 1 (e.g. CD32B, Other Immune Effector, Receptor, or Cell-free Target)
Target 2 (e.g. Intracellular Clustering, Intercellular Recruitment, Cell-free Recruitment, or Multiple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

FIG. 20

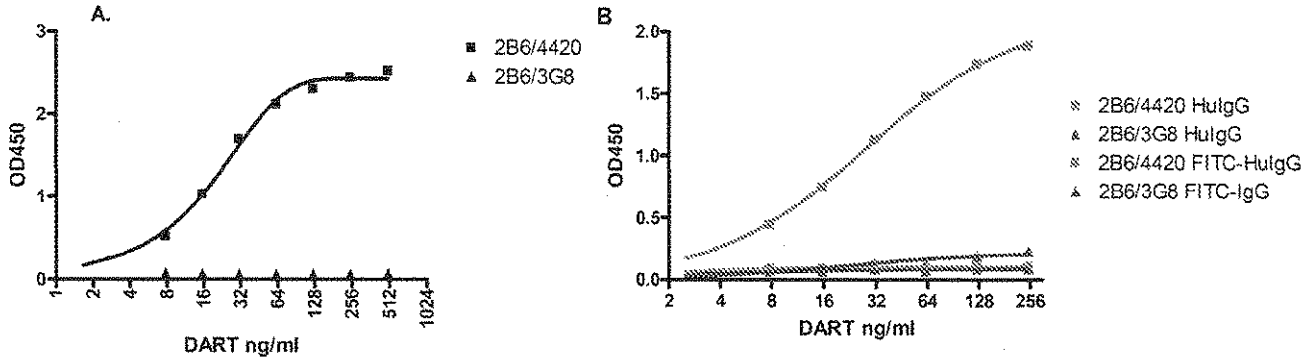


FIG. 21

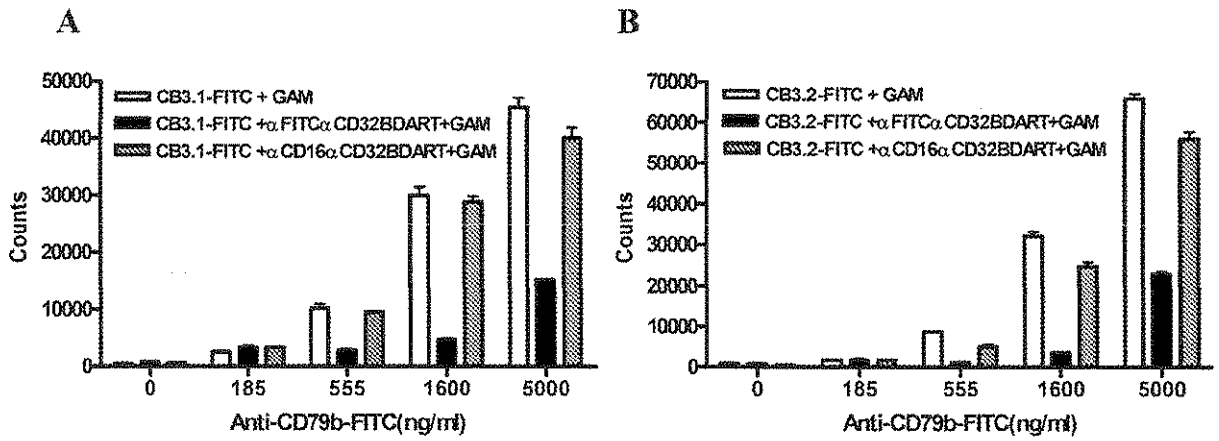


FIG. 22

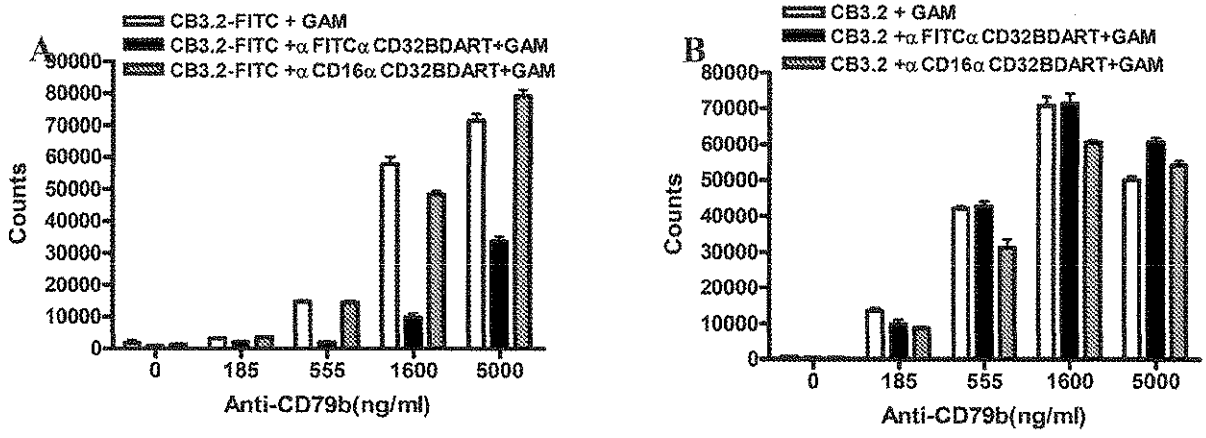
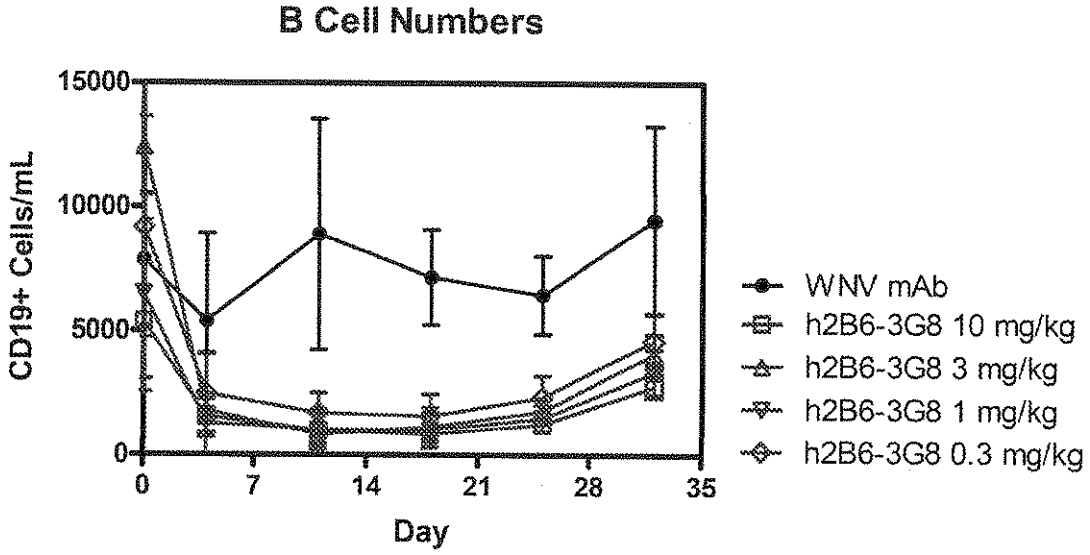


FIG. 23


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

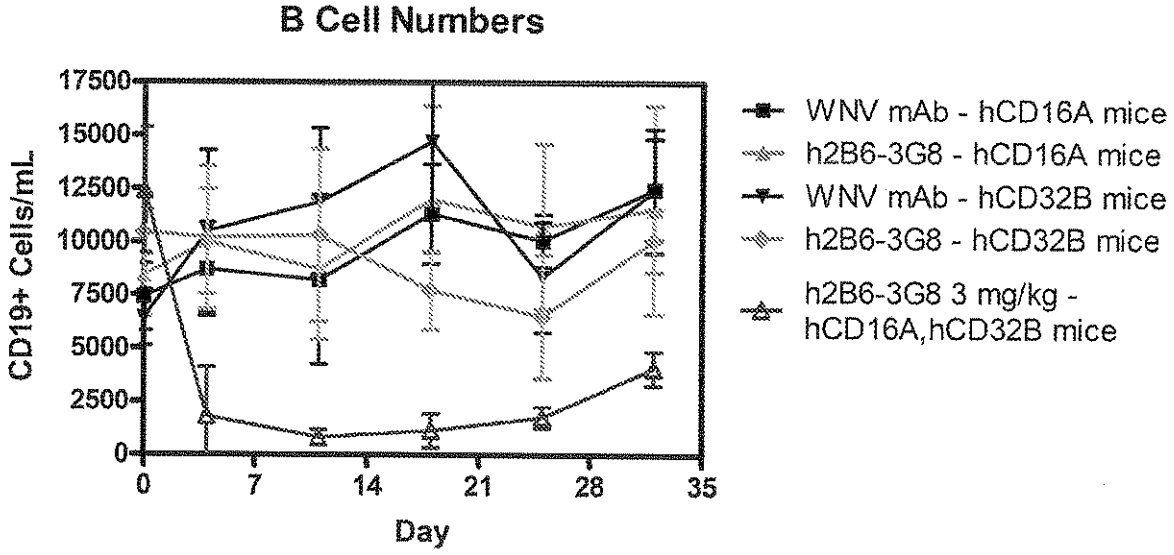

B Cell Numbers
CD19+ Cells/mL
17500
15000
12500
10000
7500
5000
2500
0
0
7
14
21
28
35
Day
WNV mAb - hCD16A mice
h2B6-3G8 - hCD16A mice
WNV mAb - hCD32B mice
h2B6-3G8 - hCD32B mice
h2B6-3G8 3 mg/kg - hCD16A,hCD32B mice

FIG. 24

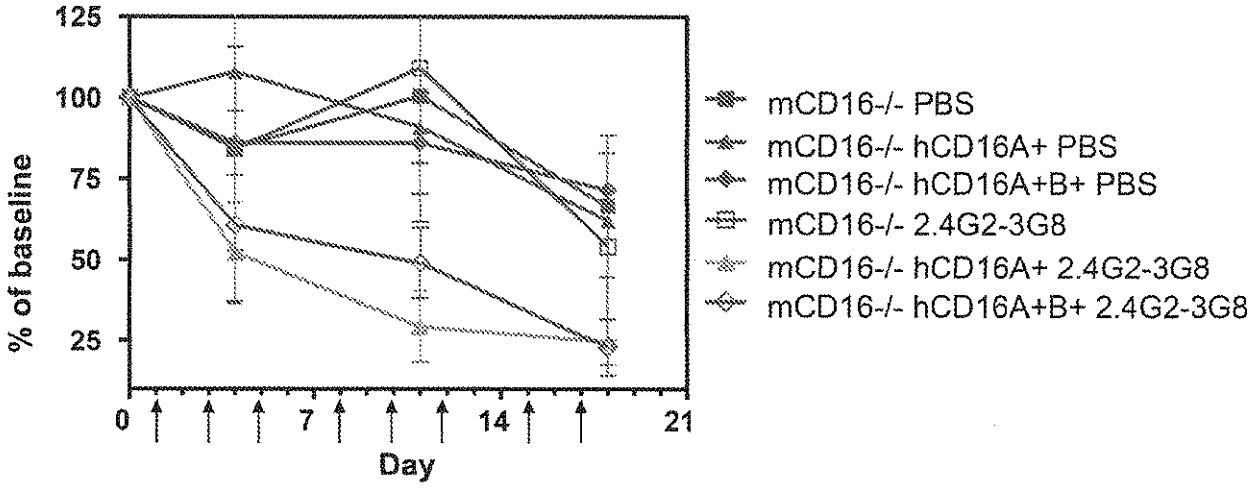

125
100
75
50
25
% of baseline
0
7
14
21
Day
mCD16-/- PBS
mCD16-/- hCD16A+ PBS
mCD16-/- hCD16A+B+ PBS
mCD16-/- 2.4G2-3G8
mCD16-/- hCD16A+ 2.4G2-3G8
mCD16-/- hCD16A+B+ 2.4G2-3G8

FIG. 25

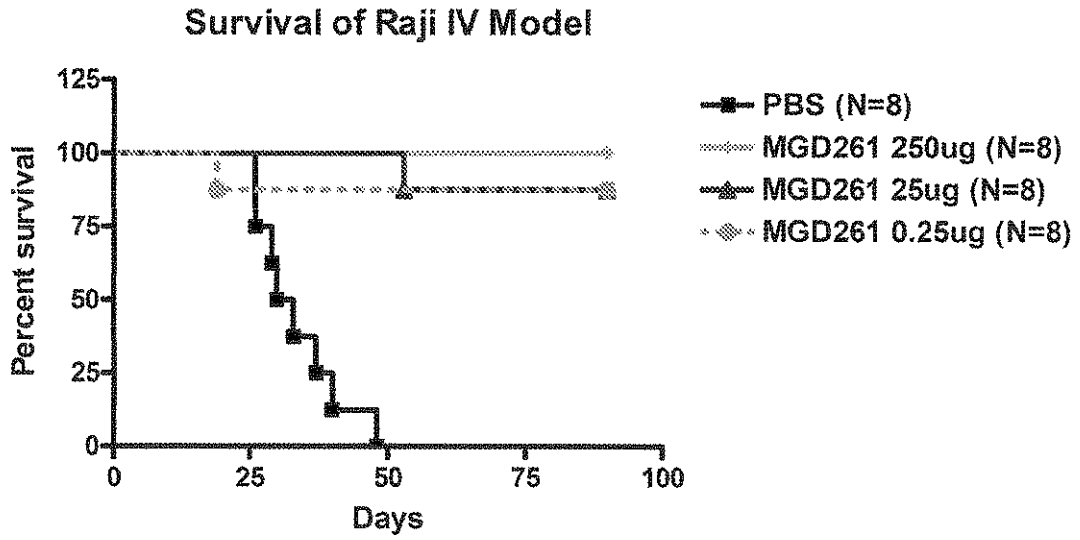

Survival of Raji IV Model
125
100
75
50
25
0
Percent survival
0
25
50
75
100
Days
PBS (N=8)
MGD261 250ug (N=8)
MGD261 25ug (N=8)
MGD261 0.25ug (N=8)

FIG. 26

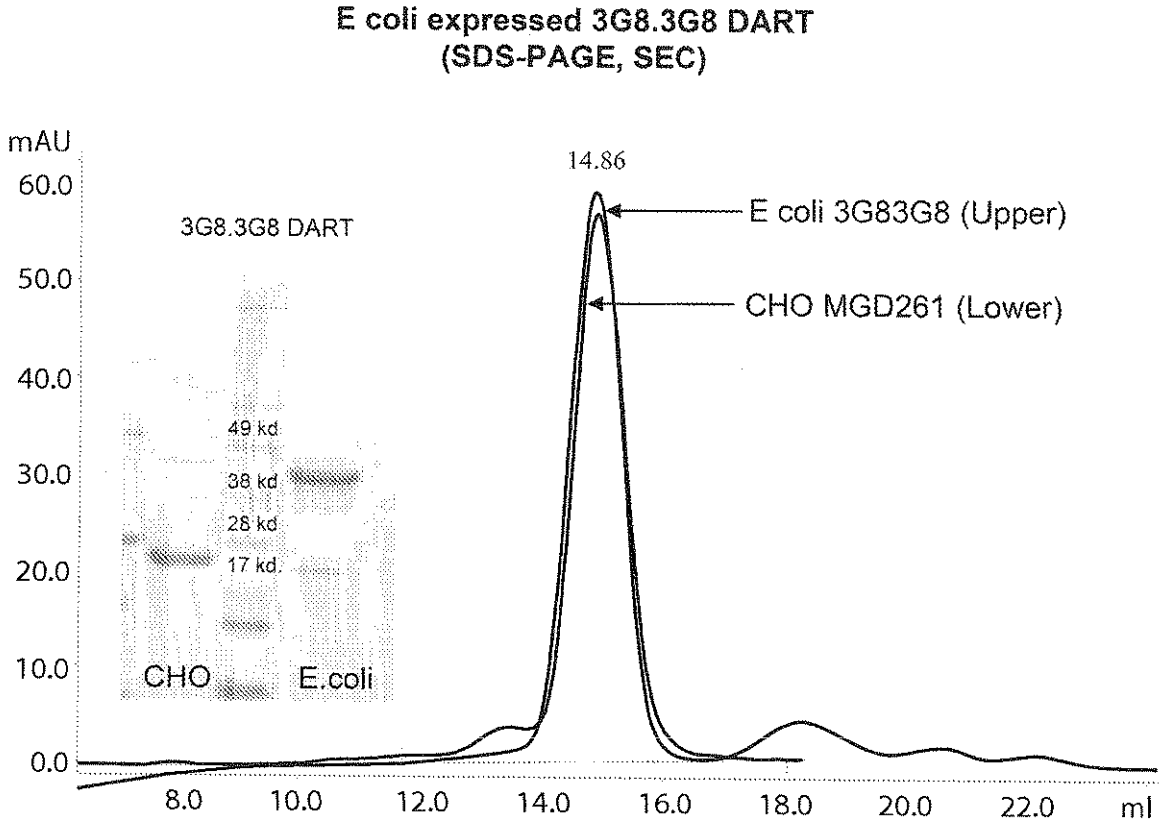

E coli expressed 3G8.3G8 DART
(SDS-PAGE, SEC)
mAU
60.0
50.0
40.0
30.0
20.0
10.0
0.0
14.86
E coli 3G83G8 (Upper)
CHO MGD261 (Lower)
3G8.3G8 DART
49 kd
38 kd
28 kd
17 kd.
CHO
E.coli
8.0
10.0
12.0
14.0
16.0
18.0
20.0
22.0
ml

FIG. 27

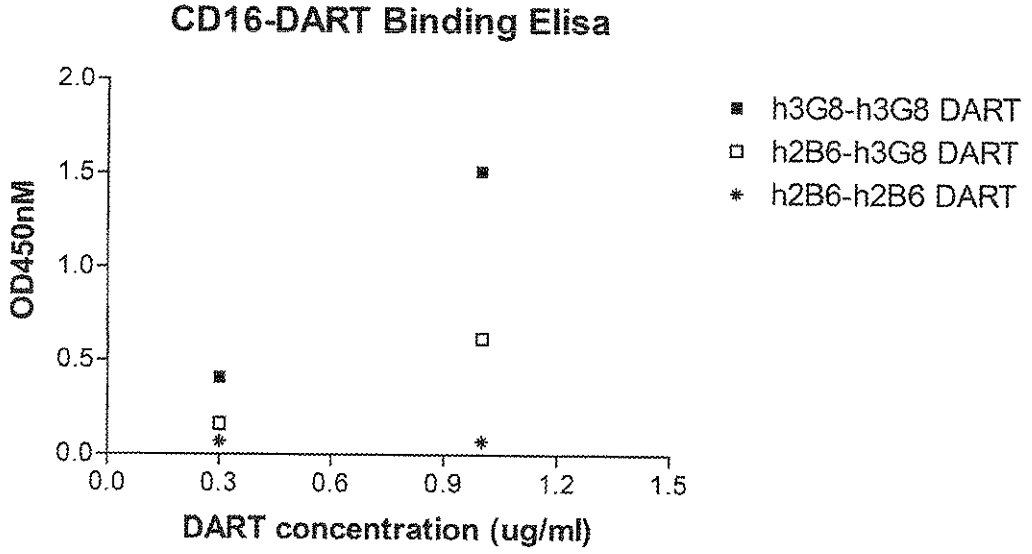

CD16-DART Binding Elisa
OD450nM
2.0
1.5
1.0
0.5
0.0
0.0
0.3
0.6
0.9
1.2
1.5
DART concentration (ug/ml)
h3G8-h3G8 DART
h2B6-h3G8 DART
h2B6-h2B6 DART

FIG. 28

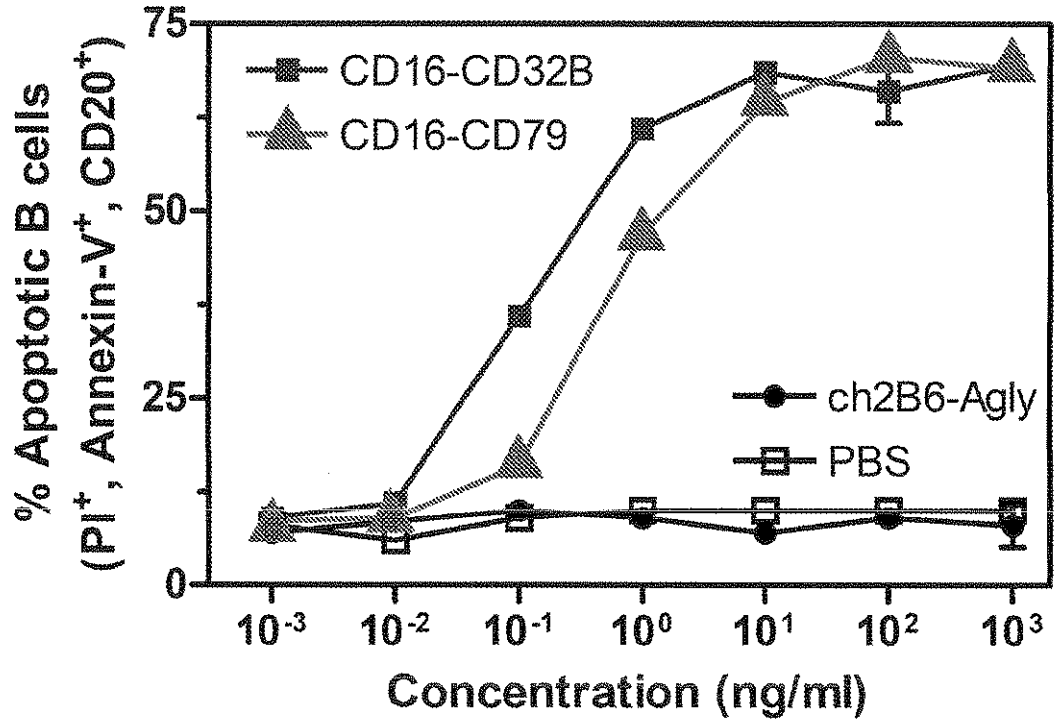

% Apoptotic B cells
(PI+, Annexin-V+, CD20+)
75
50
25
0
CD16-CD32B
CD16-CD79
ch2B6-Agly
PBS
10-3
10-2
10-1
100
101
102
103
Concentration (ng/ml)

FIG. 29

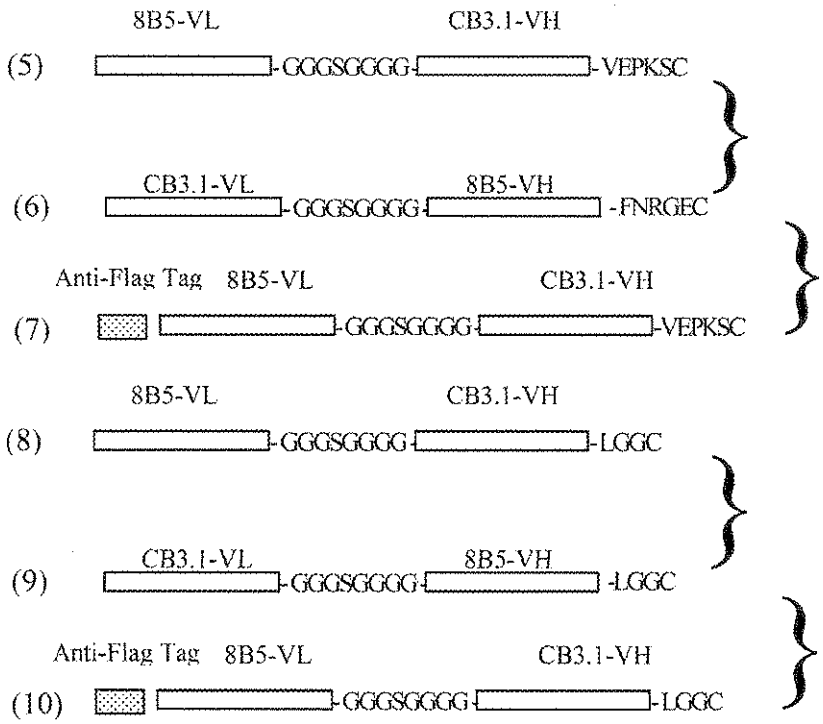

8B5-VL
CB3.1-VH
(5)
-GGGSGGGG-
-VEPKSC
CB3.1-VL
8B5-VH
(6)
-GGGSGGGG-
-FNRGEC
Anti-Flag Tag
8B5-VL
CB3.1-VH
(7)
-GGGSGGGG-
-VEPKSC
8B5-VL
CB3.1-VH
(8)
-GGGSGGGG-
-LGGC
CB3.1-VL
8B5-VH
(9)
-GGGSGGGG-
-LGGC
Anti-Flag Tag
8B5-VL
CB3.1-VH
(10)
-GGGSGGGG-
-LGGC

FIG. 30

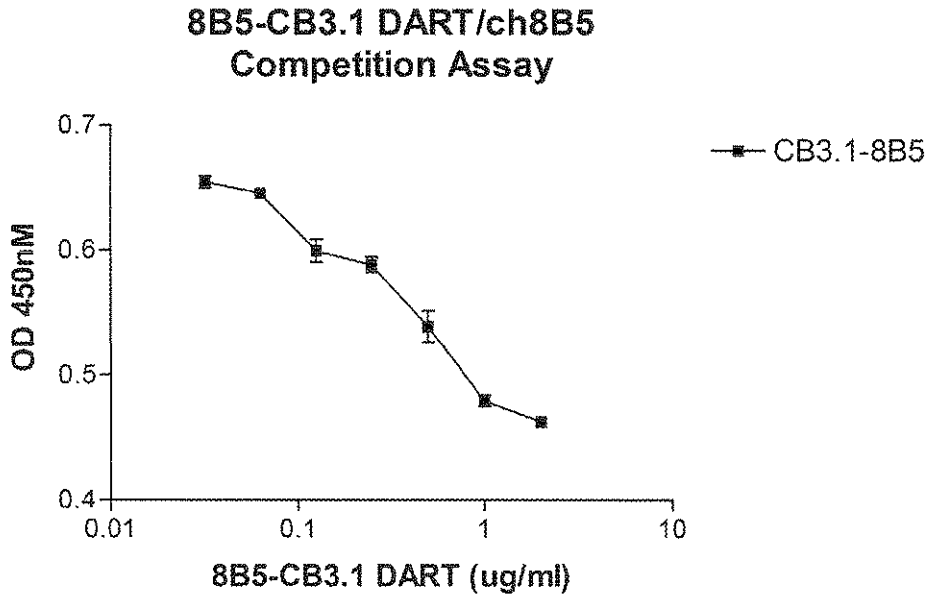

8B5-CB3.1 DART/ch8B5
Competition Assay
OD 450nM
0.7
0.6
0.5
0.4
0.01
0.1
1
10
8B5-CB3.1 DART (ug/ml)
CB3.1-8B5

FIG. 31

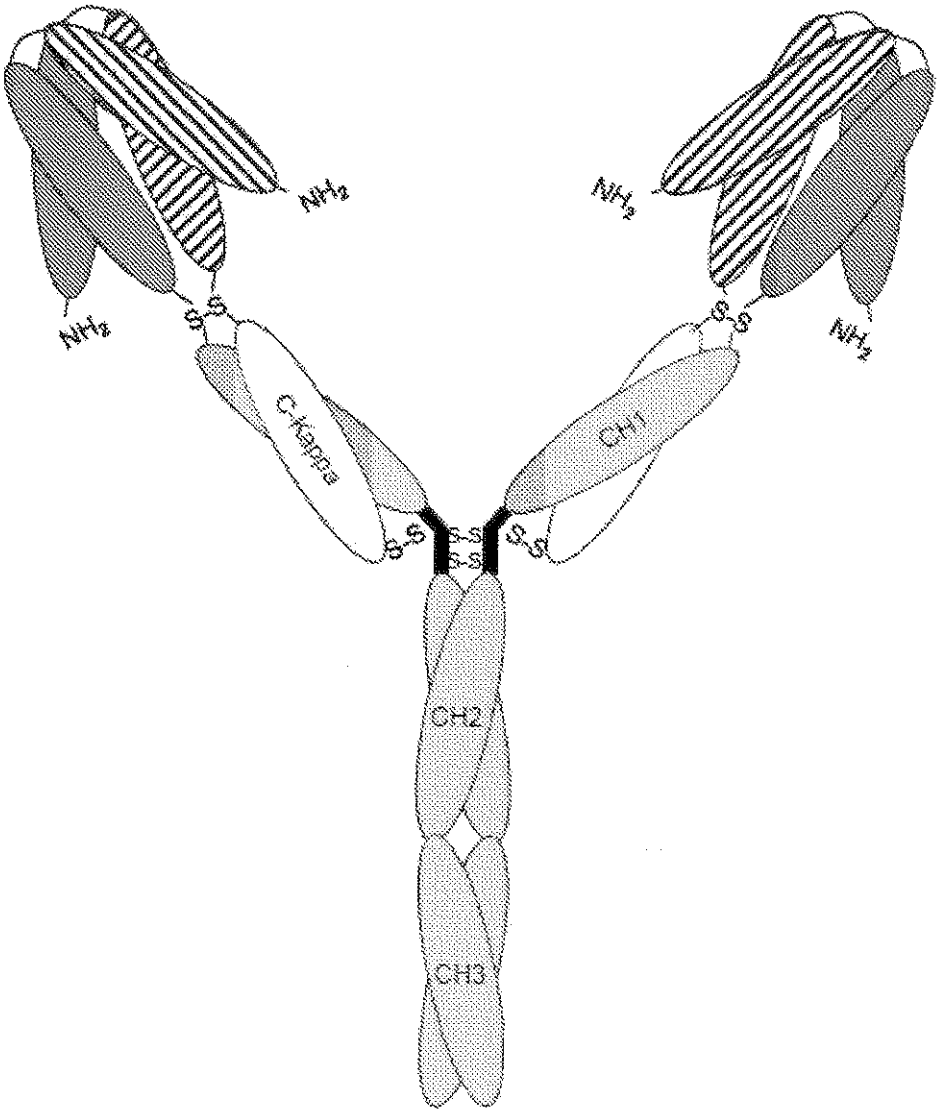
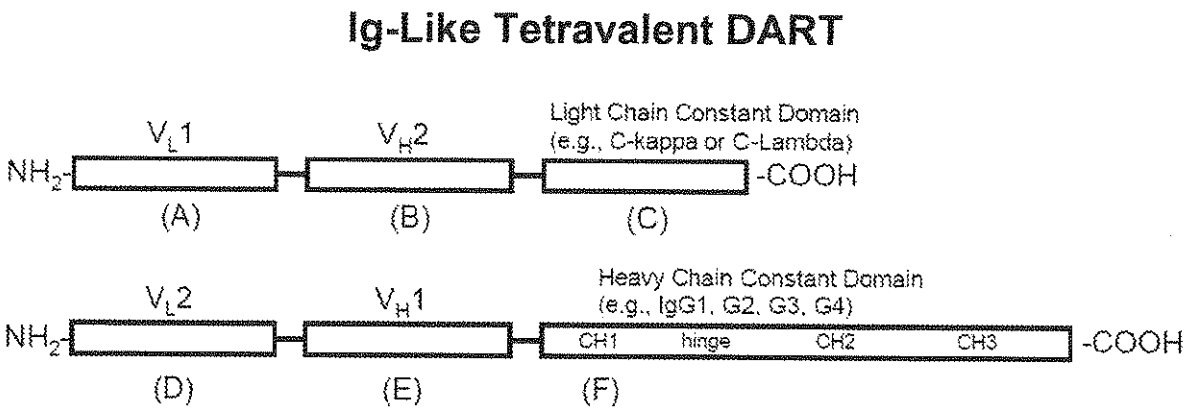


FIG. 32



(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

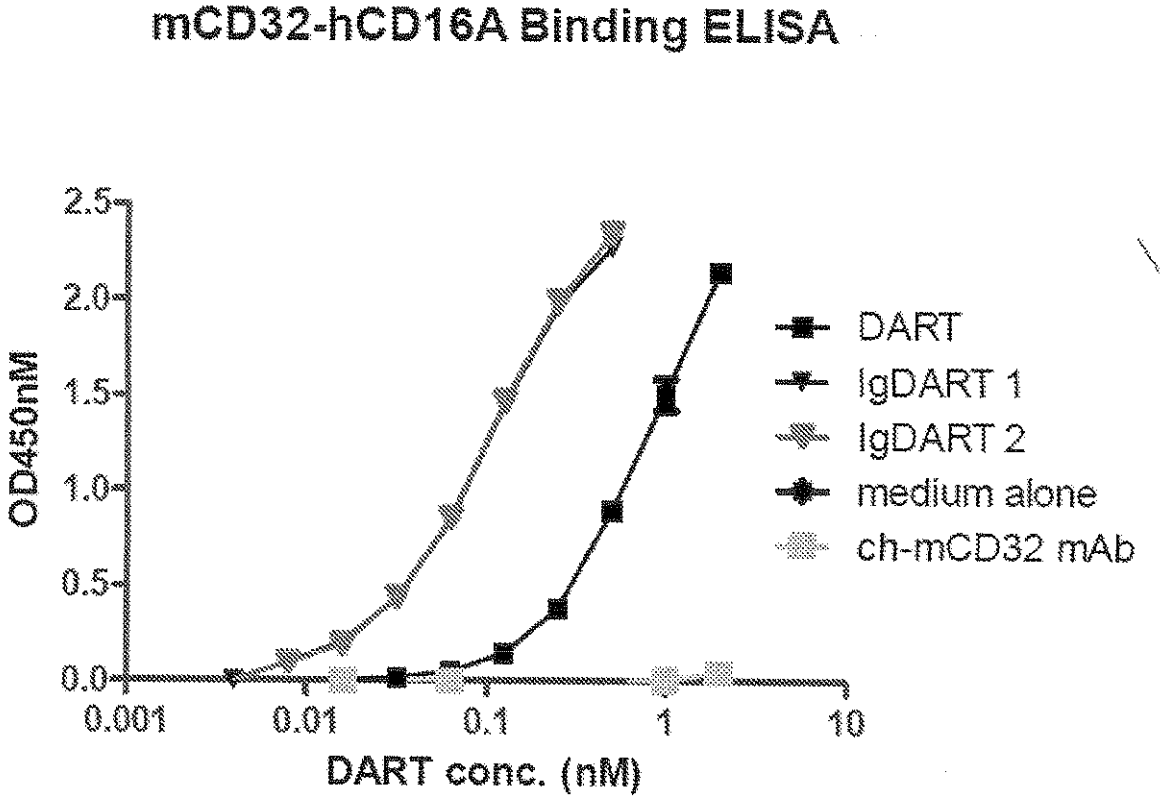


FIG. 34

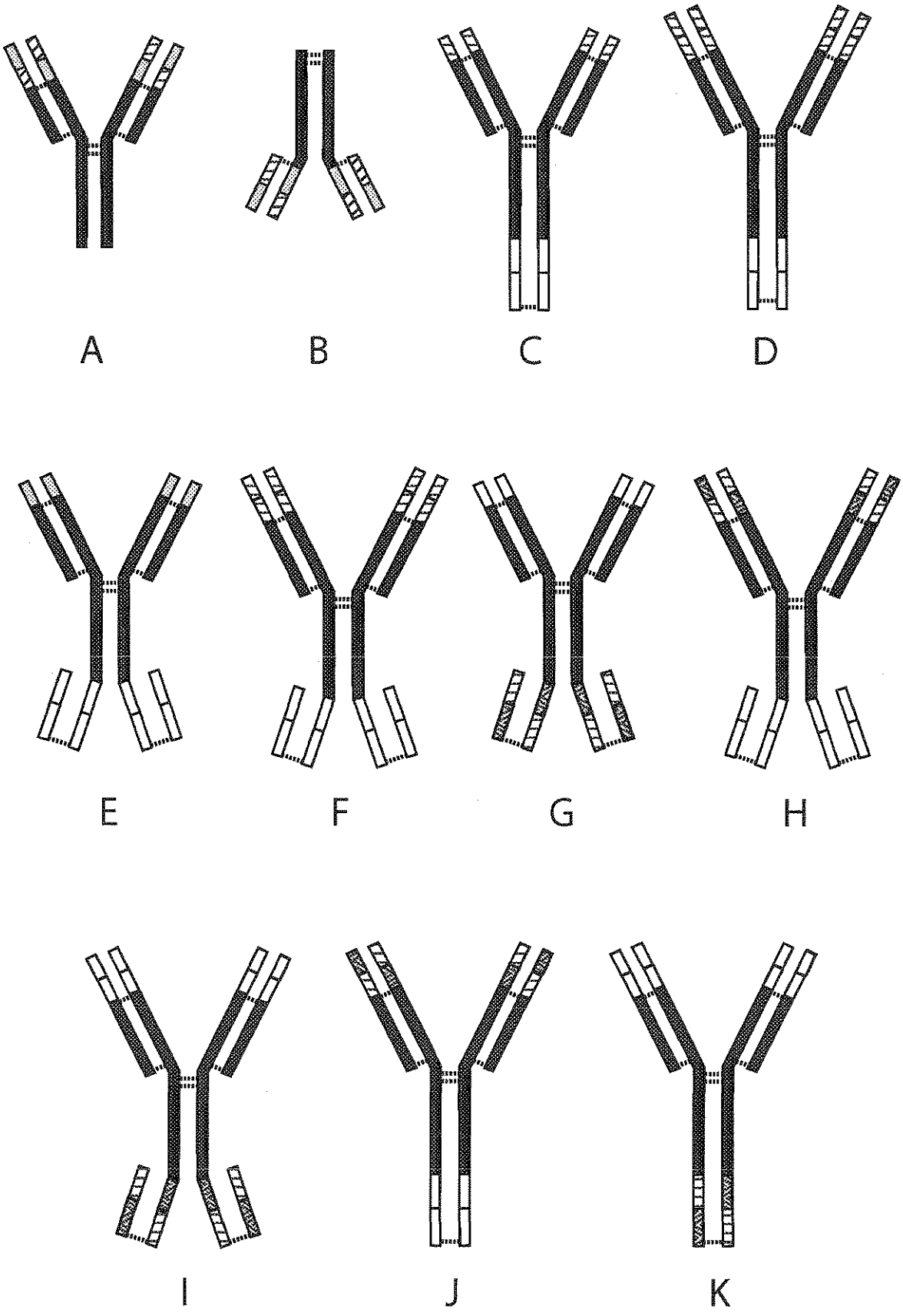


FIG. 35