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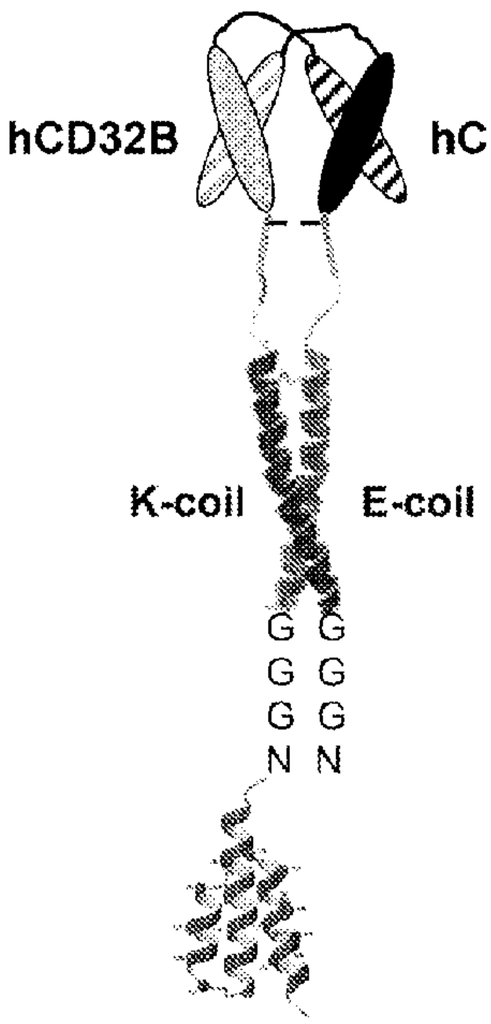
(54) Titre : DI-ANTICORPS COVALENTS ET UTILISATIONS ASSOCIEES
(54) Title: COVALENT DIABODIES AND USES THEREOF



(57) Abrégé/Abstract:
Diabody molecules and uses thereof in the treatment of a variety of diseases and disorders, including immunological disorders, infectious disease, intoxication and cancers are disclosed. The diabody molecules comprise two polypeptide chains that associate to form at least two epitope binding sites, which may recognize the same or different epitopes on the same or differing antigens. Additionally, the antigens may be from the same or different molecules. The individual polypeptide chains of the diabody molecule may be covalently bound through non-peptide bond covalent bonds, such as disulfide bonding of cysteine residues located within each polypeptide chain. The diabody molecules may further comprise an Fc region, which allows antibody-like functionality to be engineered into the molecule.

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(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,

[Continued on next page]

(54) Title: COVALENT DIABODIES AND USES THEREOF

(57) Abstract: Diabody molecules and uses thereof in the treatment of a variety of diseases and disorders, including immunological disorders, infectious disease, intoxication and cancers are disclosed. The diabody molecules comprise two polypeptide chains that associate to form at least two epitope binding sites, which may recognize the same or different epitopes on the same or differing antigens. Additionally, the antigens may be from the same or different molecules. The individual polypeptide chains of the diabody molecule may be covalently bound through non-peptide bond covalent bonds, such as disulfide bonding of cysteine residues located within each polypeptide chain. The diabody molecules may further comprise an Fc region, which allows antibody-like functionality to be engineered into the molecule.

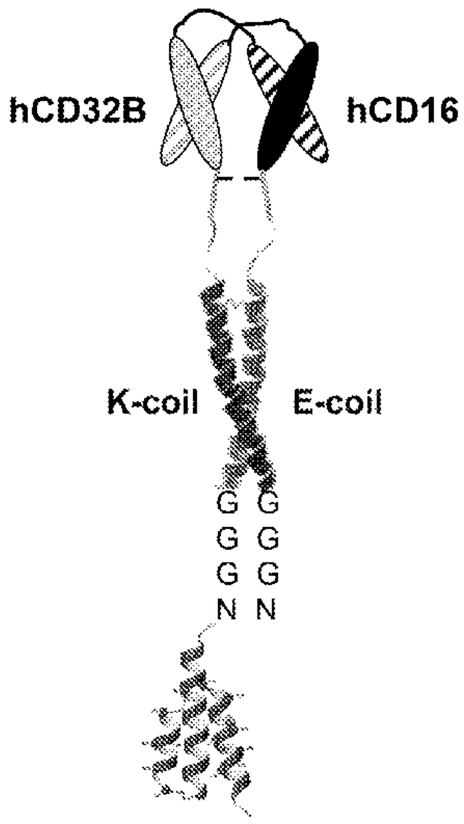

FIG. 45

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EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

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— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

CLAIMS:

1. A diabody complex able to bind serum albumin, wherein said diabody complex comprises three polypeptide chains each comprising an N-terminus and a C-terminus, wherein:

(A) said first and second polypeptide chains are covalently bonded to one another, and each comprise, in the N-terminal to C-terminal direction, a light chain variable domain linked to a heavy chain variable domain, wherein:

(1) said light chain variable domain of said first polypeptide chain associates with said heavy chain variable domain of said second polypeptide chain to form a binding site specific for a first epitope; and

(2) said light chain variable domain of said second polypeptide chain associates with said heavy chain variable domain of said first polypeptide chain to form a binding site specific for a second epitope;

(B) said first polypeptide chain comprises a heterodimer formation promoting polypeptide portion, said heterodimer formation promoting polypeptide portion being positioned C-terminal to said heavy chain variable domain of said first polypeptide chain, and being an E-coil or a K-coil; wherein said E-coil is 4 heptameric repeats of EVAALEK (SEQ ID NO: 299), and said K-coil is 4 heptameric repeats of KVAALKE (SEQ ID NO: 300); and

(C) said third polypeptide chain comprises a polypeptide portion of a protein able to bind to said serum albumin and a heterodimer formation promoting polypeptide portion that is:

(1) an E-coil if said first polypeptide chain comprises a K-coil, or

(2) a K-coil if said first polypeptide chain comprises an E-coil;

wherein said heterodimer formation promoting polypeptide portion of said first and third polypeptide chains are complexed together to thereby form said diabody complex, and

said diabody complex is able to simultaneously bind to said first epitope, to said second epitope and to said serum albumin.

2. The diabody complex of claim 1, wherein on said third polypeptide, said polypeptide portion of said protein able to bind to said serum albumin is located N-terminal to said heterodimer formation promoting polypeptide portion.

3. The diabody complex of claim 1, wherein on said third polypeptide, said polypeptide portion of said protein able to bind to said serum albumin is located C-terminal to said heterodimer formation promoting polypeptide portion.

4. The diabody complex of any one of claims 1-3, wherein said protein able to bind to said serum albumin is streptococcal protein G and said polypeptide portion thereof is an albumin-binding domain (ABD) of said streptococcal protein G.

5. The diabody complex of claim 4, wherein said albumin-binding domain (ABD) of said streptococcal protein G is albumin-binding domain 3 (ABD3) of protein G of Streptococcus strain G148.

6. The diabody complex of claim 1, wherein said diabody complex exhibits an *in vivo* serum half-life greater than 2 hours.

7. The diabody complex of any one of claims 1-6, wherein said diabody complex binds a Natural Killer Group 2D (NKG2D) receptor, a T cell receptor (TCR) binding domain or a hapten.

8. The diabody complex of claim 7, wherein said diabody complex additionally binds to a tumor-associated antigen.

9. The diabody complex of claim 8, wherein said tumor-associated antigen is a breast cancer antigen, an ovarian cancer antigen, a prostate cancer antigen, a cervical cancer antigen, a pancreatic carcinoma antigen, a lung cancer antigen, a bladder cancer antigen, a colon cancer antigen, a testicular cancer antigen, a glioblastoma cancer antigen, an antigen associated with a B cell malignancy, an antigen associated with multiple myeloma, an antigen associated with non-Hodgkin's lymphoma, or an antigen associated with chronic lymphocytic leukemia.

10. The diabody complex of claim 9, wherein said tumor-associated antigen is A33; A Disintegrin and Metalloprotease-Domain Containing Protein 9 (ADAM-9); CD166 antigen (also known as activated leukocyte cell adhesion molecule (ALCAM)); cyclin B1; cancer testes gene antigen (also known as B melanoma antigen BAGE); beta-catenin; cancer antigen 125 (CA125); Carboxypeptidase M; CD5; CD19; CD20; CD22; CD23; CD25; CD27; CD28; CD32B; CD36; CD40; CD45; CD46; CD56; CD79a; CD79b; CD103; CD154; CDK4; CEA; CTLA4; Cytokeratin 8; EGF-R; an Ephrin receptor; ErbB1; ErbB3; ErbB4; HLA-Cw6 molecule (GAGE-1); HLA-Cw6 molecule (GAGE-2); GD2; GD3; GM2; gp100; HER-2/neu; human papillomavirus-E6; human papillomavirus-E7; Integrin Alpha-V-Beta-6; JAM-3; KID3; KID31; Ep-CAM (KSA (17-1A)); N-linked carbohydrate epitope (LUCA-2); melanoma-associated cancer-testis gene-1 (MAGE-1); melanoma-associated cancer-testis gene-3 (MAGE-3); melanoma antigen recognized by T cells (MART); Mucin 1, cell surface associated (MUC-1); Interferon regulatory factor 4 (MUM-1); N-acetylglucosaminyltransferase; Oncostatin M (Oncostatin Receptor Beta); p15; pathogenicity island-encoded protein A (PipA); PSA; Prostate-Specific Membrane Antigen (PSMA); B7-H3 (RAAG10); ROR1; Squamous Cell Carcinoma Antigen Recognized by T cells (SART); sTn; TES7; the TNF-α receptor; the TNF-ß receptor; the TNF-γ receptor; the Transferrin Receptor or the VEGF receptor.

11. The diabody complex of claim 10, wherein said tumor-associated antigen is HER-2/neu or CD32B.

12. A pharmaceutical composition that comprises the diabody complex of claim 7 or 8, and a pharmaceutically acceptable carrier.

13. The pharmaceutical composition of claim 12 for use in the treatment of cancer.

14. The pharmaceutical composition of claim 13, wherein said use is in the treatment of breast cancer, ovarian cancer, prostate cancer, cervical cancer, pancreatic carcinoma, lung cancer, bladder cancer, colon cancer, testicular cancer, glioblastoma cancer, a B cell malignancy, multiple myeloma, non-Hodgkin's lymphoma, or chronic lymphocytic leukemia.

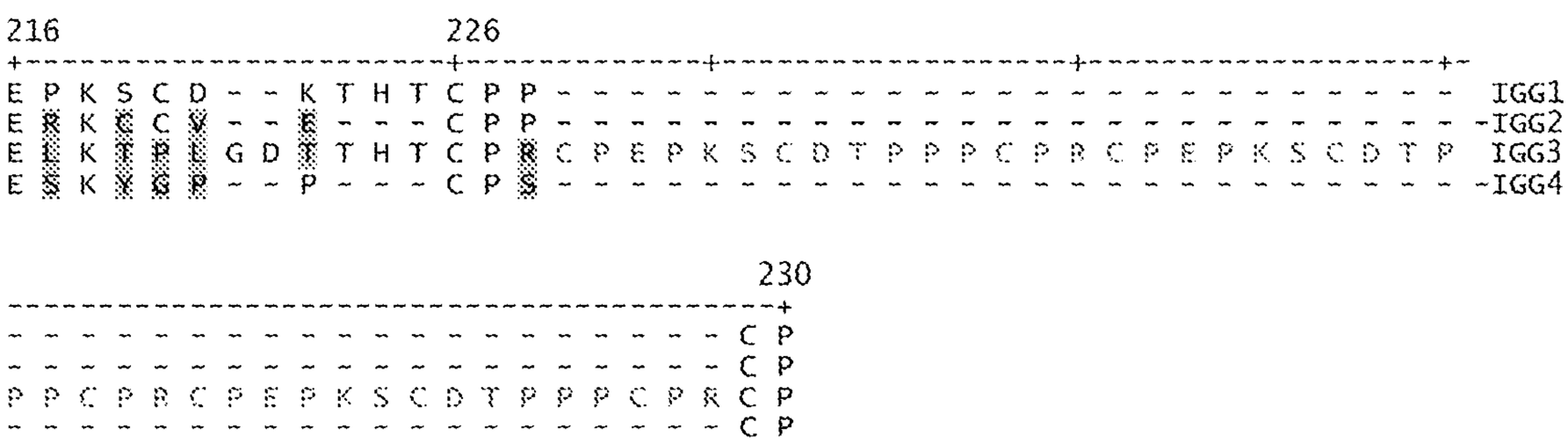

216
226
230
E P K S C D - - K T H T C P P - IGG1
E R K C C V - - E - - - C P P -IGG2
E L K T P L G D T T H T C P R C P E P K S C D T P P P C P R C P E P K S C D T P IGG3
E S K Y G P - - P - - - C P S -IGG4
C P
C P
P P C P R C P E P K S C D T P P P C P R C P
C P

FIG. 1A

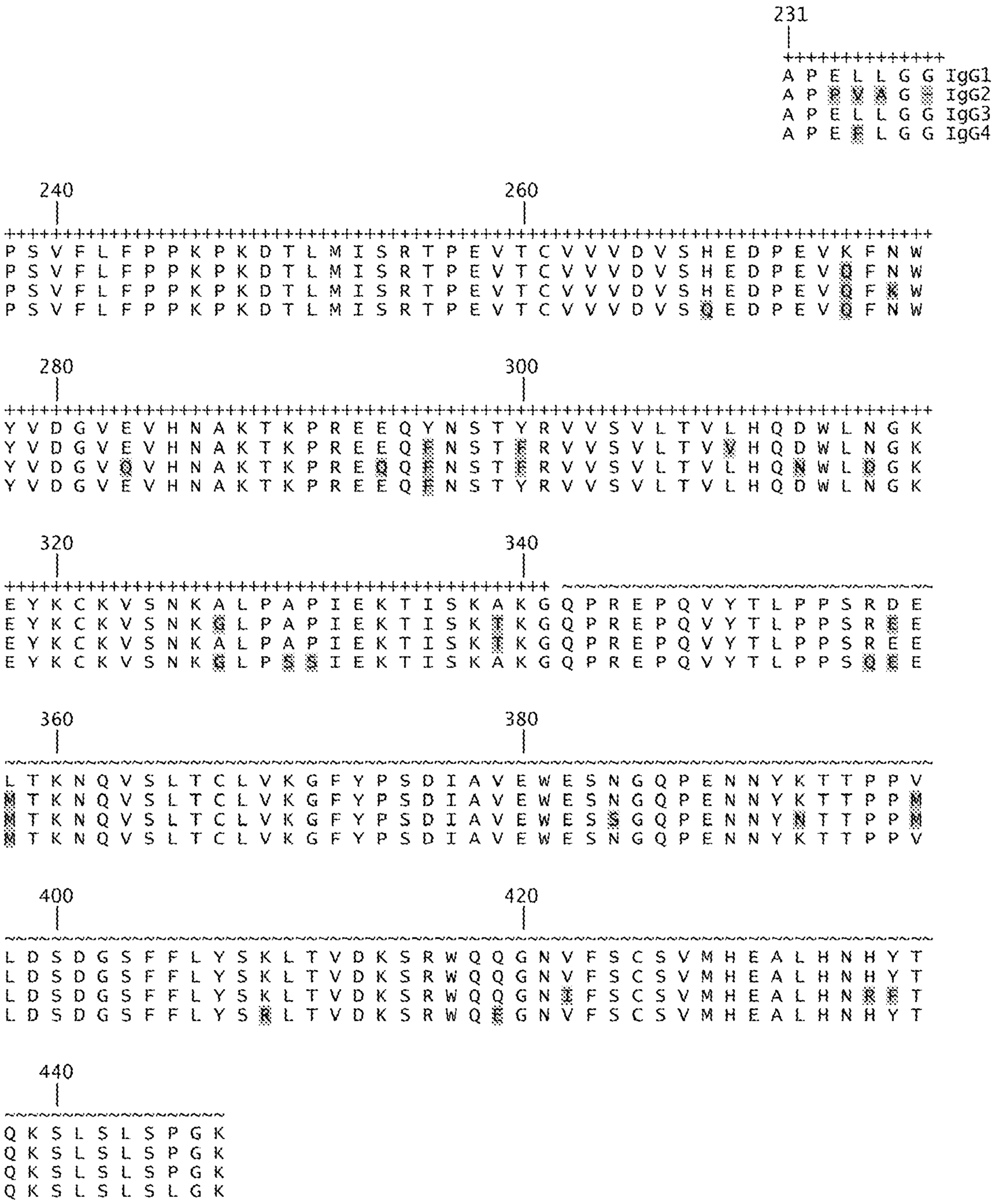

231
APELLGG IgG1
APPVAG IgG2
APELLGG IgG3
APESLGG IgG4
240
260
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFKW
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW
280
300
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
YVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGK
YVDGVQVHNAKTKPREQQFNSTFRVVSVLTVLHQNWLDGK
YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGK
320
340
EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE
EYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREE
EYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSREE
EYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE
360
380
LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
MTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPM
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
400
420
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
LDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFT
LDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYT
440
QKSLSLSPGK
QKSLSLSPGK
QKSLSLSPGK
QKSLSLSLGK

FIG. 1B

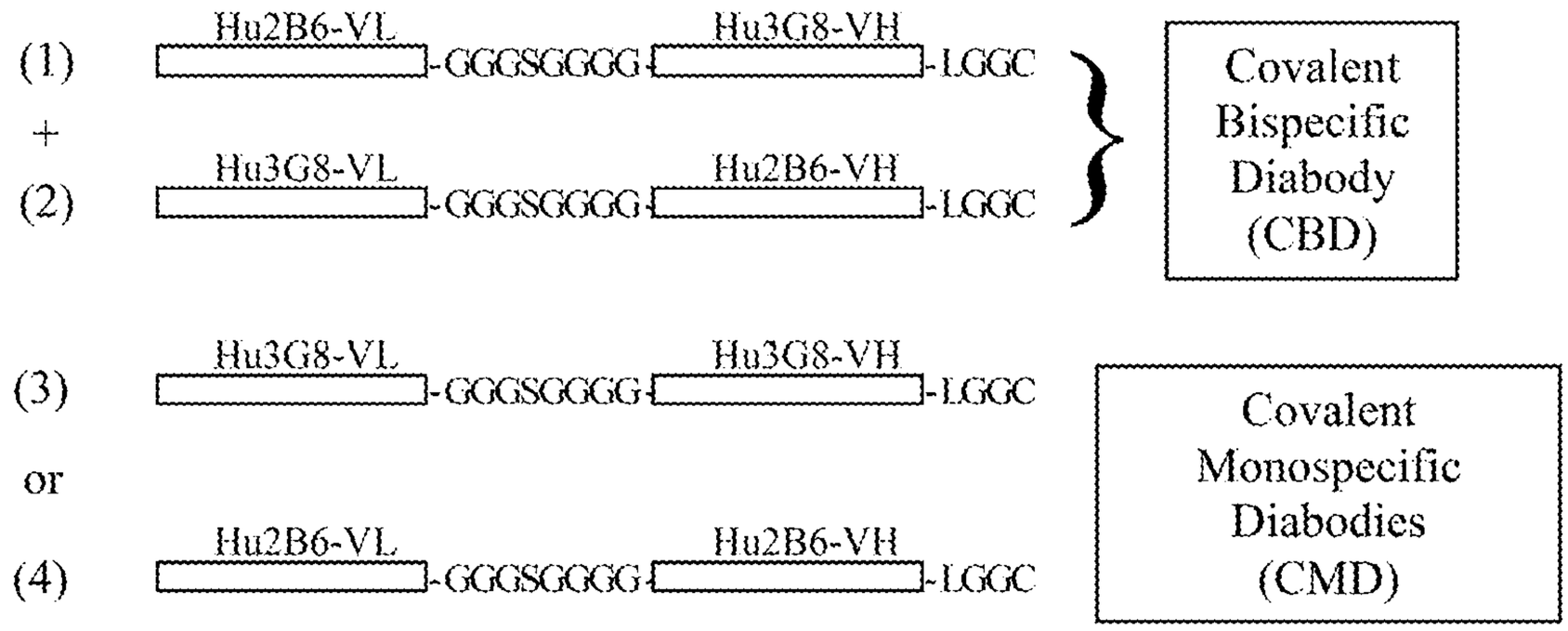

(1)
Hu2B6-VL
-GGGSGGGG-
Hu3G8-VH
-LGGC
+
(2)
Hu3G8-VL
-GGGSGGGG-
Hu2B6-VH
-LGGC
Covalent Bispecific Diabody (CBD)
(3)
Hu3G8-VL
-GGGSGGGG-
Hu3G8-VH
-LGGC
or
(4)
Hu2B6-VL
-GGGSGGGG-
Hu2B6-VH
-LGGC
Covalent Monospecific Diabodies (CMD)

FIG. 2

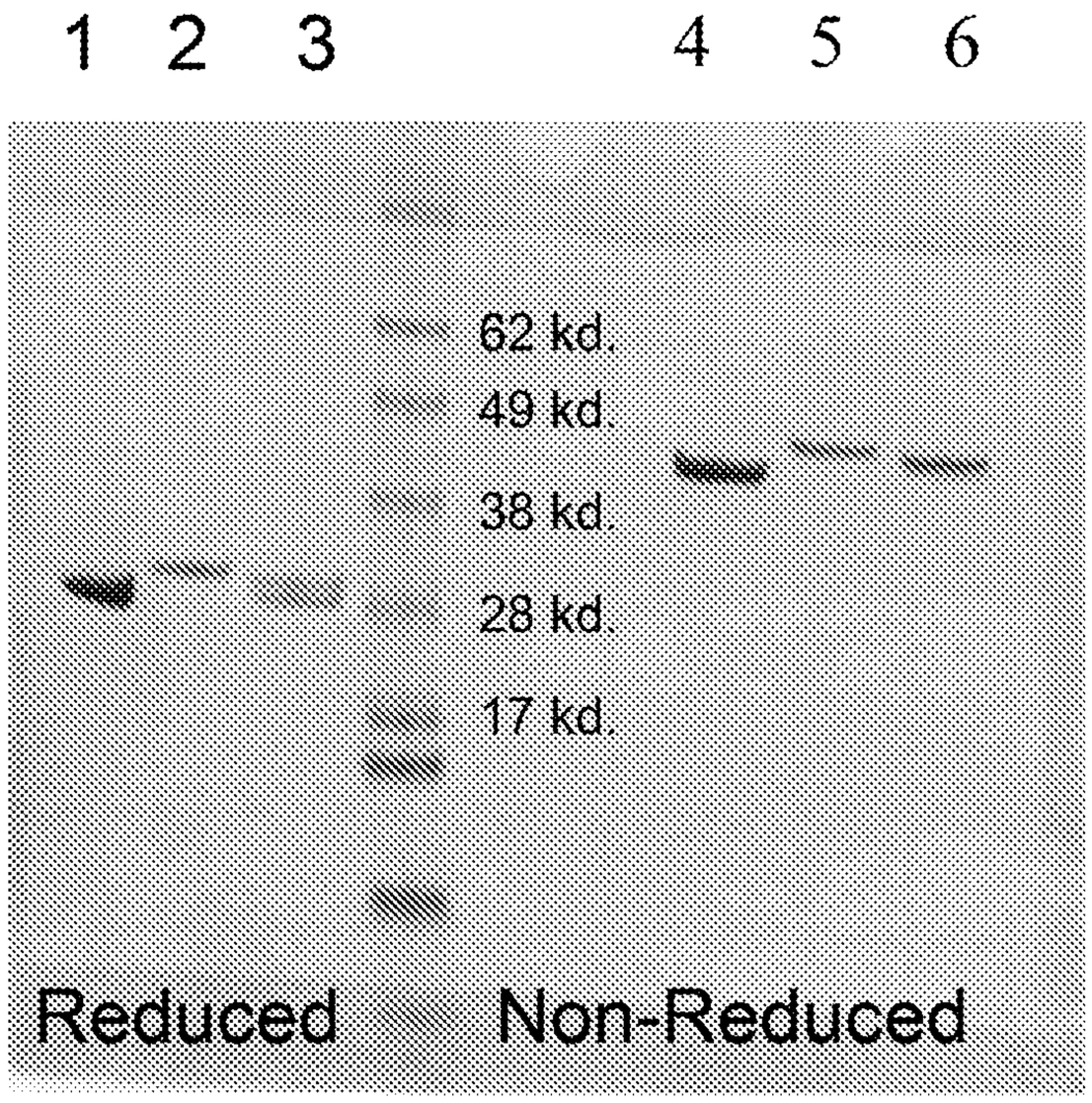


FIG. 3

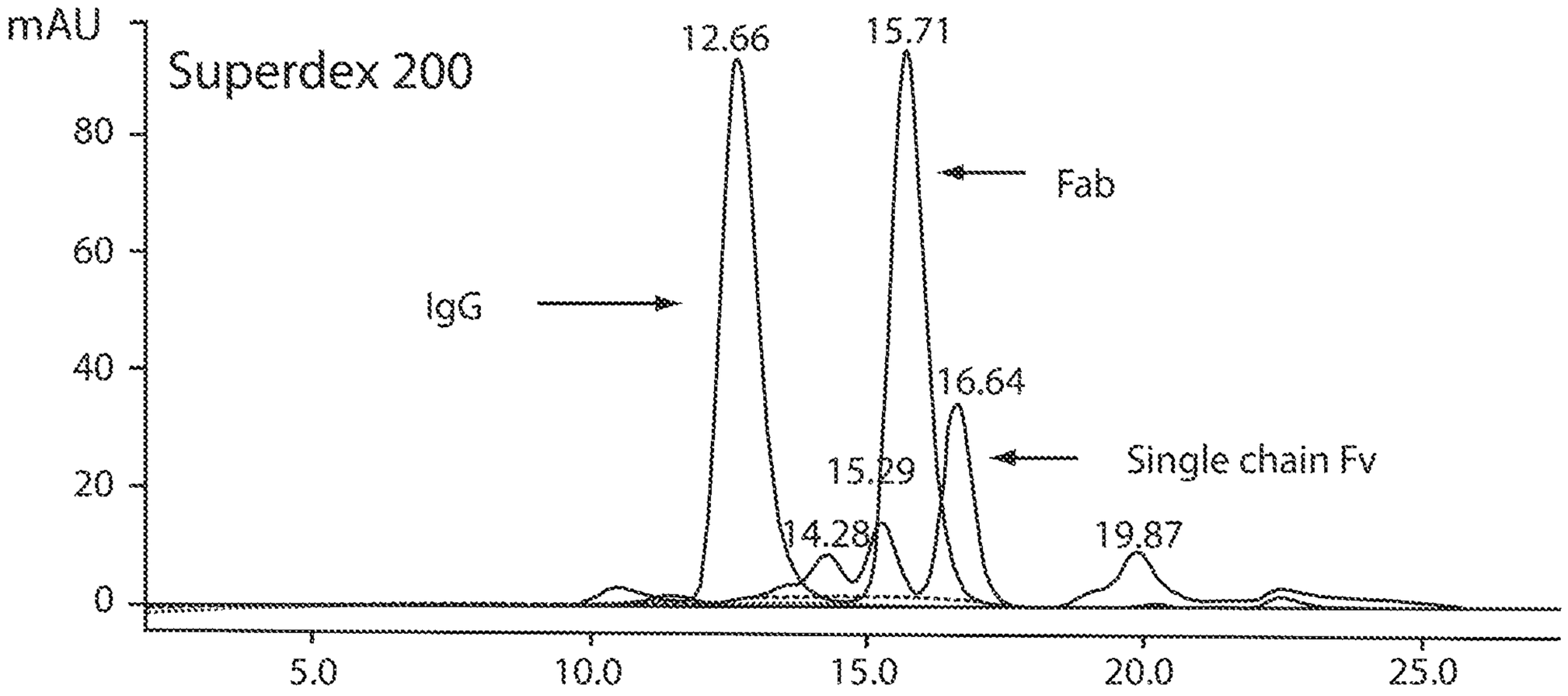

mAU
Superdex 200
80
60
40
20
0
5.0
10.0
15.0
20.0
25.0
12.66
15.71
16.64
15.29
14.28
19.87
IgG
Fab
Single chain Fv

FIG. 4A

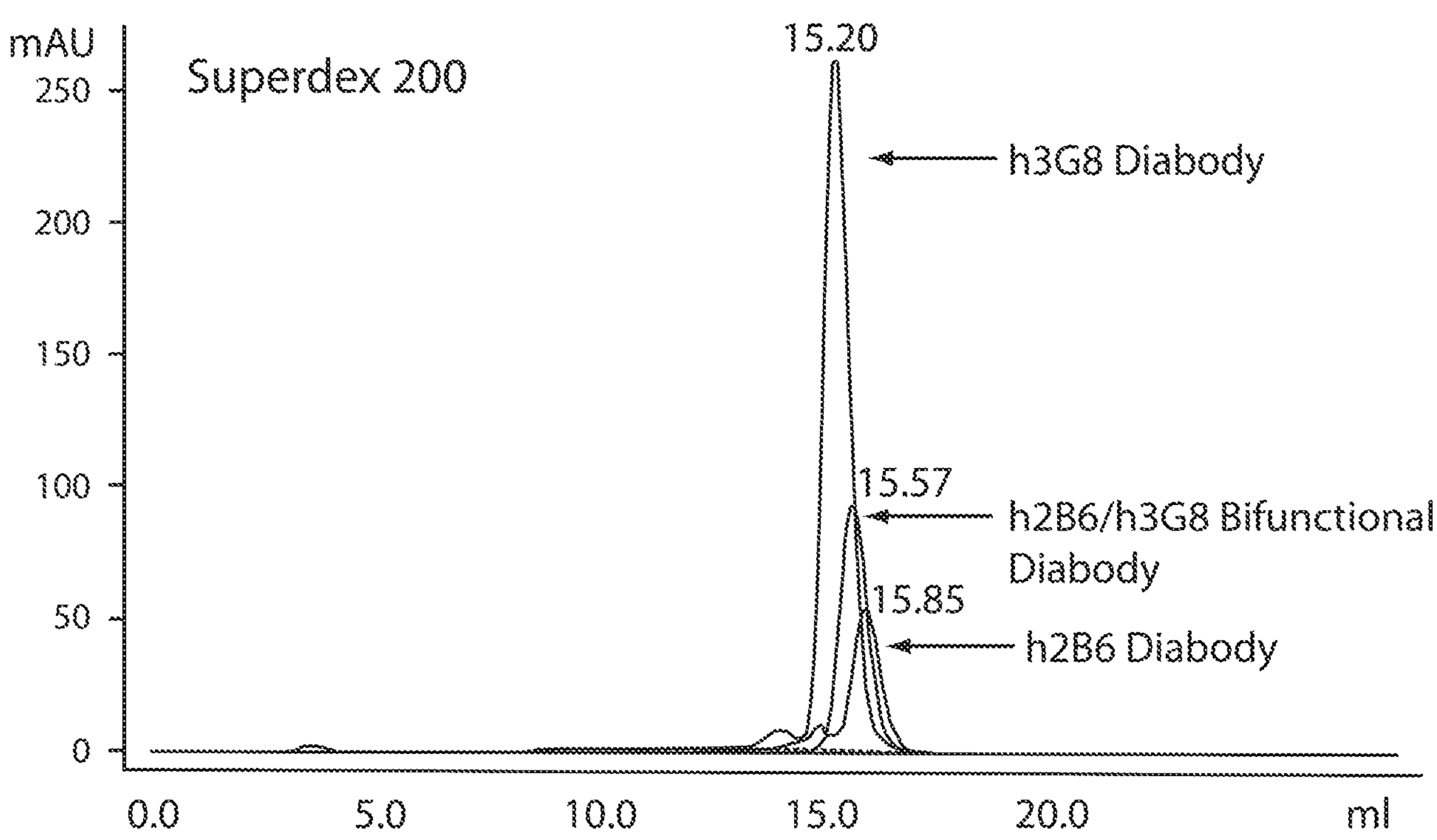

mAU
Superdex 200
250
200
150
100
50
0
0.0
5.0
10.0
15.0
20.0
ml
15.20
h3G8 Diabody
15.57
h2B6/h3G8 Bifunctional Diabody
15.85
h2B6 Diabody

FIG. 4B

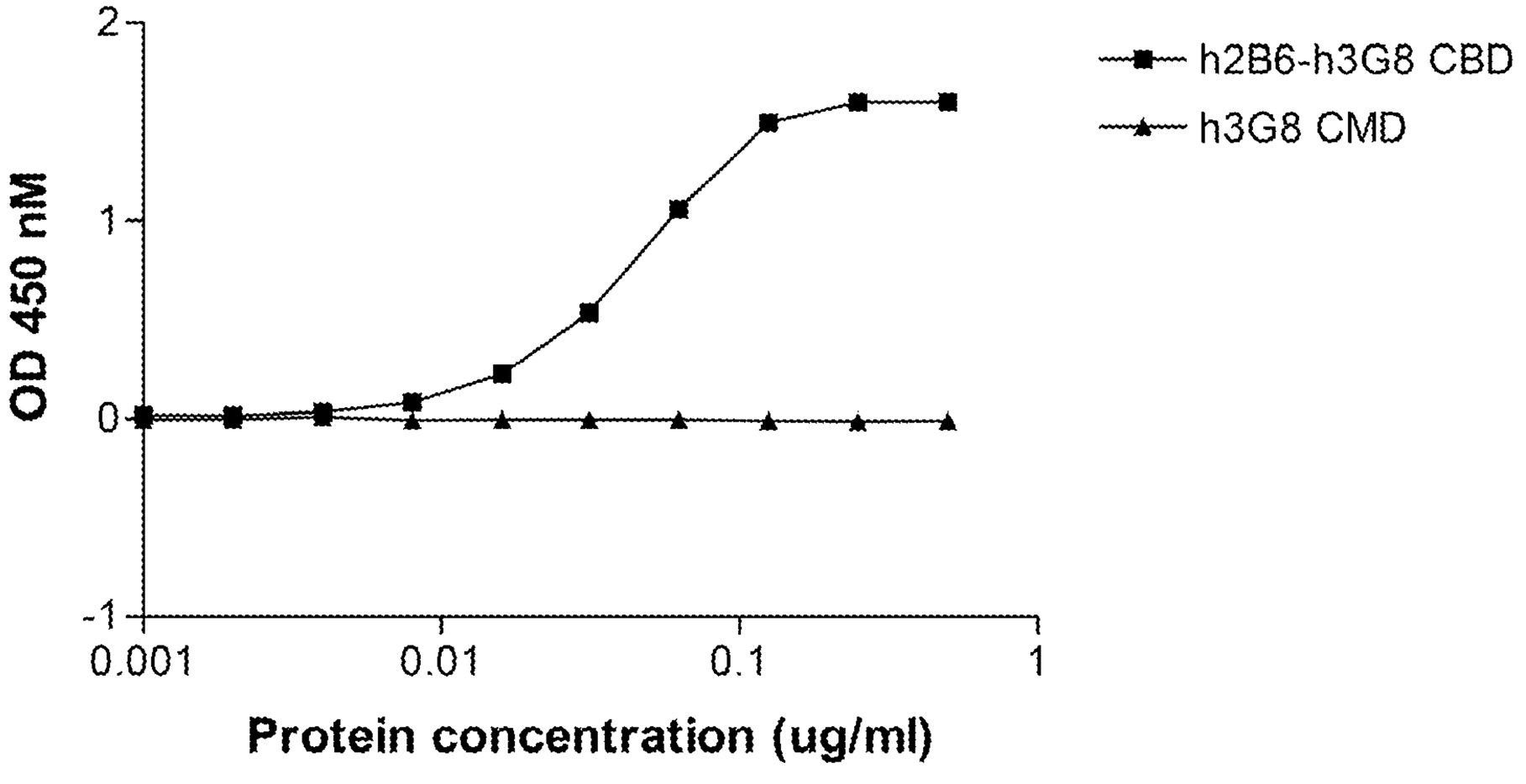

h2B6-h3G8 CBD
h3G8 CMD
OD 450 nM
2
1
0
-1
0.001
0.01
0.1
1
Protein concentration (ug/ml)

FIG. 5

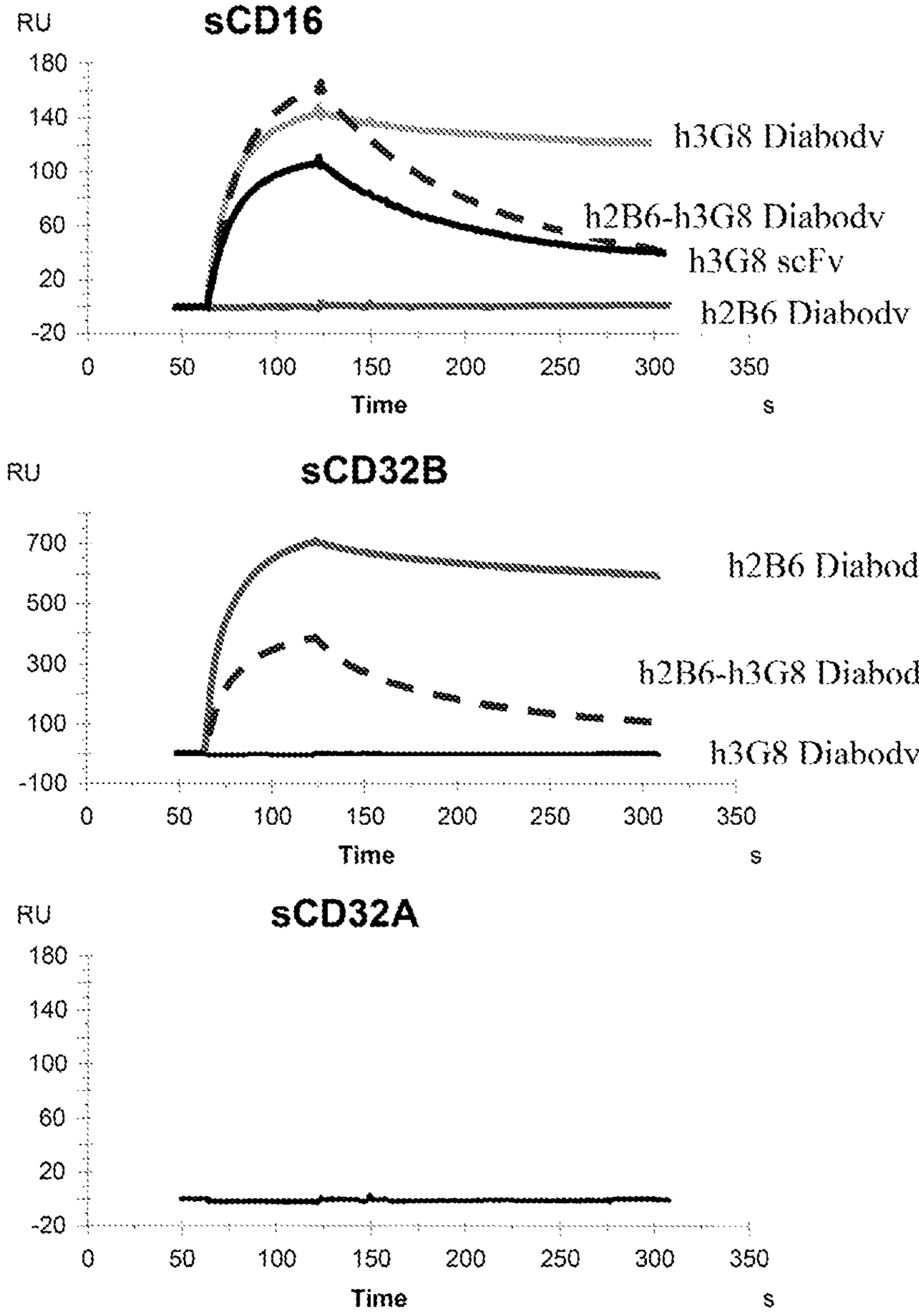


FIG. 6A

FIG. 6B

FIG. 6C

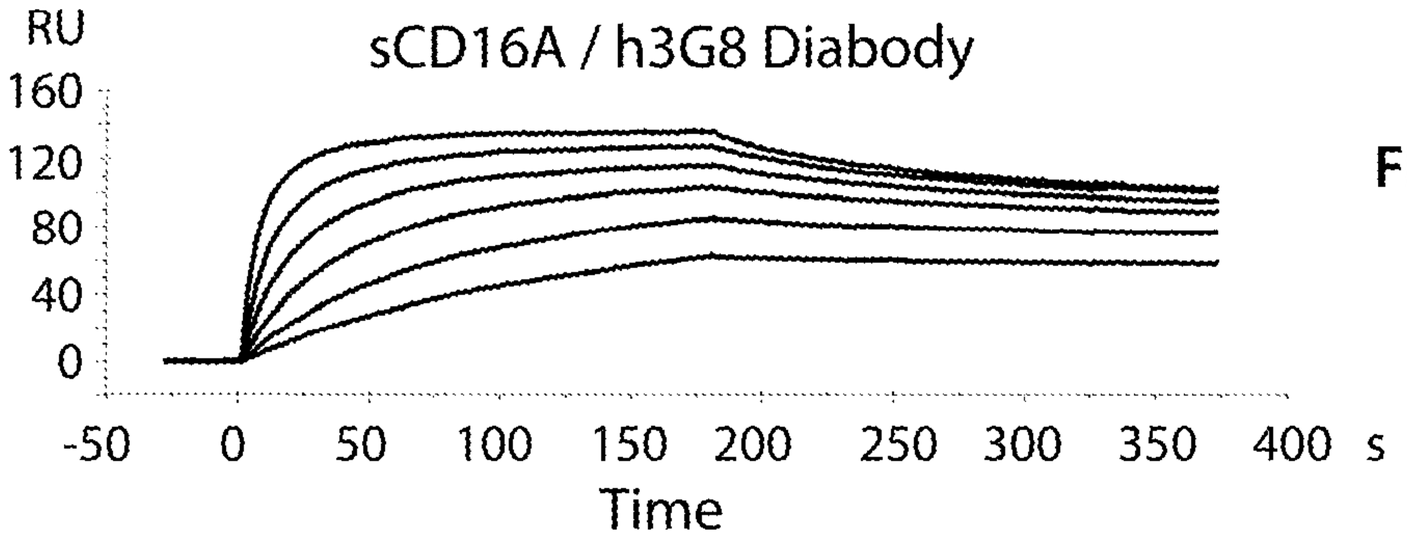


FIG. 7A

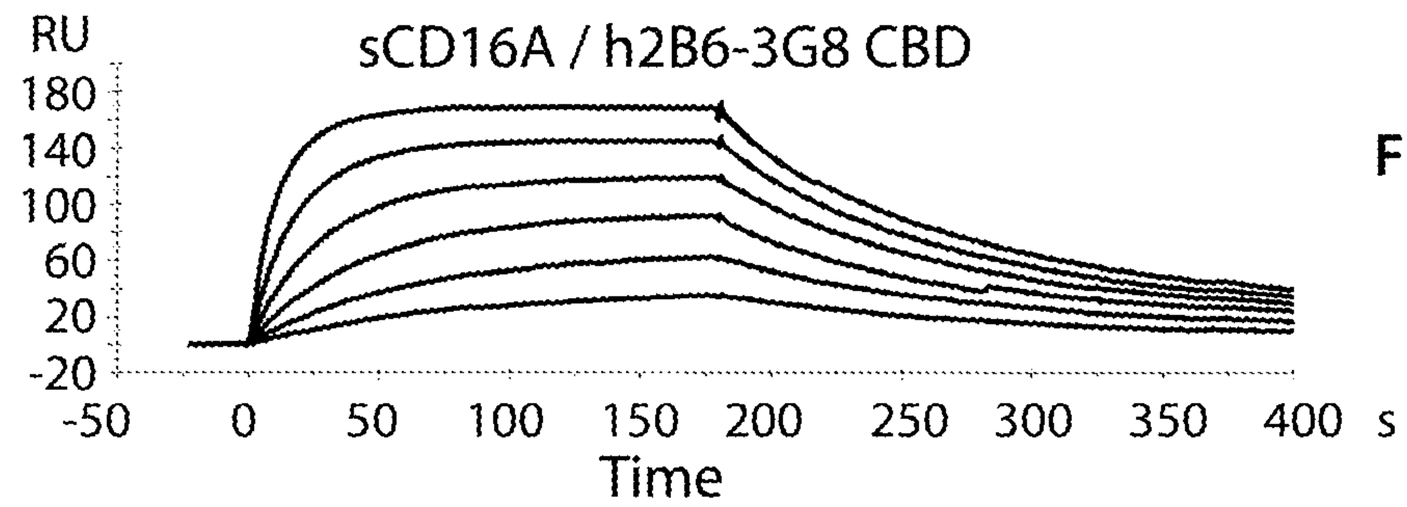


FIG. 7B

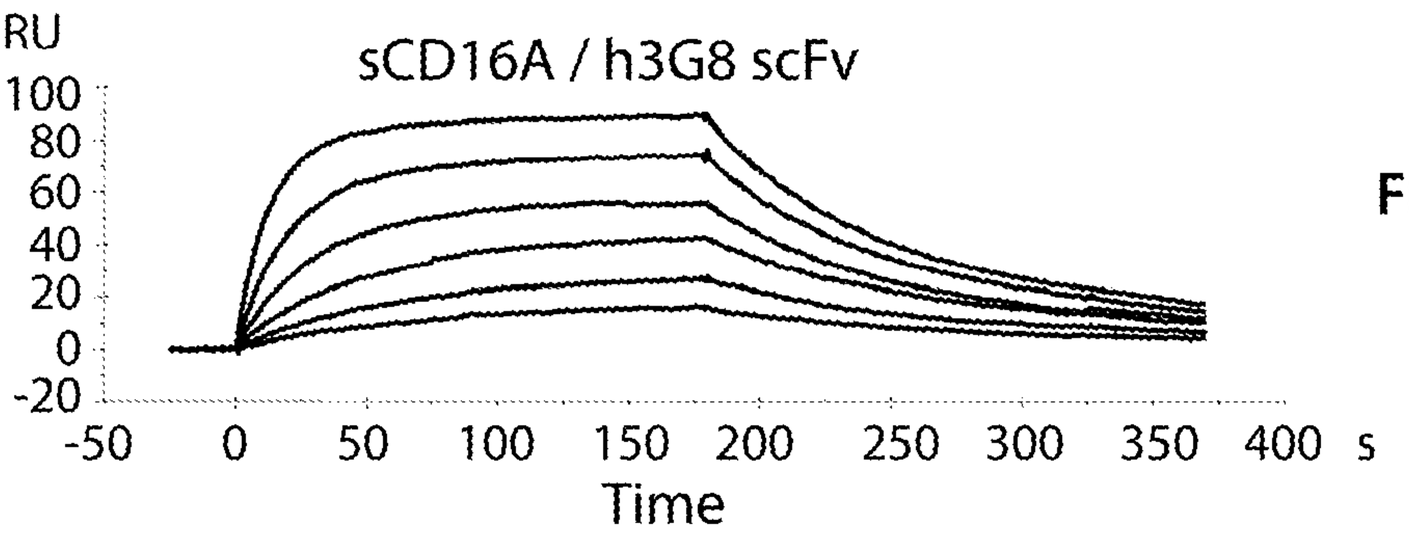


FIG. 7C

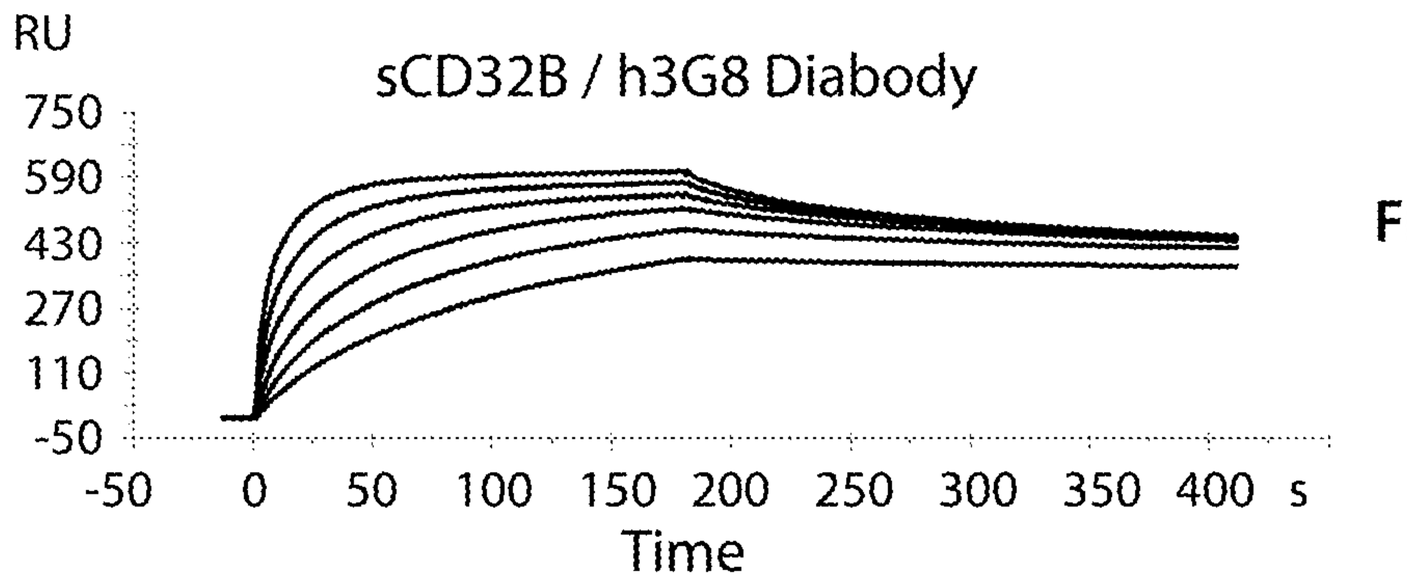


FIG. 7D

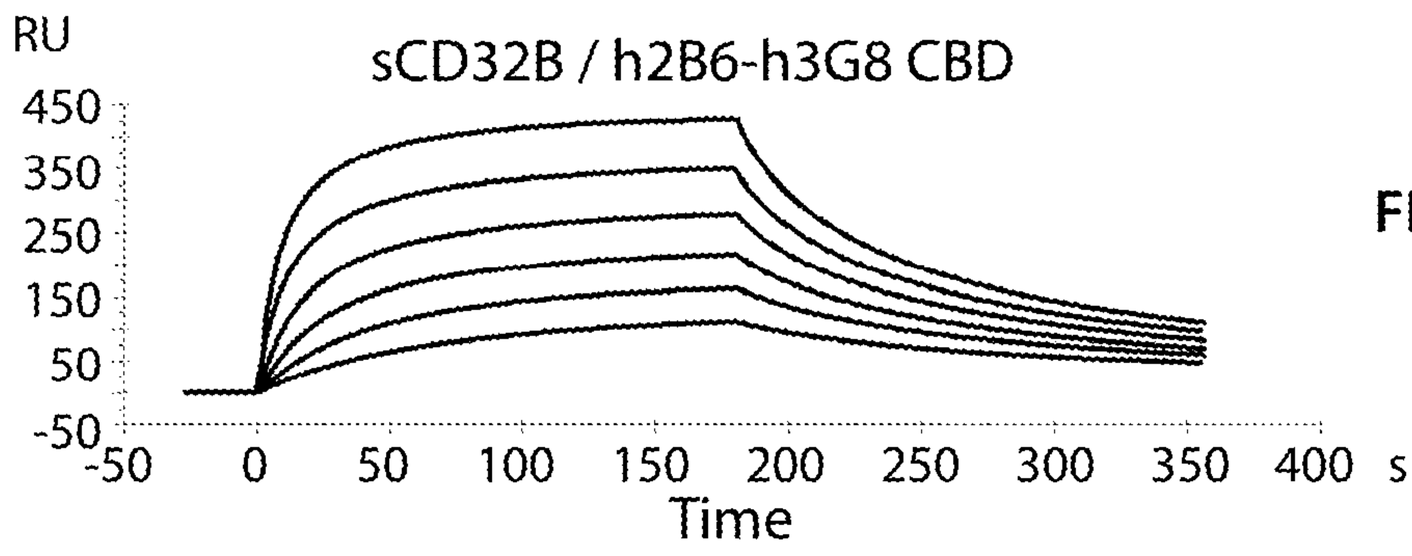


FIG. 7E

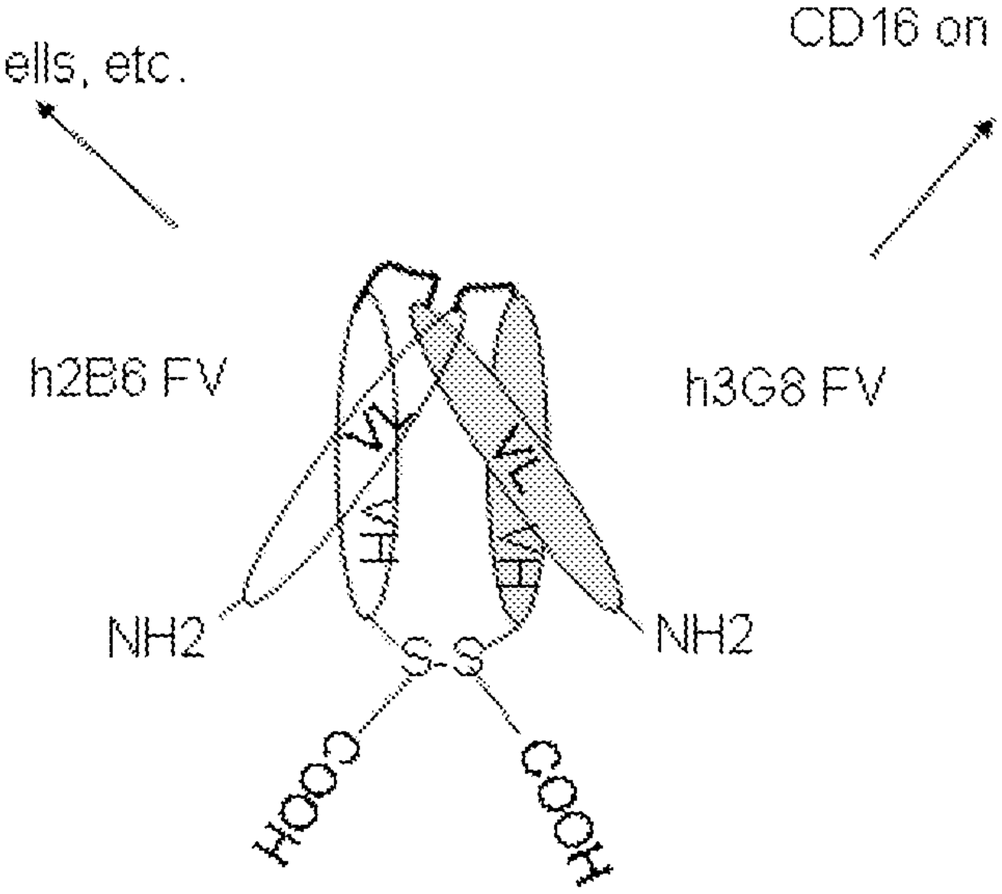
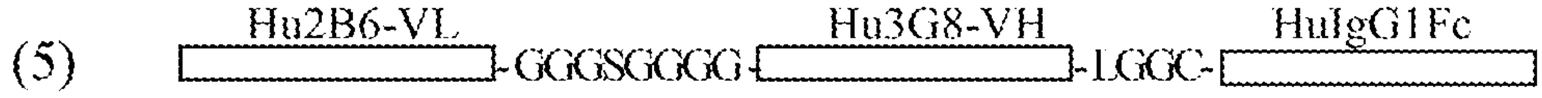
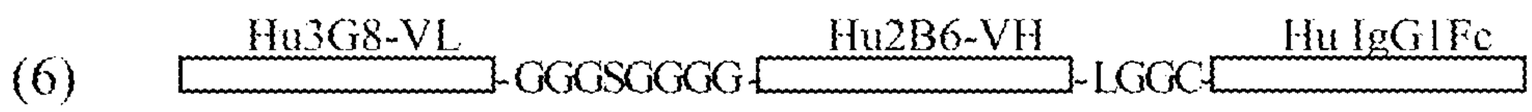

CD32B on B cells, etc.
CD16 on NK, MΦ, PMN
h2B6 FV
h3G8 FV
VL
VH
VH
VL
NH2
NH2
S-S
HOOC
COOH

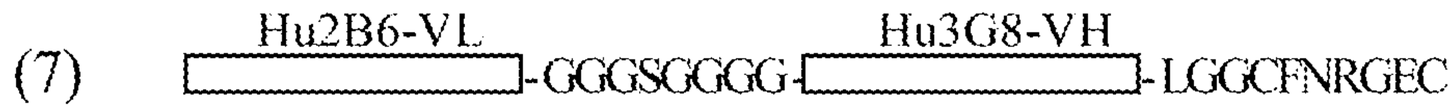
FIG. 8


(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

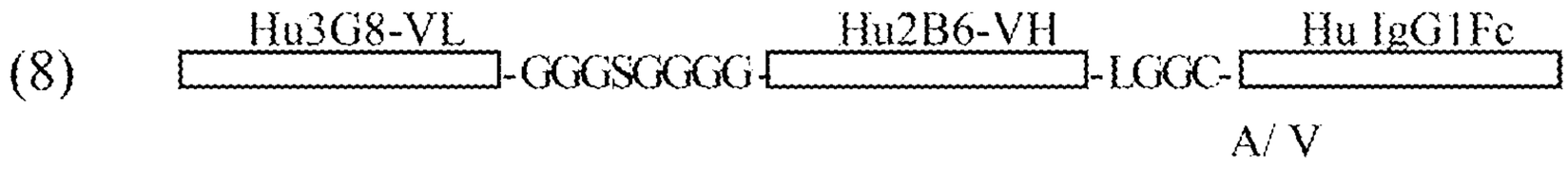

(8)
Hu3G8-VL
-GGGSGGGG-
Hu2B6-VH
-LGGC-
Hu IgG1Fc
A/ V

FIG. 9

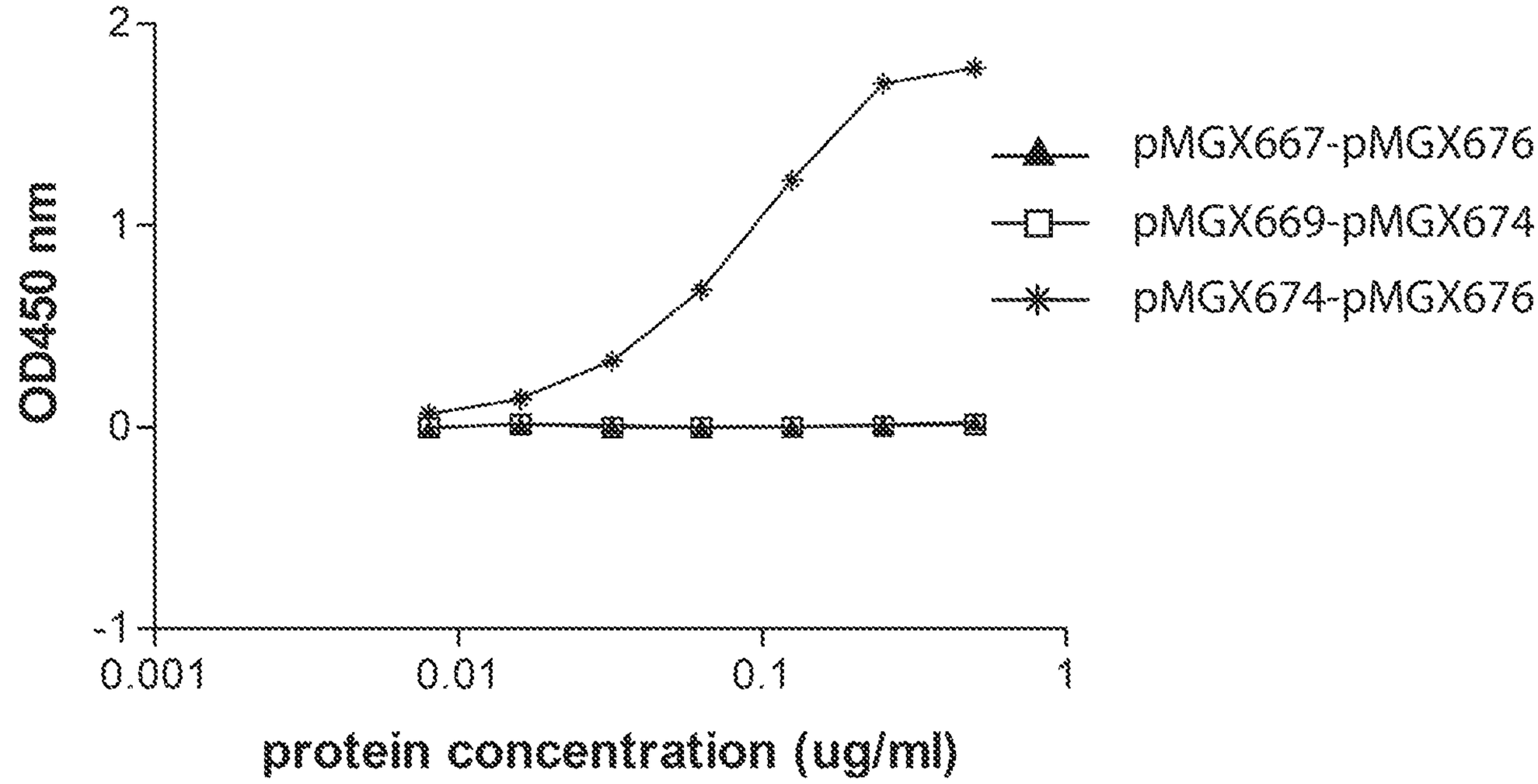

OD450 nm
2
1
0
-1
0.001
0.01
0.1
1
protein concentration (ug/ml)
pMGX667-pMGX676
pMGX669-pMGX674
pMGX674-pMGX676

FIG. 10

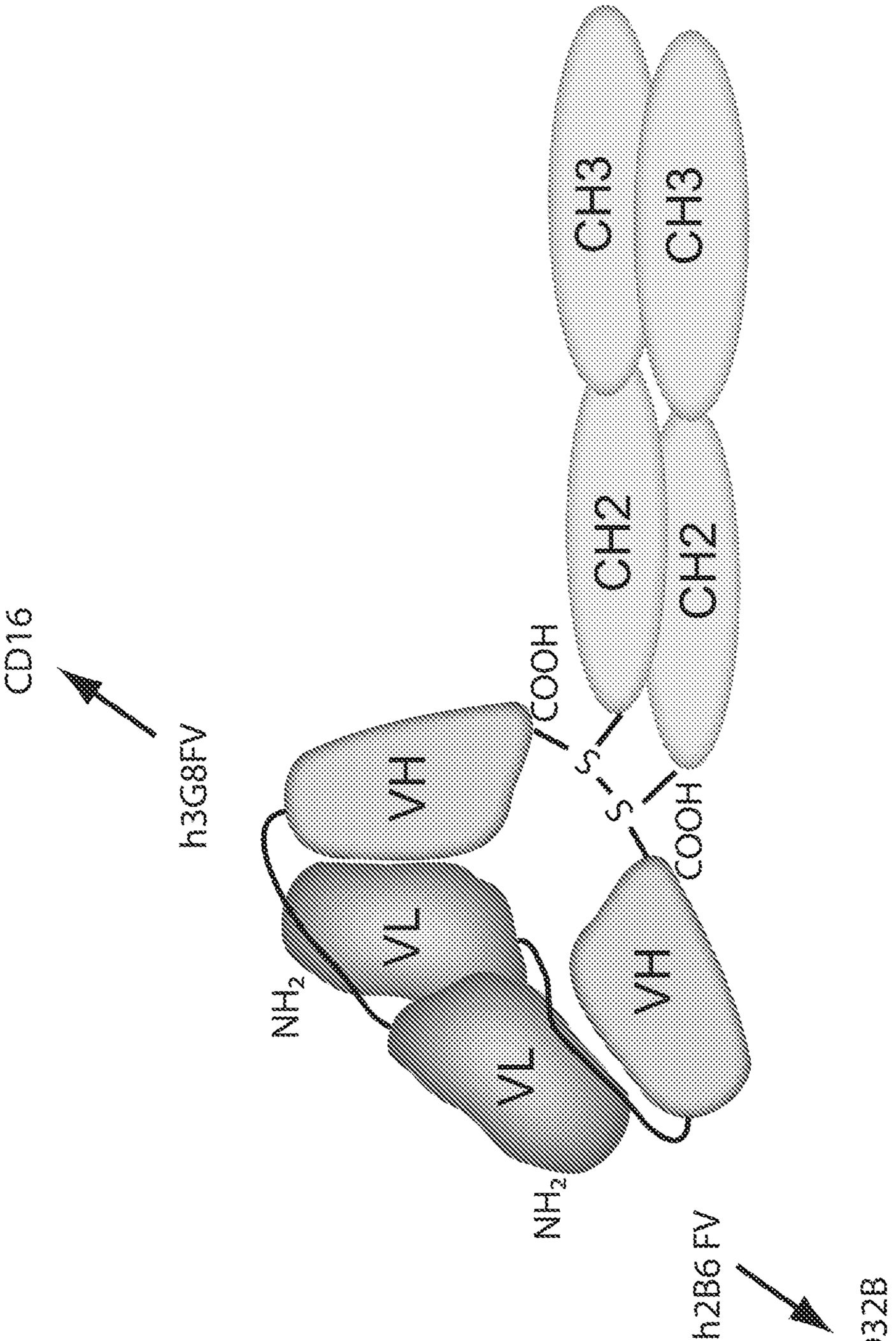

CD16
h3G8FV
COOH
CH3
CH3
CH2
CH2
VH
VL
VL
VH
NH_2
NH_2
S-S
COOH
h2B6 FV
D32B

FIG. 11

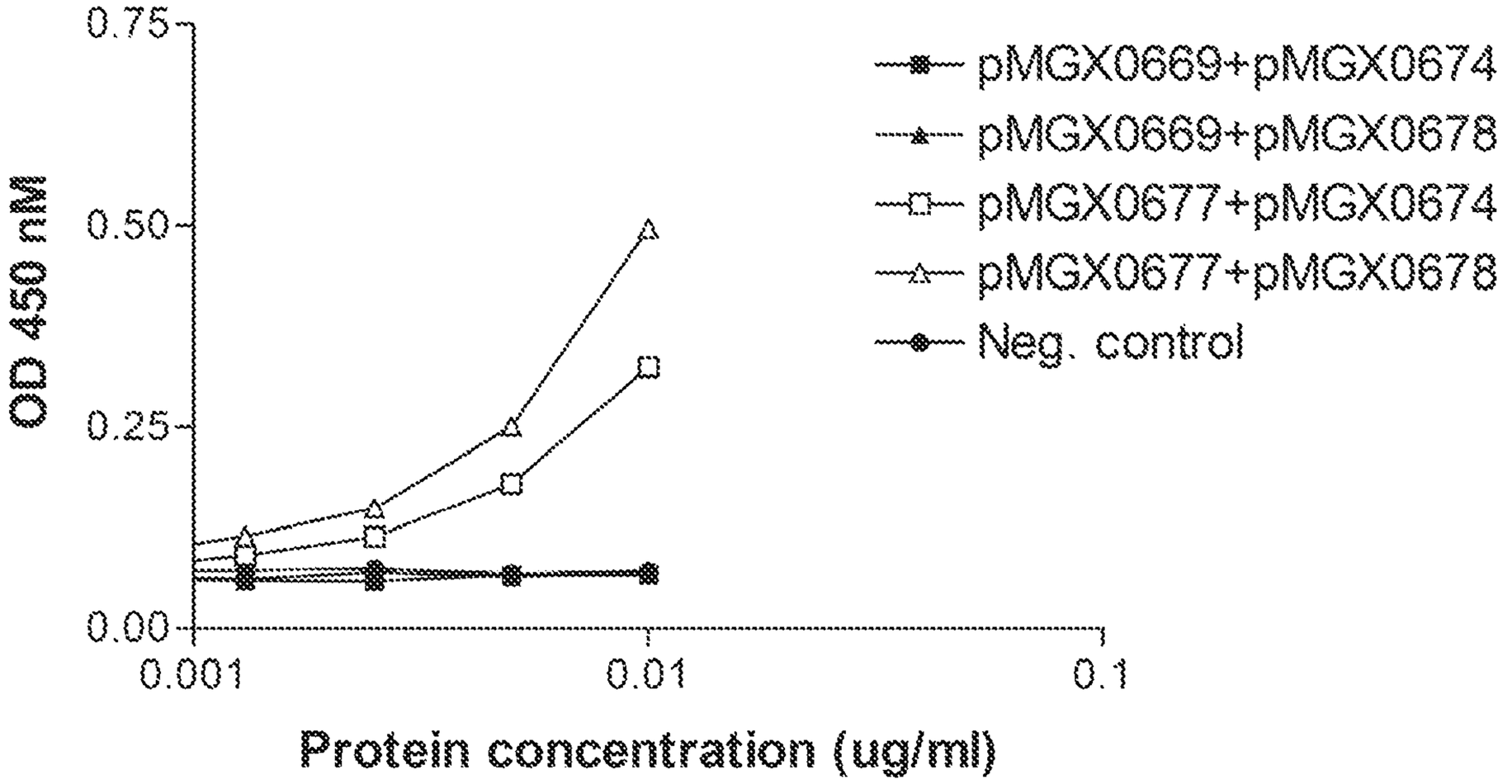

0.75
0.50
0.25
0.00
OD 450 nM
0.001
0.01
0.1
Protein concentration (ug/ml)
pMGX0669+pMGX0674
pMGX0669+pMGX0678
pMGX0677+pMGX0674
pMGX0677+pMGX0678
Neg. control

FIG. 12

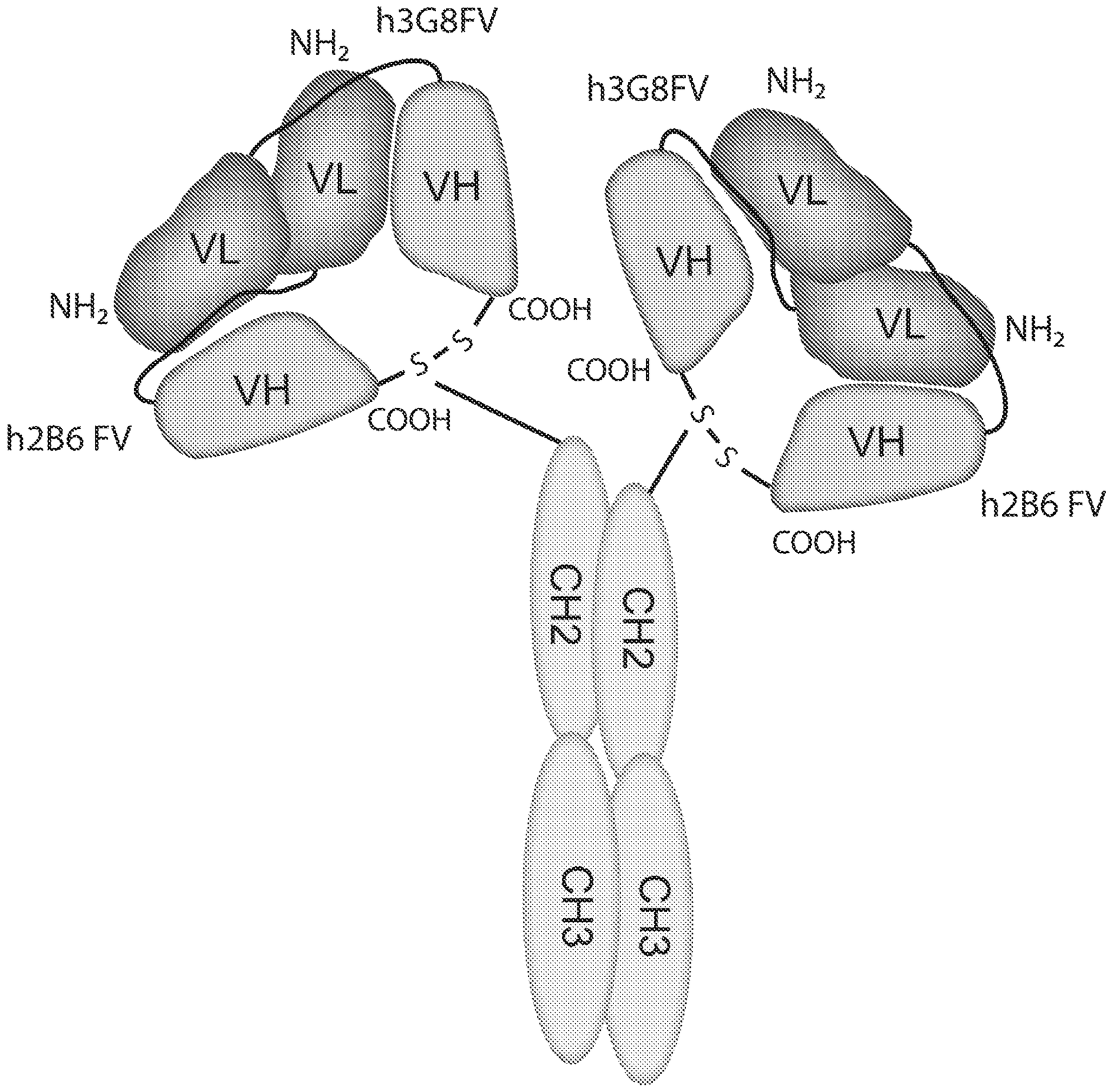

h3G8FV
NH2
VL
VH
VL
NH2
COOH
S-S
VH
COOH
h2B6 FV
h3G8FV
NH2
VL
VH
VL
NH2
COOH
S-S
VH
h2B6 FV
COOH
CH2
CH2
CH3
CH3

FIG. 13

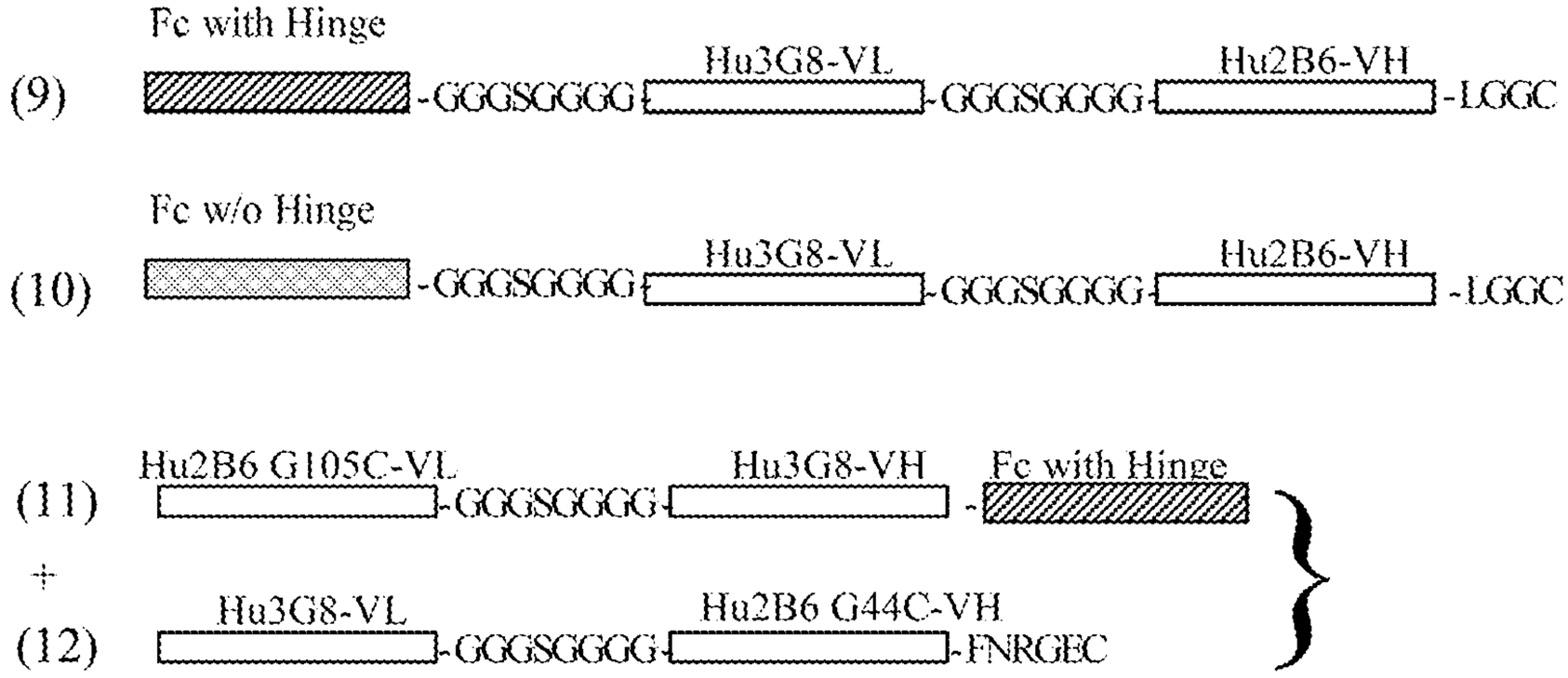

Fc with Hinge
(9)
-GGGSGGGG
Hu3G8-VL
-GGGSGGGG
Hu2B6-VH
-LGGC
Fc w/o Hinge
(10)
-GGGSGGGG
Hu3G8-VL
-GGGSGGGG
Hu2B6-VH
-LGGC
Hu2B6 G105C-VL
(11)
-GGGSGGGG
Hu3G8-VH
-
Fc with Hinge
+
Hu3G8-VL
(12)
-GGGSGGGG
Hu2B6 G44C-VH
-FNRGEC

FIG. 14

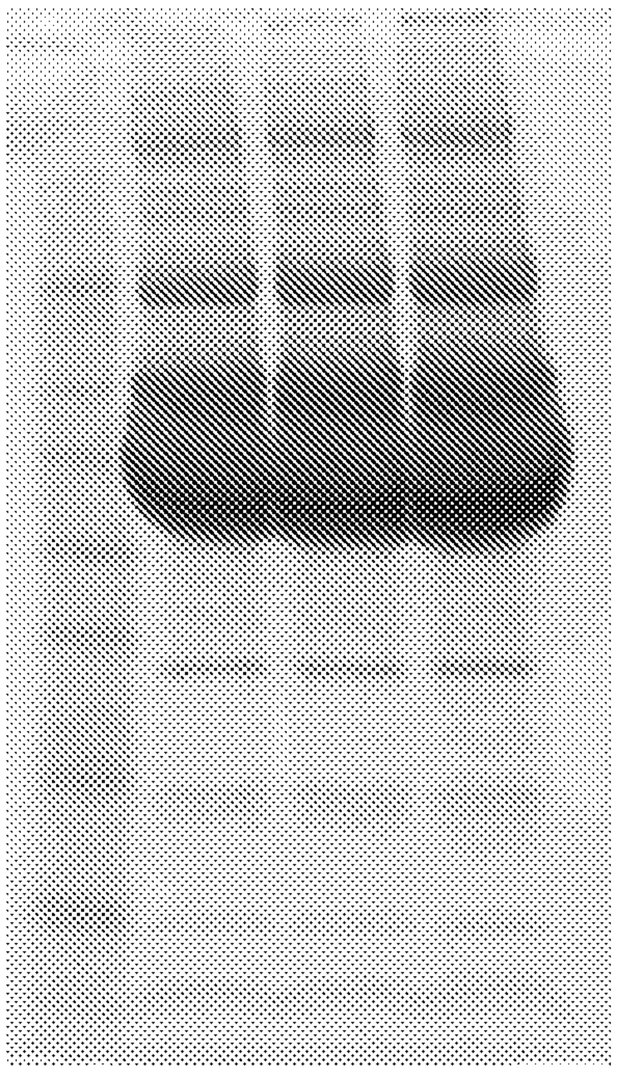


FIG. 15A

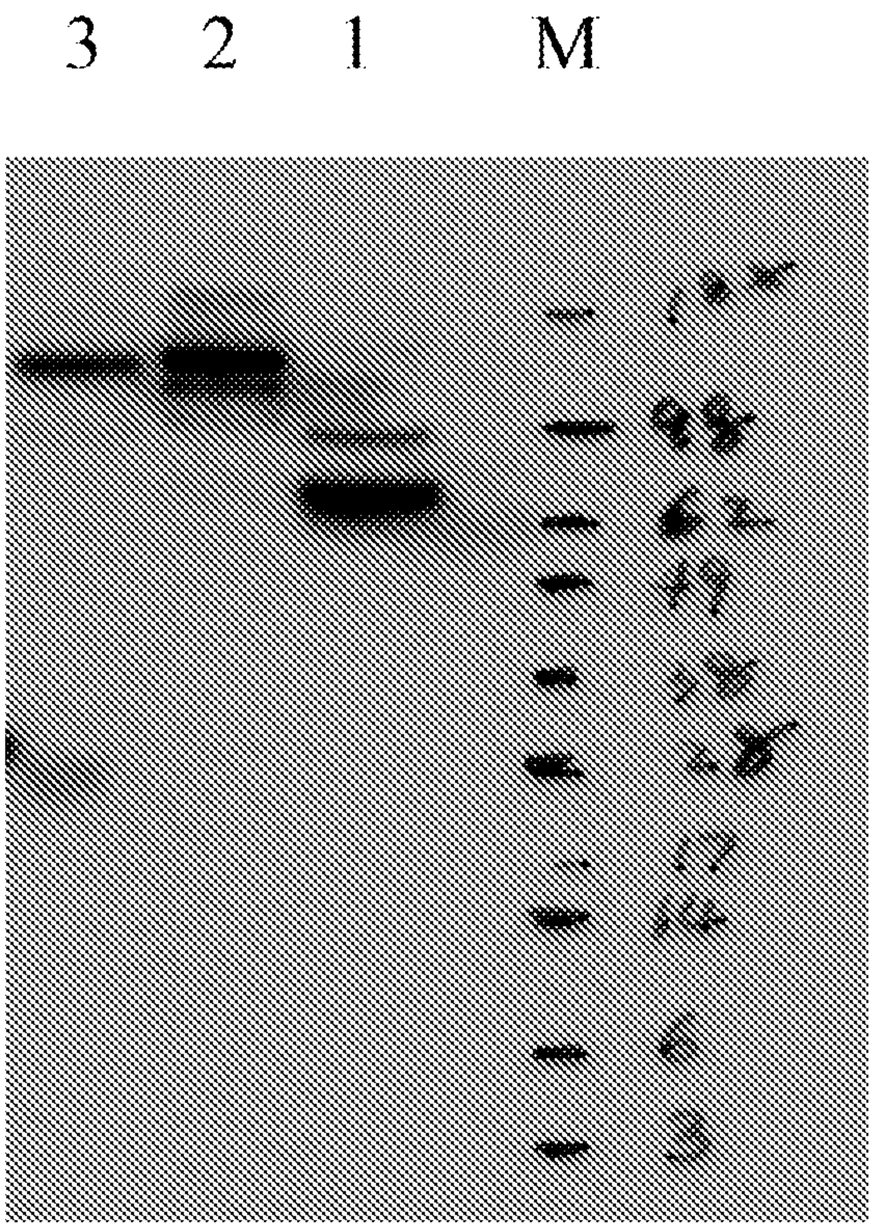


FIG. 15B

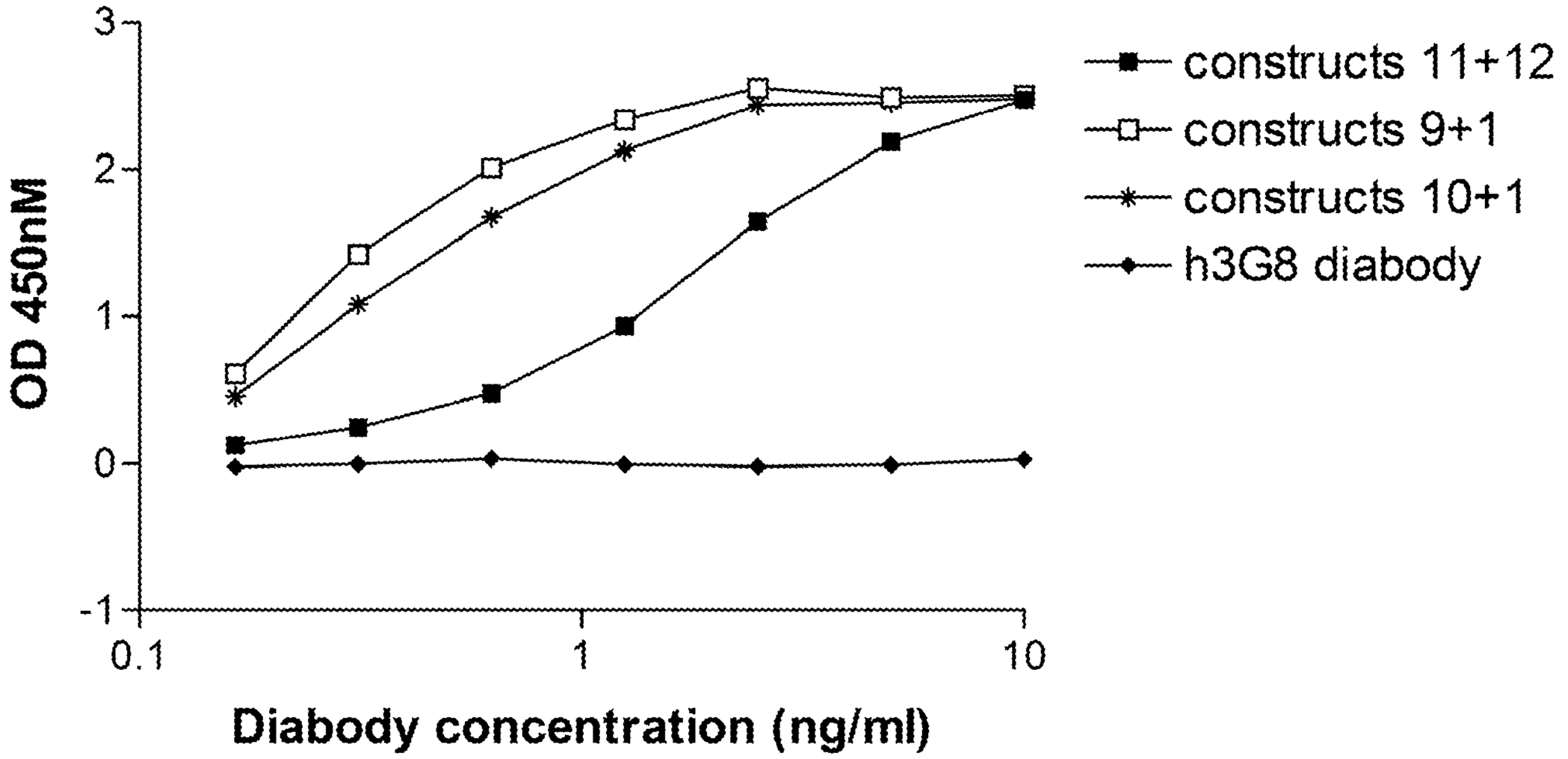

3
2
1
0
-1
OD 450nM
0.1
1
10
Diabody concentration (ng/ml)
constructs 11+12
constructs 9+1
constructs 10+1
h3G8 diabody

FIG. 16

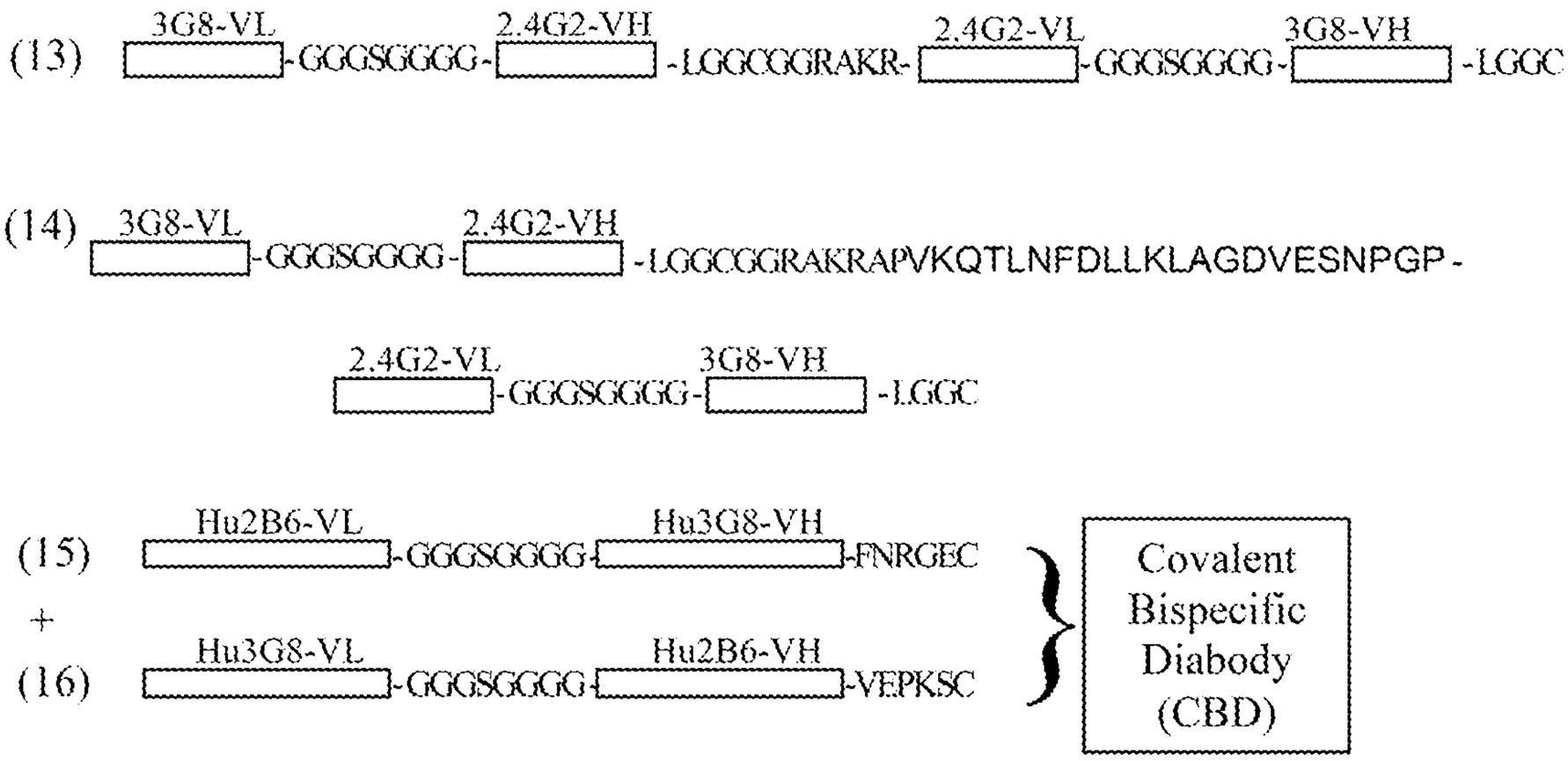

(13) 3G8-VL -GGGSGGGG- 2.4G2-VH -LGGCGGRAKR- 2.4G2-VL -GGGSGGGG- 3G8-VH -LGGC
(14) 3G8-VL -GGGSGGGG- 2.4G2-VH -LGGCGGRAKRAPVKQTLNFDLLKLAGDVESNPGP-
2.4G2-VL -GGGSGGGG- 3G8-VH -LGGC
(15) Hu2B6-VL -GGGSGGGG- Hu3G8-VH -FNRGEC
+
(16) Hu3G8-VL -GGGSGGGG- Hu2B6-VH -VEPKSC
Covalent Bispecific Diabody (CBD)

FIG. 17

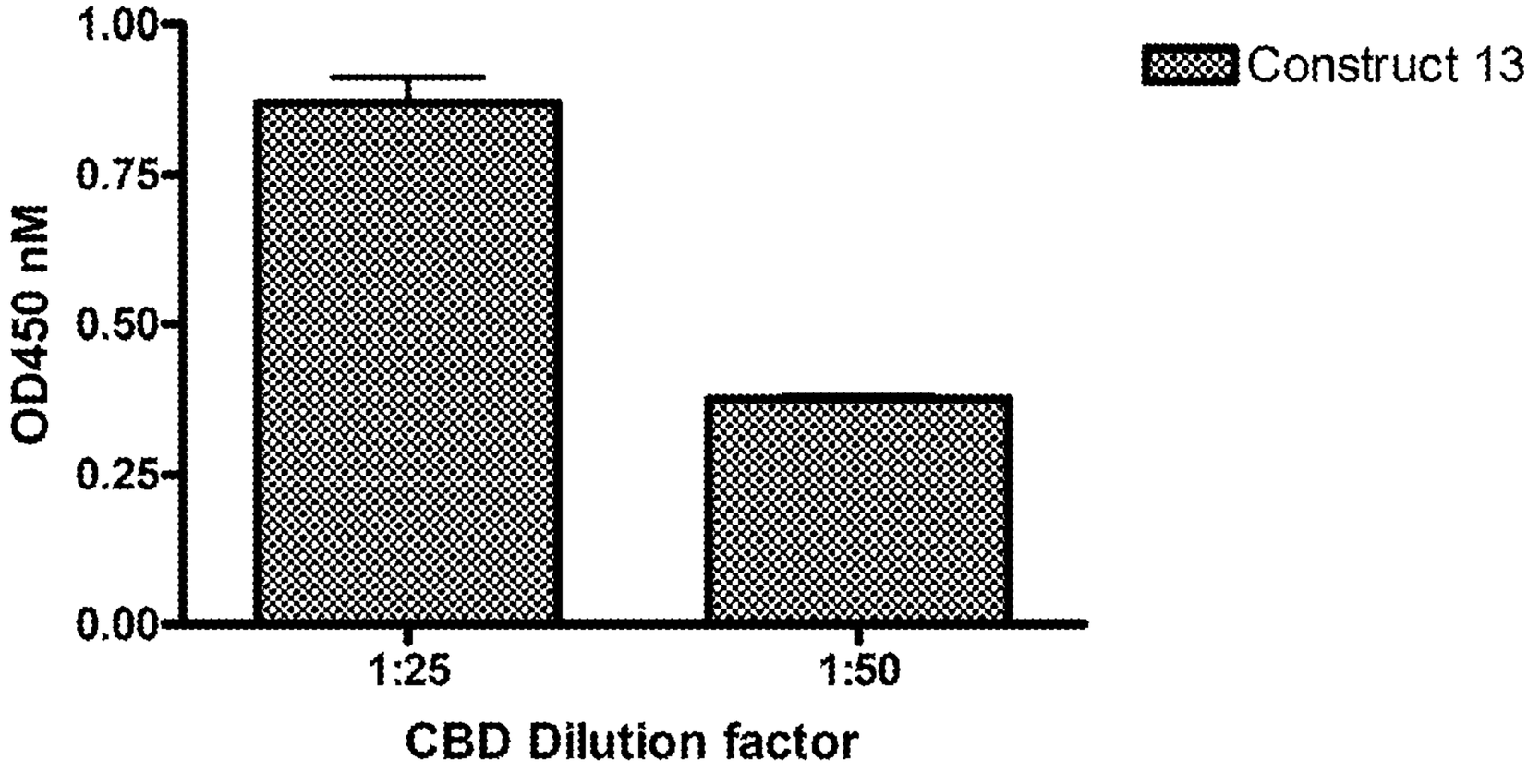

1.00
0.75
0.50
0.25
0.00
OD450 nM
1:25
1:50
CBD Dilution factor
Construct 13

FIG. 18

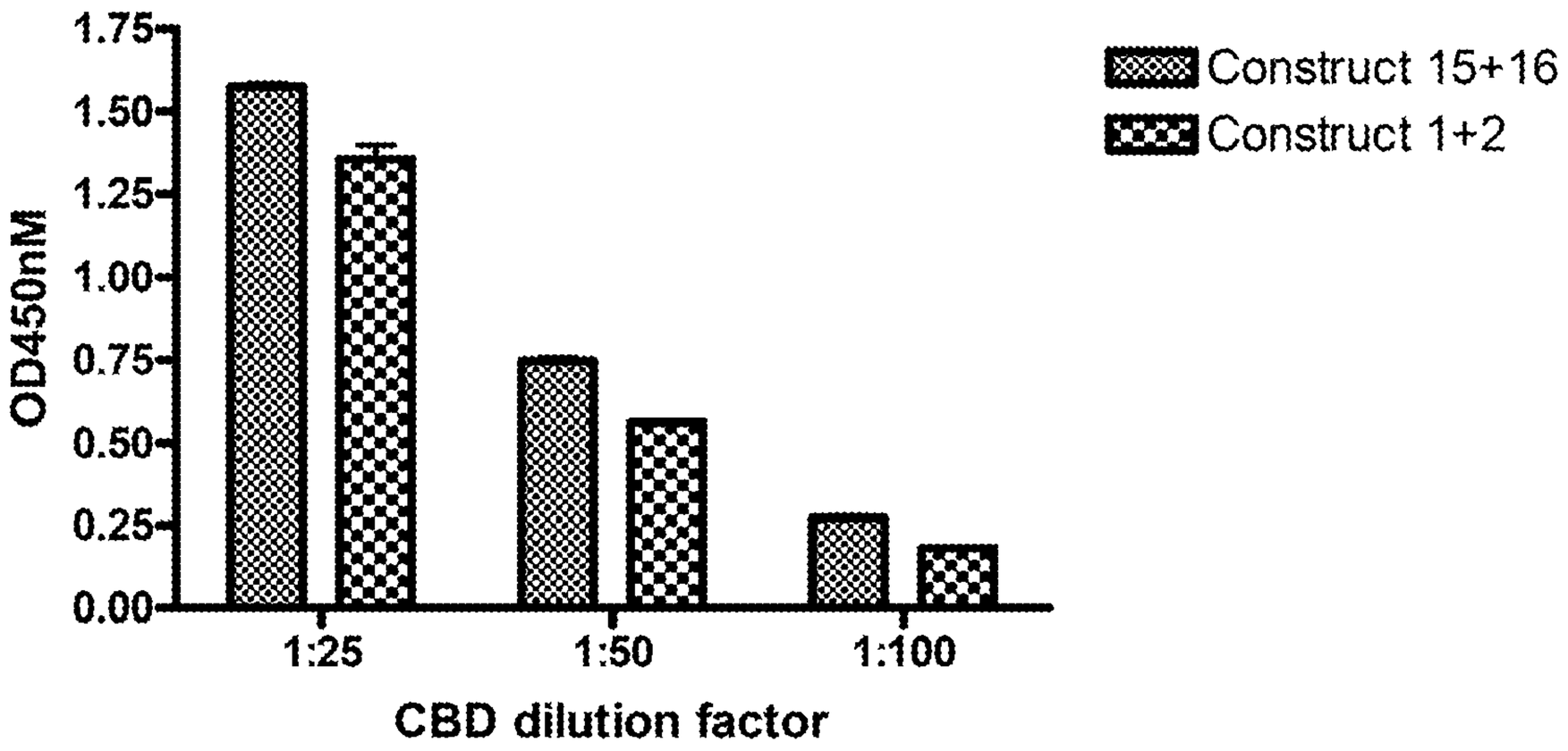

Construct 15+16
Construct 1+2
OD450nM
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
1:25
1:50
1:100
CBD dilution factor

FIG. 19

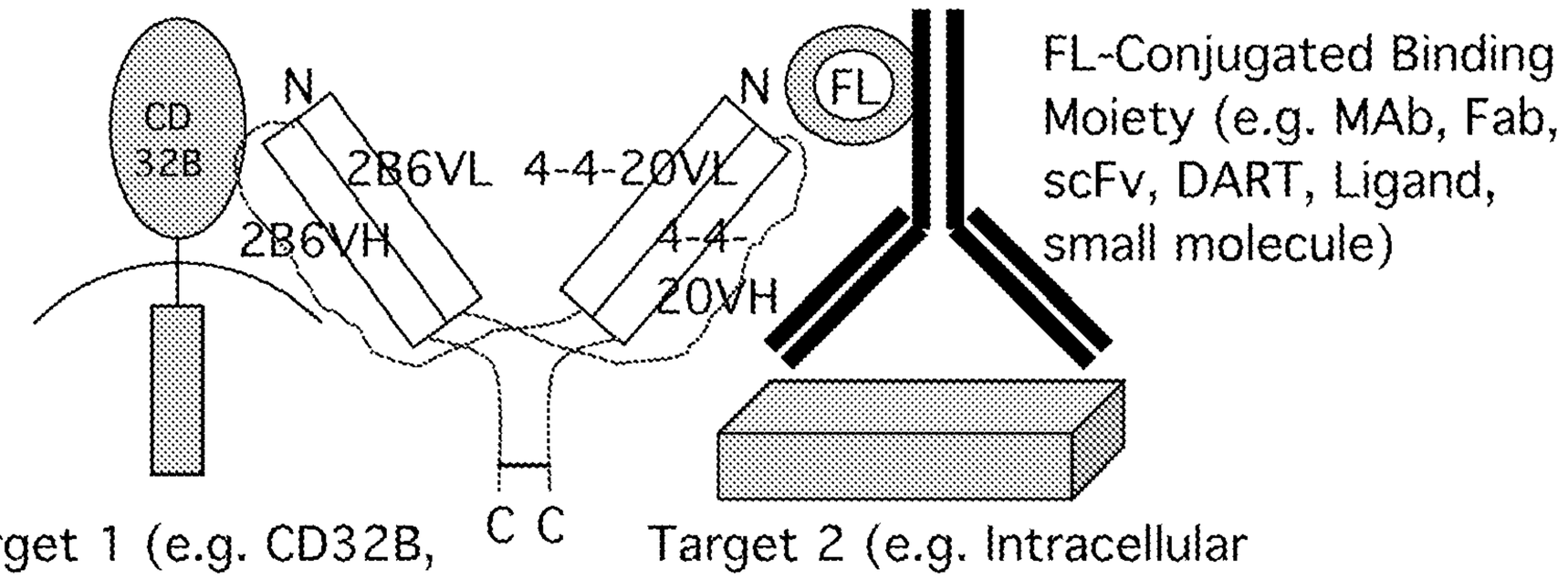

CD
32B
N
2B6VL
2B6VH
N
FL
4-4-20VL
4-4-
20VH
FL-Conjugated Binding
Moiety (e.g. MAb, Fab,
scFv, DART, Ligand,
small molecule)
C C
Target 1 (e.g. CD32B,
Other Immune
Effector, Receptor, or
Cell-free Target)
Target 2 (e.g. Intracellular
Clustering, Intercellular
Recruitment, Cell-free
Recruitment, or Multiple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

FIG. 20

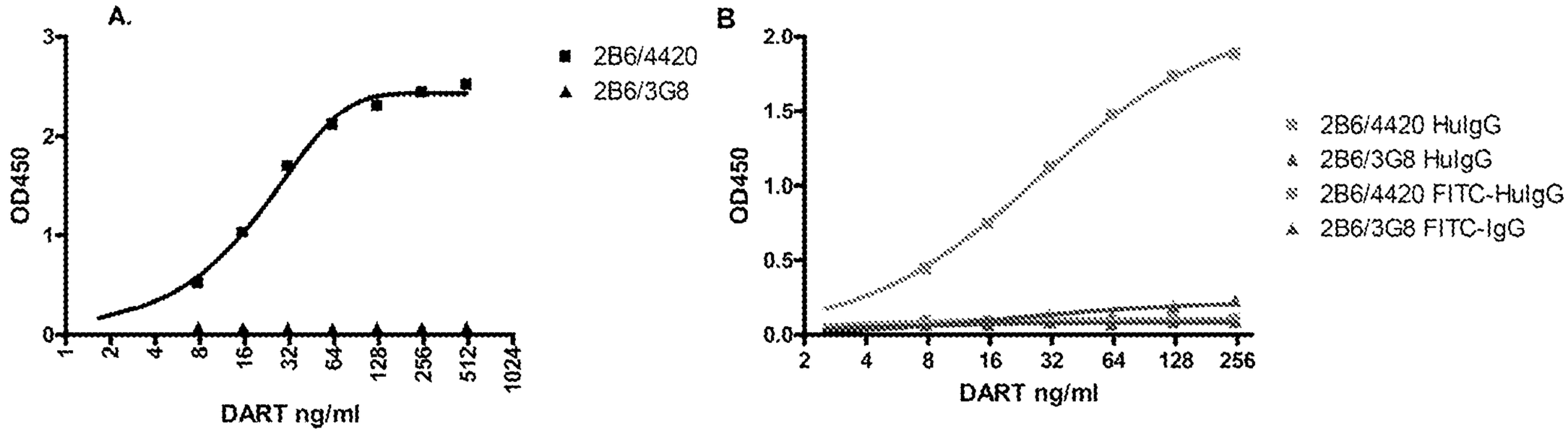


FIG. 21

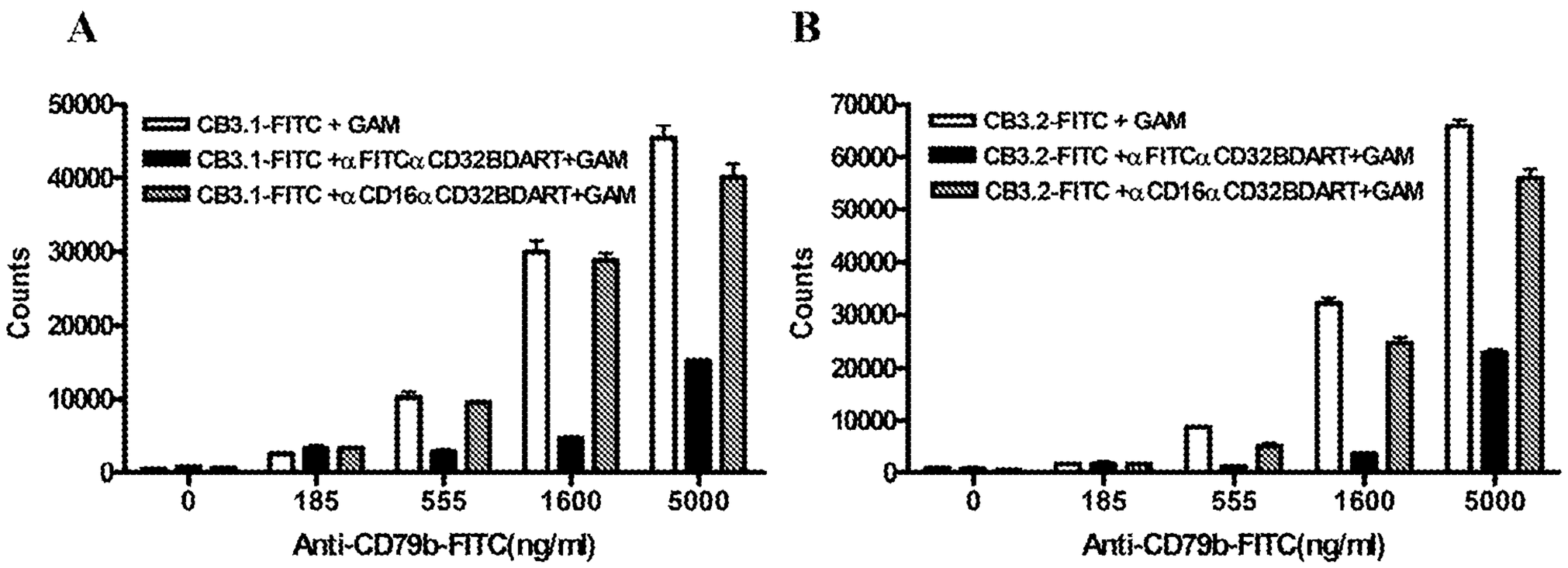


FIG. 22

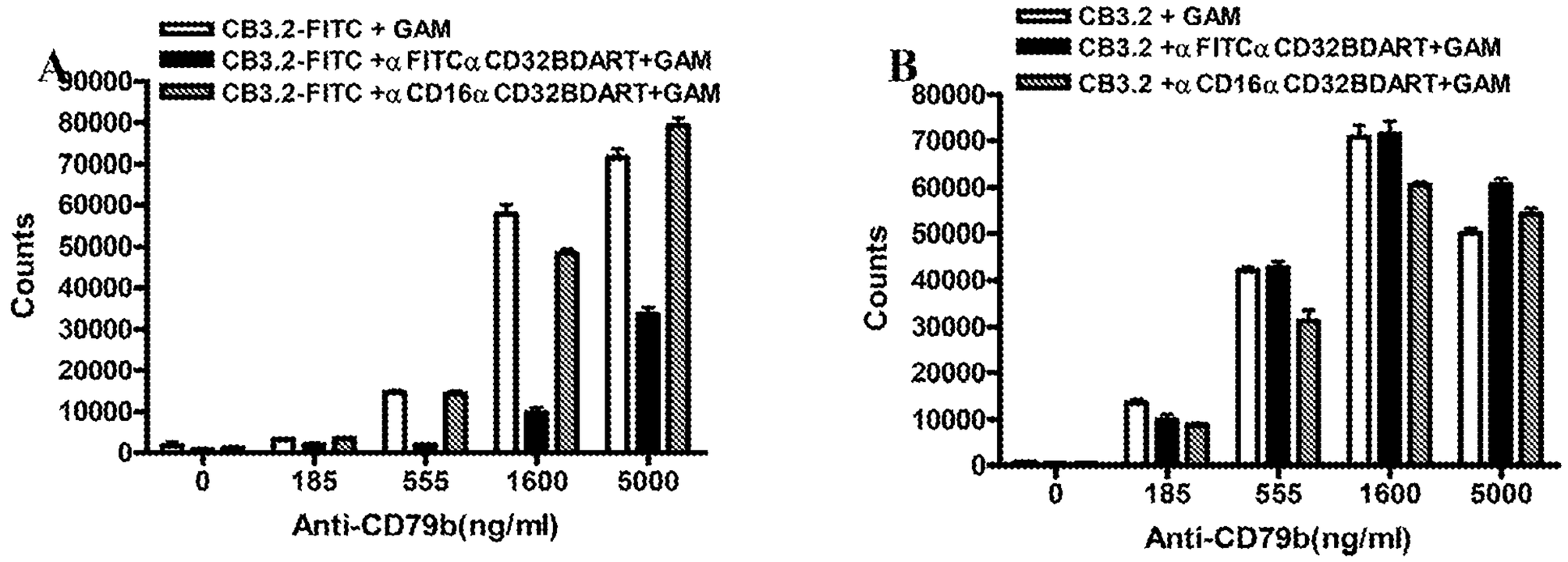
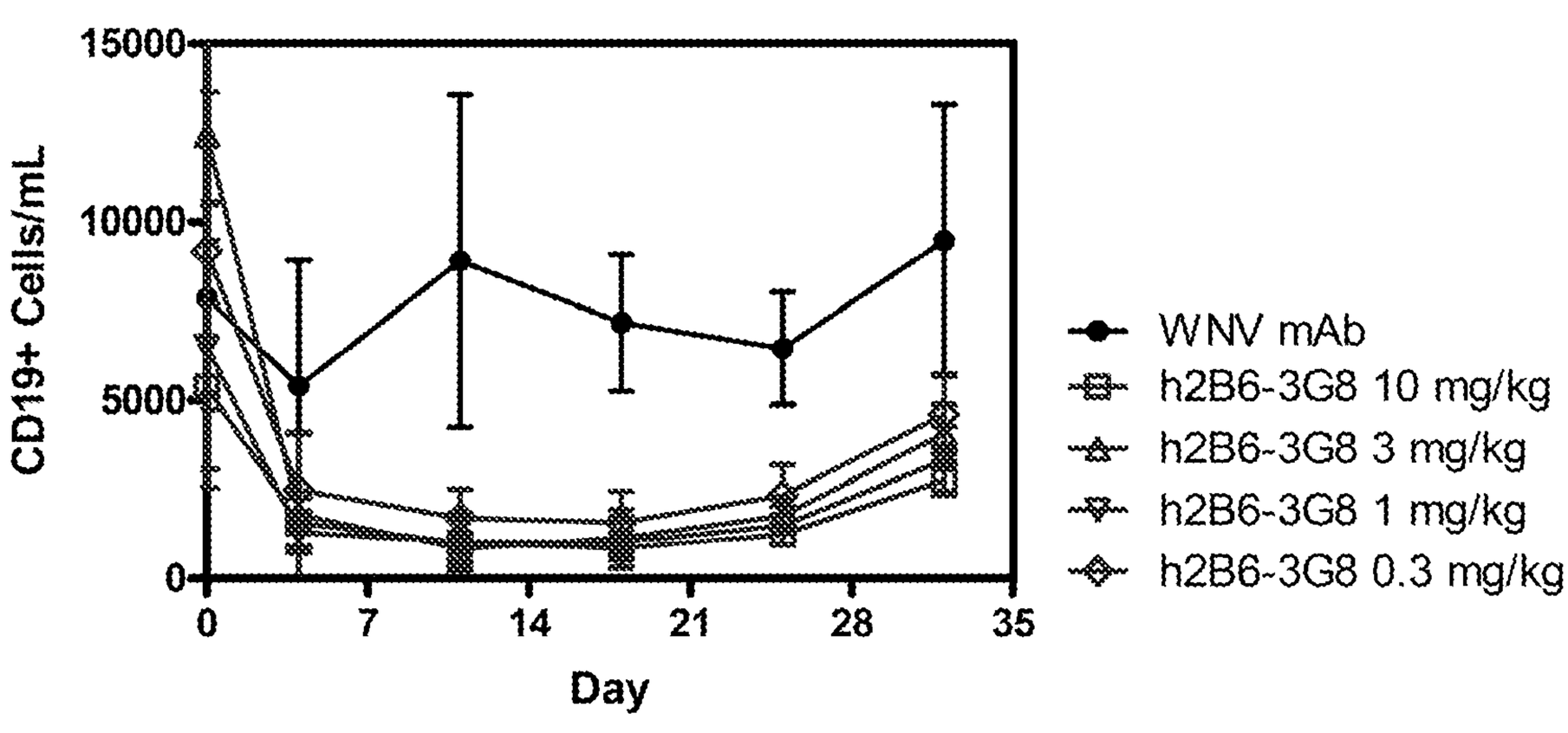


FIG. 23


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

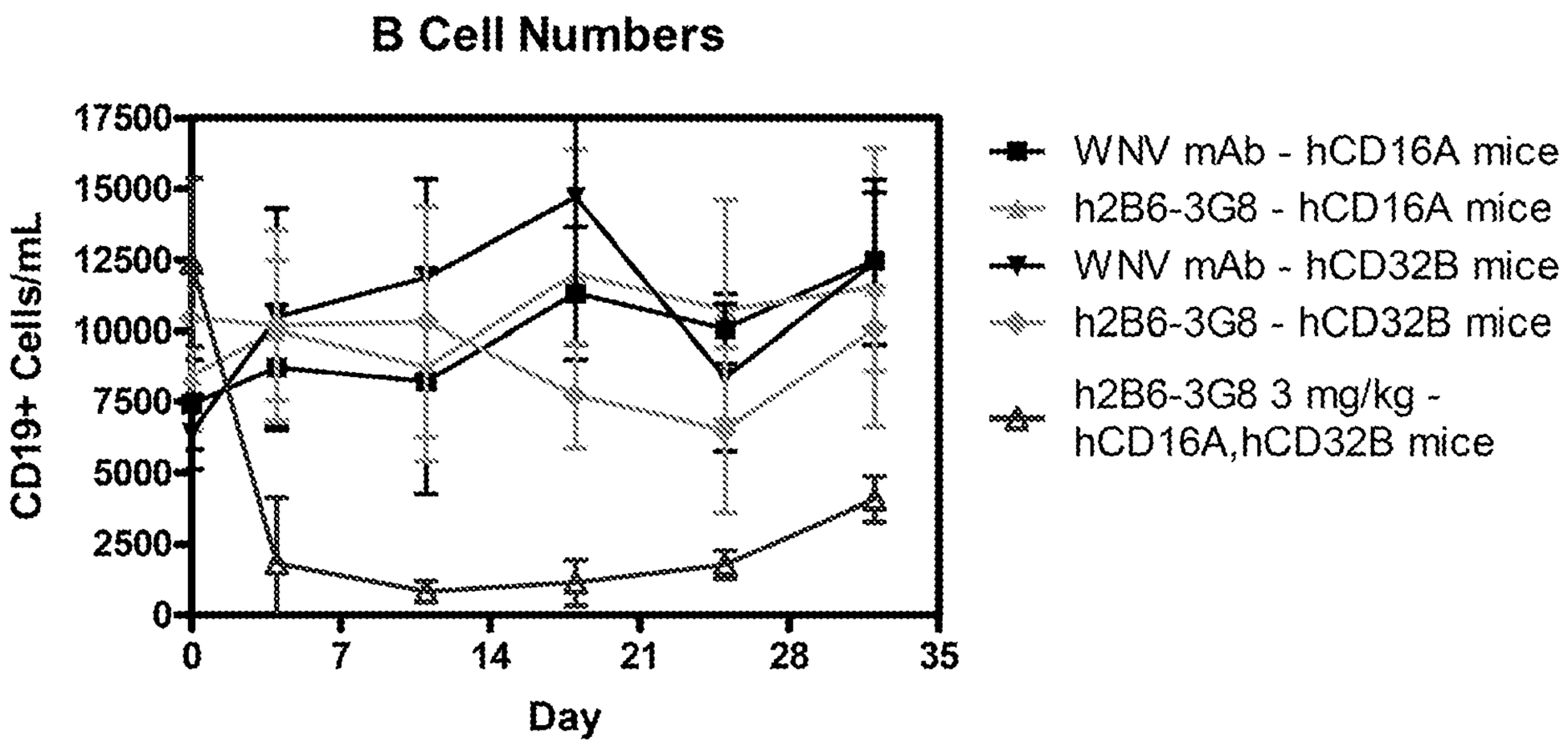

B Cell Numbers
CD19+ Cells/mL
17500
15000
12500
10000
7500
5000
2500
0
0
7
14
21
28
35
Day
WNV mAb - hCD16A mice
h2B6-3G8 - hCD16A mice
WNV mAb - hCD32B mice
h2B6-3G8 - hCD32B mice
h2B6-3G8 3 mg/kg - hCD16A,hCD32B mice

FIG. 24

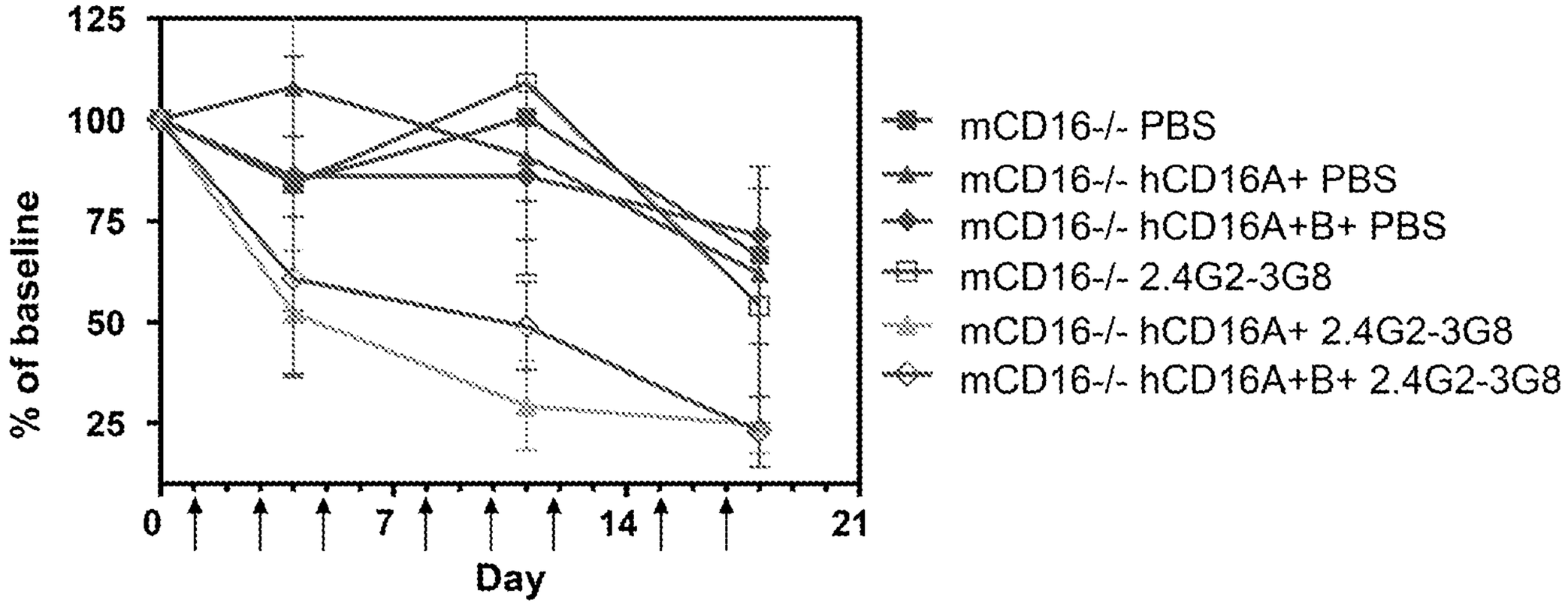

125
100
75
50
25
% of baseline
0
7
14
21
Day
mCD16-/- PBS
mCD16-/- hCD16A+ PBS
mCD16-/- hCD16A+B+ PBS
mCD16-/- 2.4G2-3G8
mCD16-/- hCD16A+ 2.4G2-3G8
mCD16-/- hCD16A+B+ 2.4G2-3G8

FIG. 25

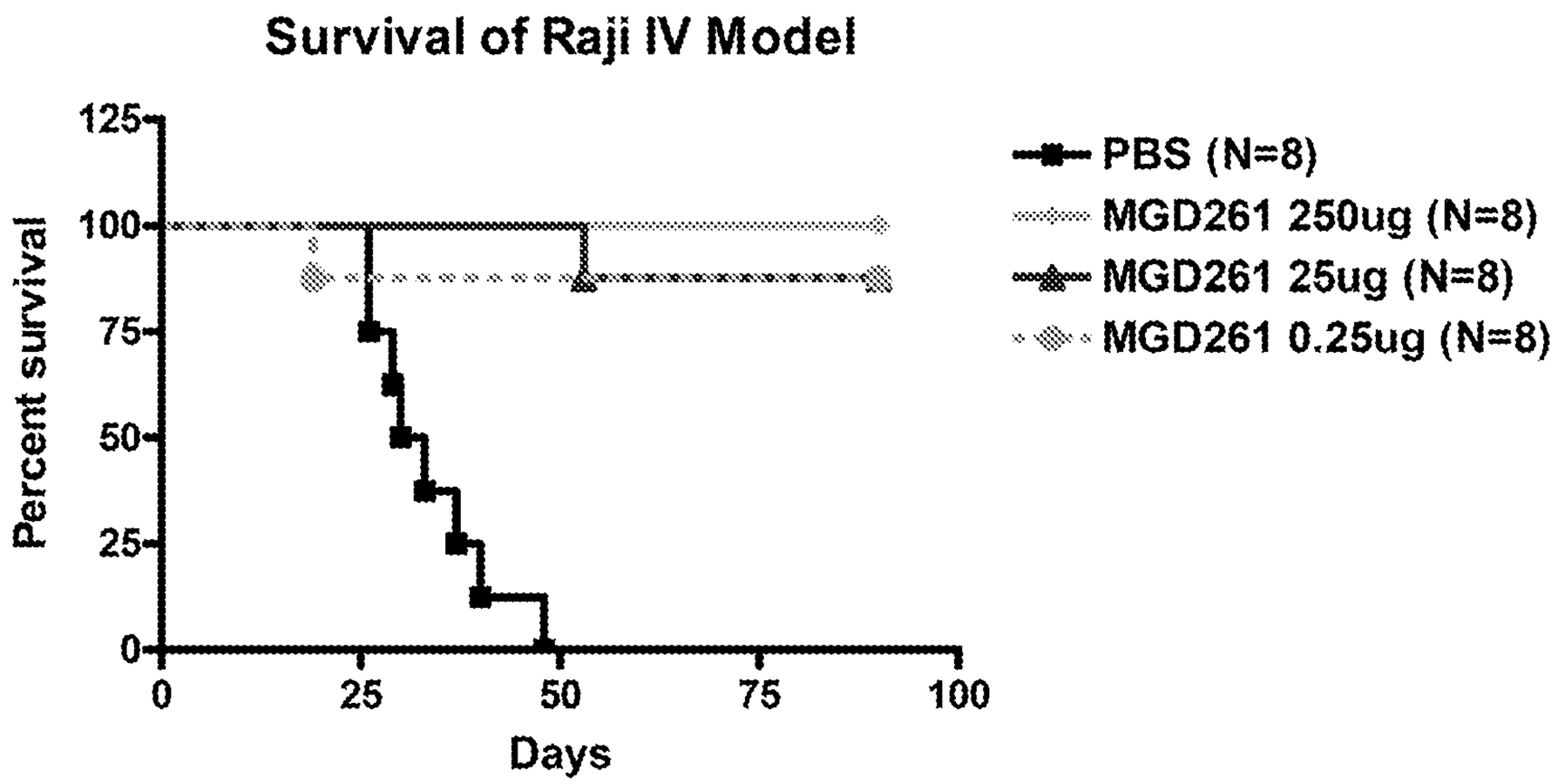

Survival of Raji IV Model
Percent survival
125
100
75
50
25
0
0
25
50
75
100
Days
PBS (N=8)
MGD261 250ug (N=8)
MGD261 25ug (N=8)
MGD261 0.25ug (N=8)

FIG. 26

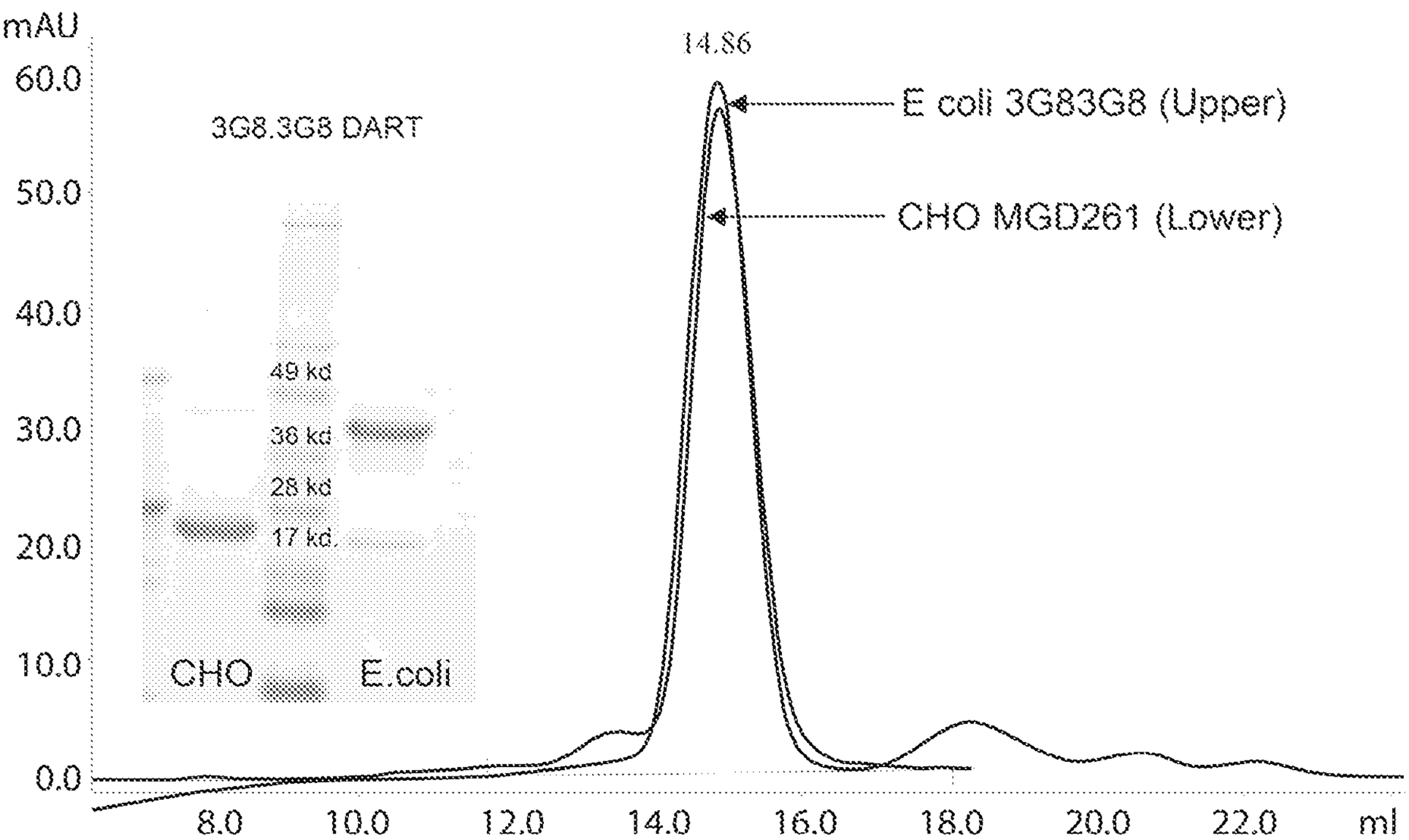

E coli expressed 3G8.3G8 DART
(SDS-PAGE, SEC)
mAU
14.86
60.0
50.0
40.0
30.0
20.0
10.0
0.0
3G8.3G8 DART
49 kd
38 kd
28 kd
17 kd.
CHO
E.coli
E coli 3G83G8 (Upper)
CHO MGD261 (Lower)
8.0
10.0
12.0
14.0
16.0
18.0
20.0
22.0
ml

FIG. 27

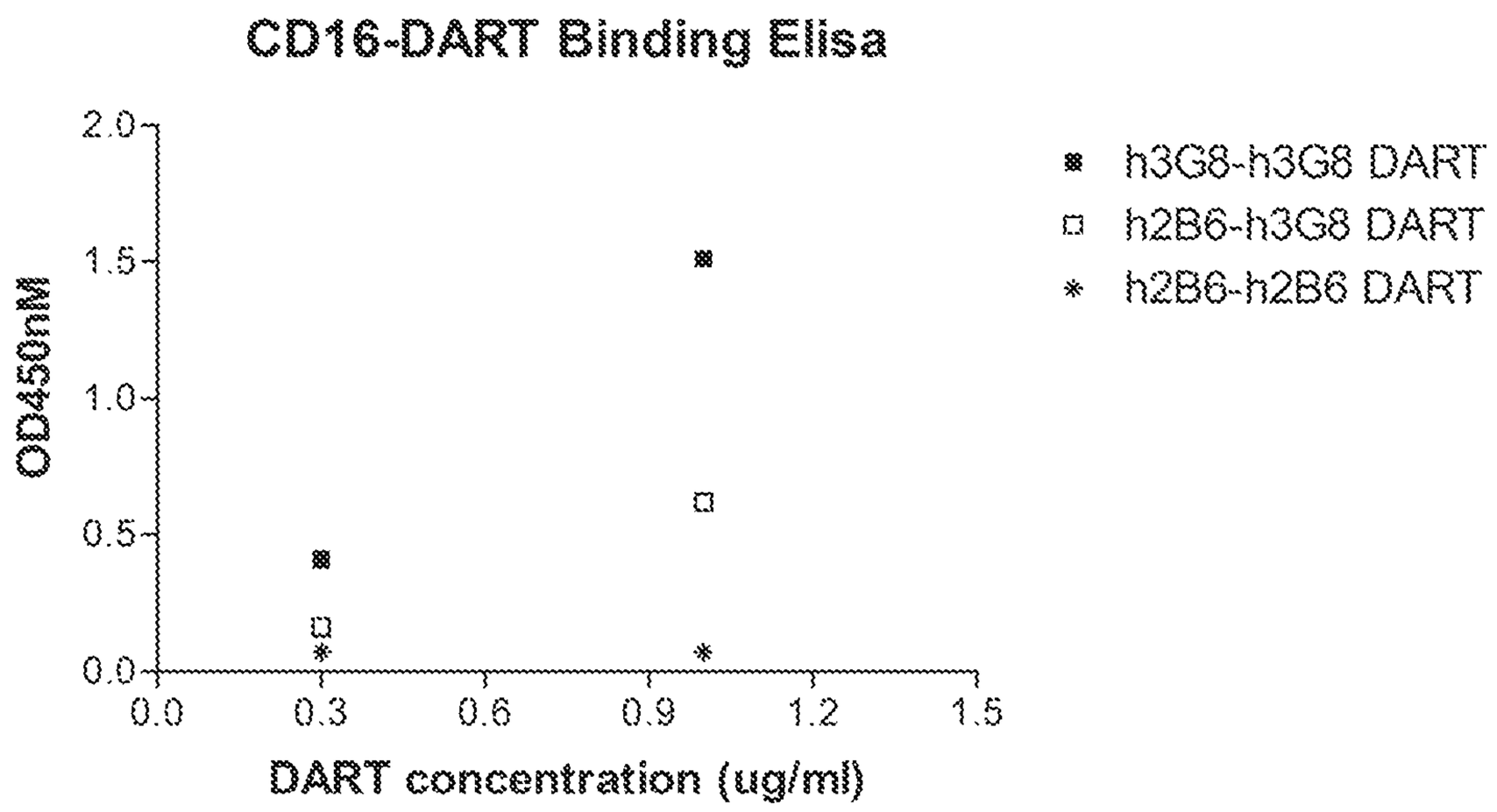

CD16-DART Binding Elisa
OD450nM
2.0
1.5
1.0
0.5
0.0
0.0
0.3
0.6
0.9
1.2
1.5
DART concentration (ug/ml)
h3G8-h3G8 DART
h2B6-h3G8 DART
h2B6-h2B6 DART

FIG. 28

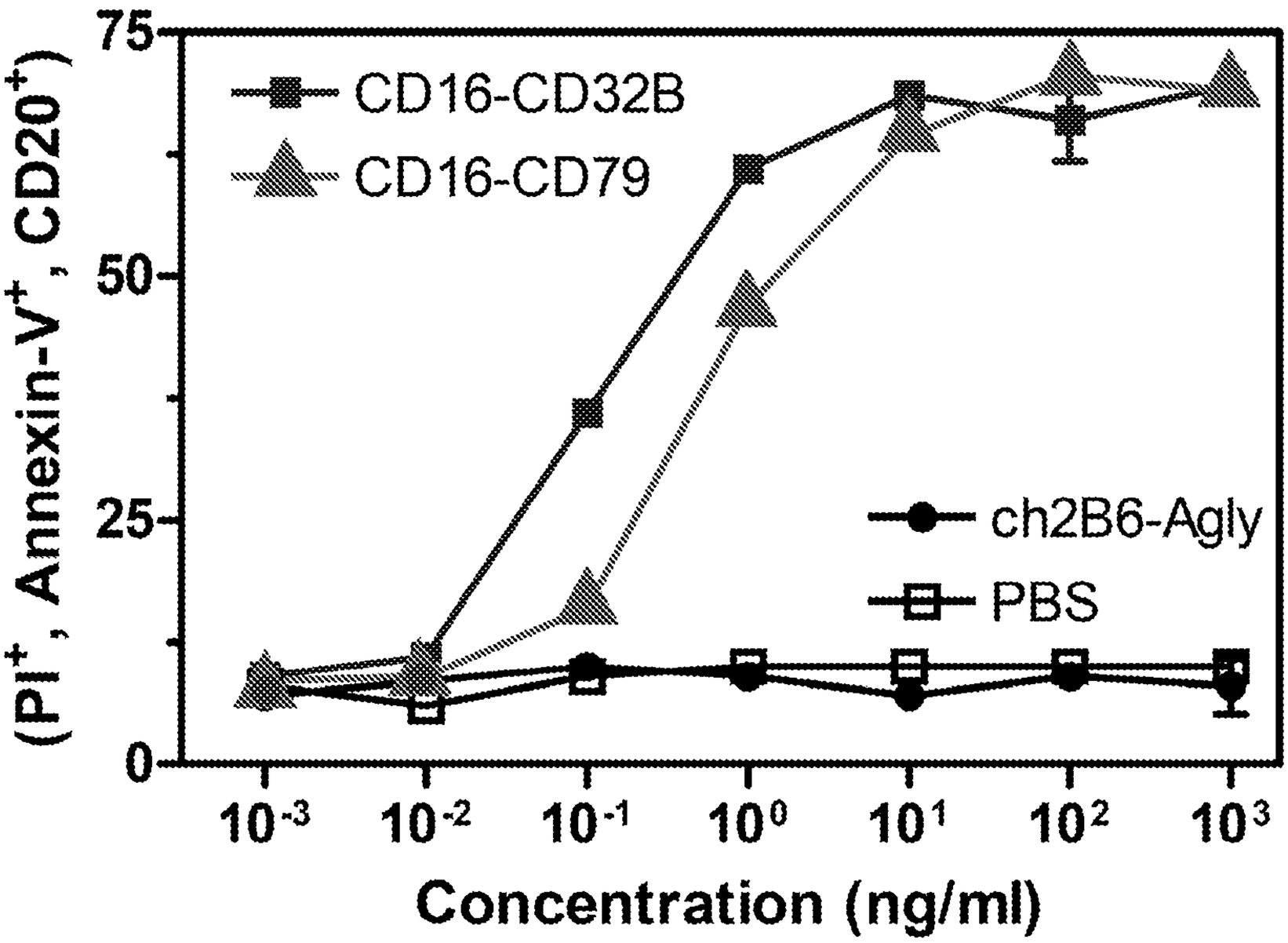

% Apoptotic B cells
(PI+, Annexin-V+, CD20+)
75
50
25
0
CD16-CD32B
CD16-CD79
ch2B6-Agly
PBS
10-3
10-2
10-1
100
101
102
103
Concentration (ng/ml)

FIG. 29

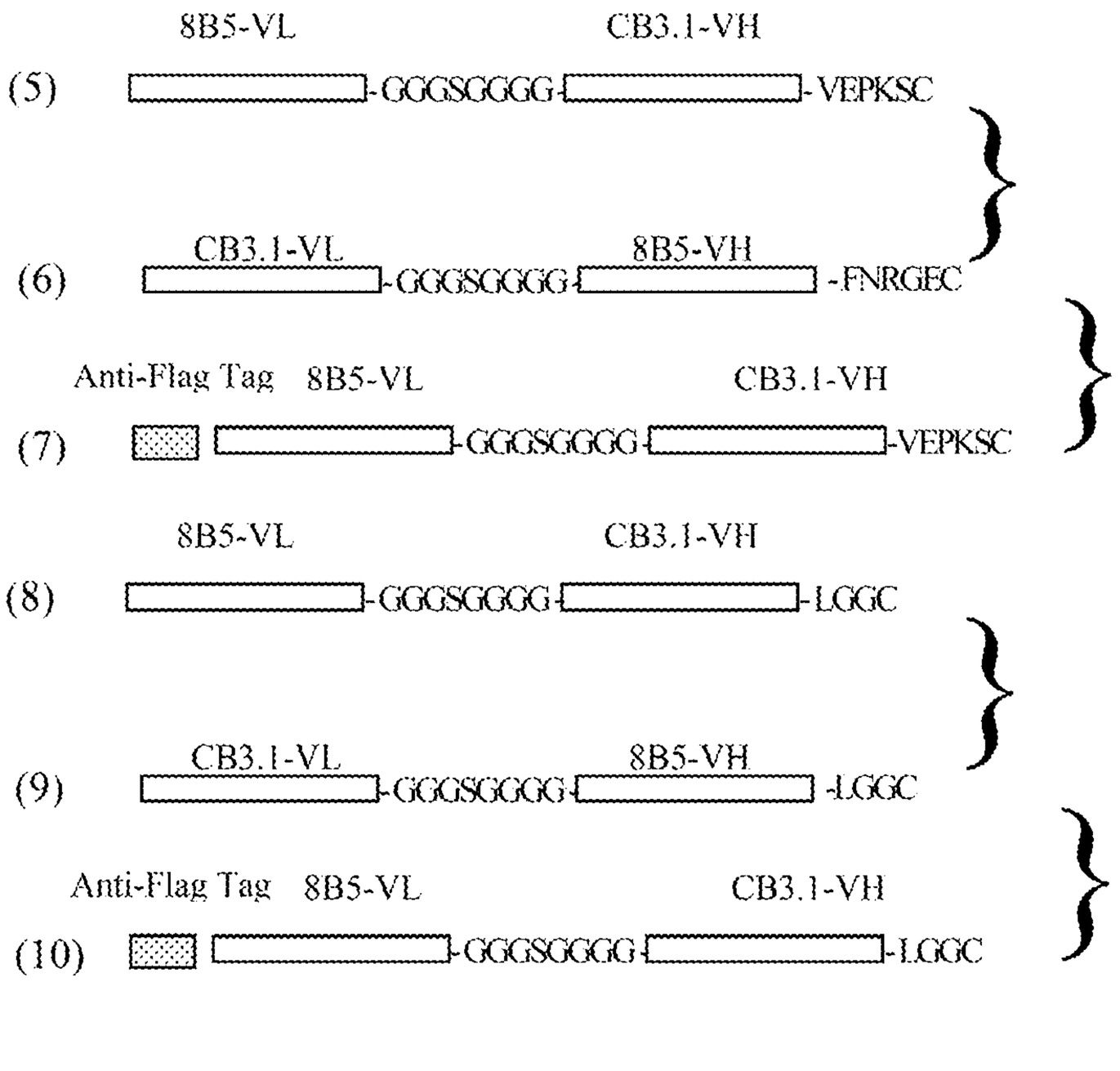

8B5-VL
CB3.1-VH
(5)
-GGGSGGGG-
-VEPKSC
CB3.1-VL
8B5-VH
(6)
-GGGSGGGG-
-FNRGEC
Anti-Flag Tag
8B5-VL
CB3.1-VH
(7)
-GGGSGGGG-
-VEPKSC
8B5-VL
CB3.1-VH
(8)
-GGGSGGGG-
-LGGC
CB3.1-VL
8B5-VH
(9)
-GGGSGGGG-
-LGGC
Anti-Flag Tag
8B5-VL
CB3.1-VH
(10)
-GGGSGGGG-
-LGGC

FIG. 30

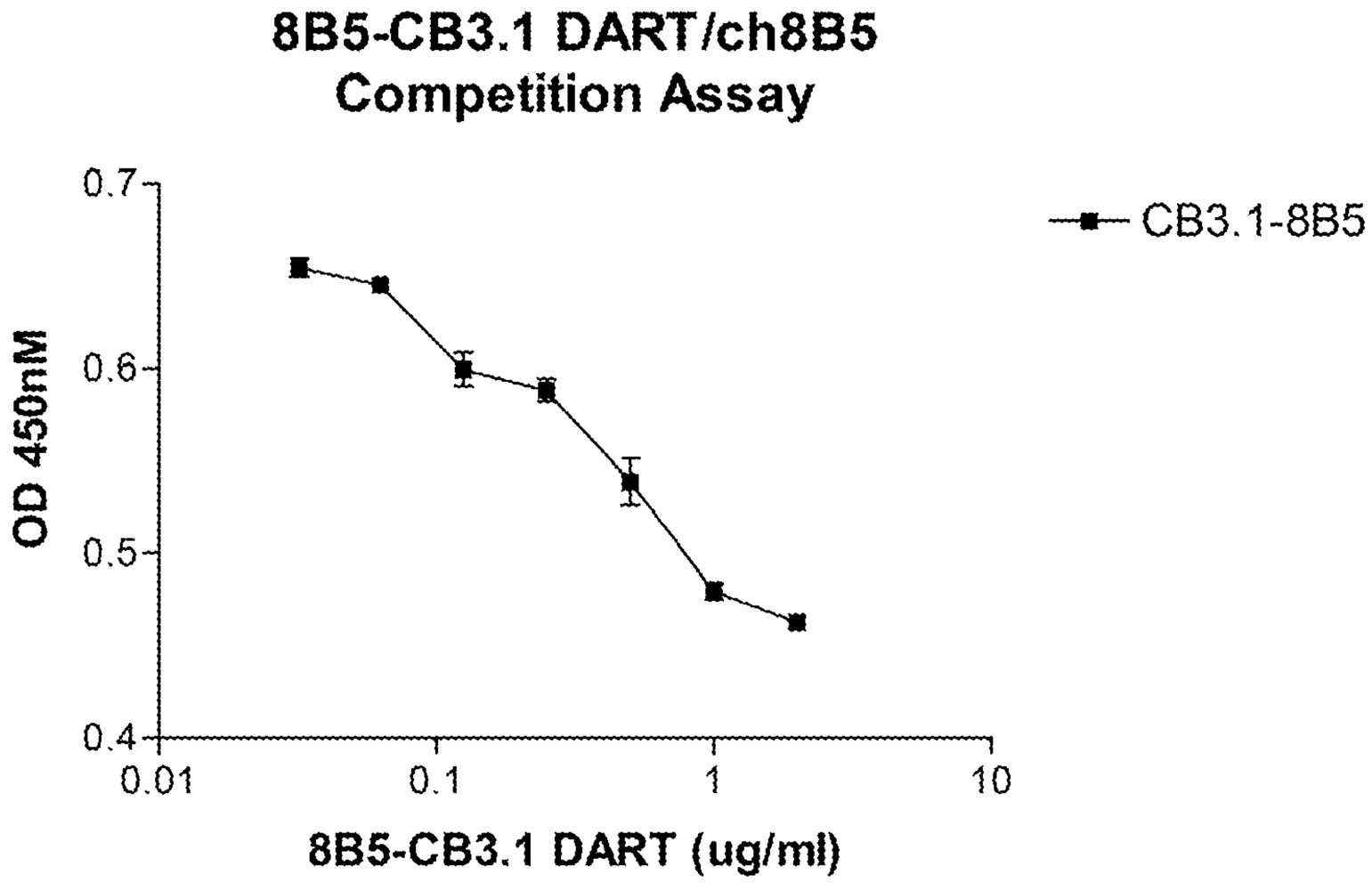

8B5-CB3.1 DART/ch8B5
Competition Assay
OD 450nM
0.7
0.6
0.5
0.4
0.01
0.1
1
10
8B5-CB3.1 DART (ug/ml)
CB3.1-8B5

FIG. 31

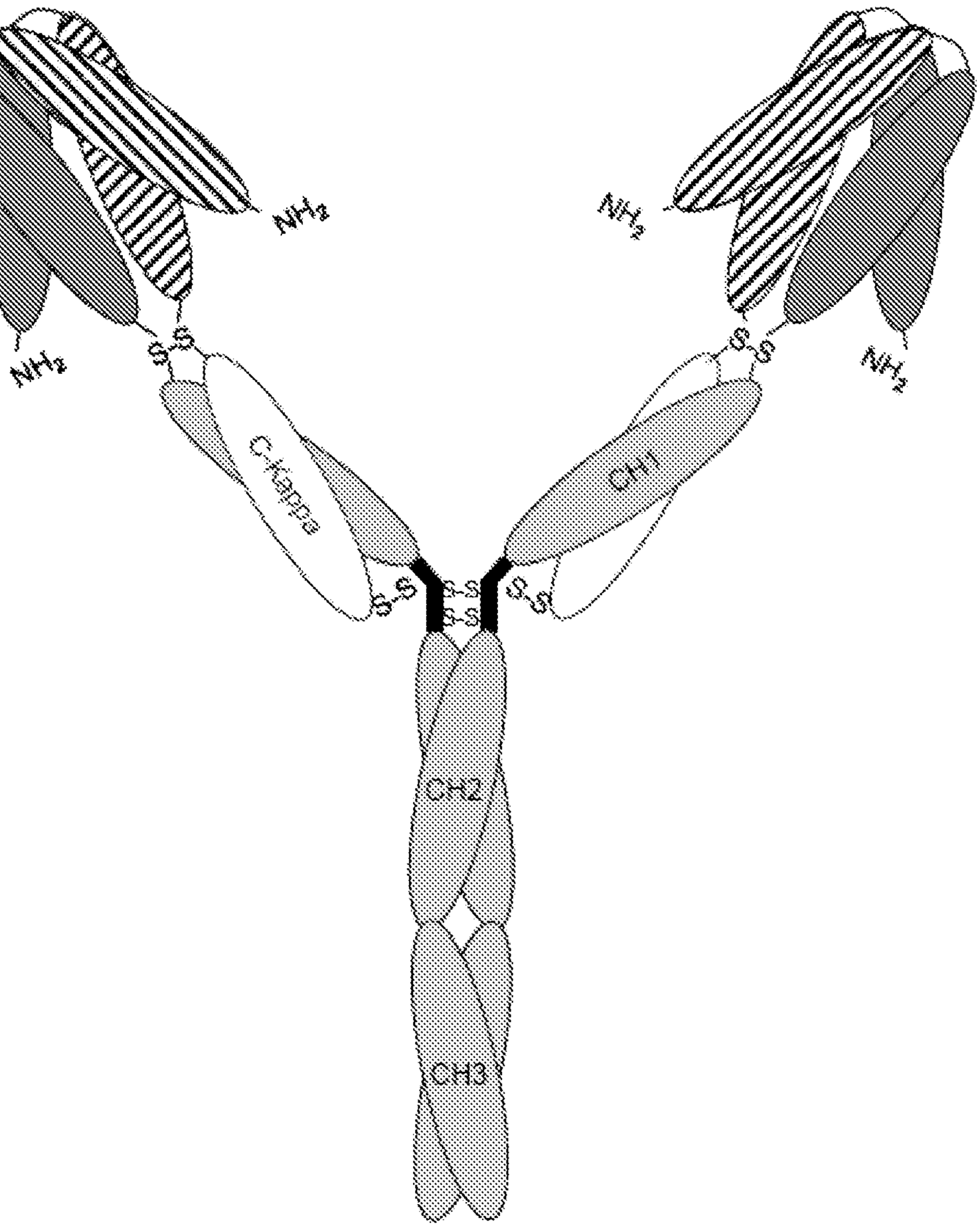
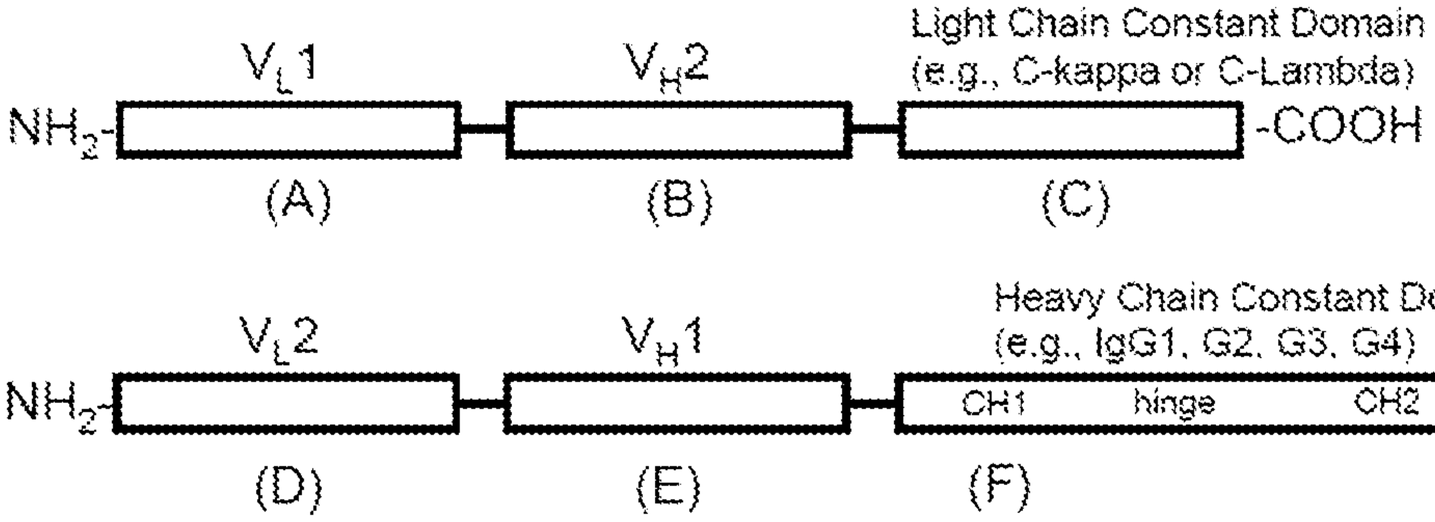


FIG. 32

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

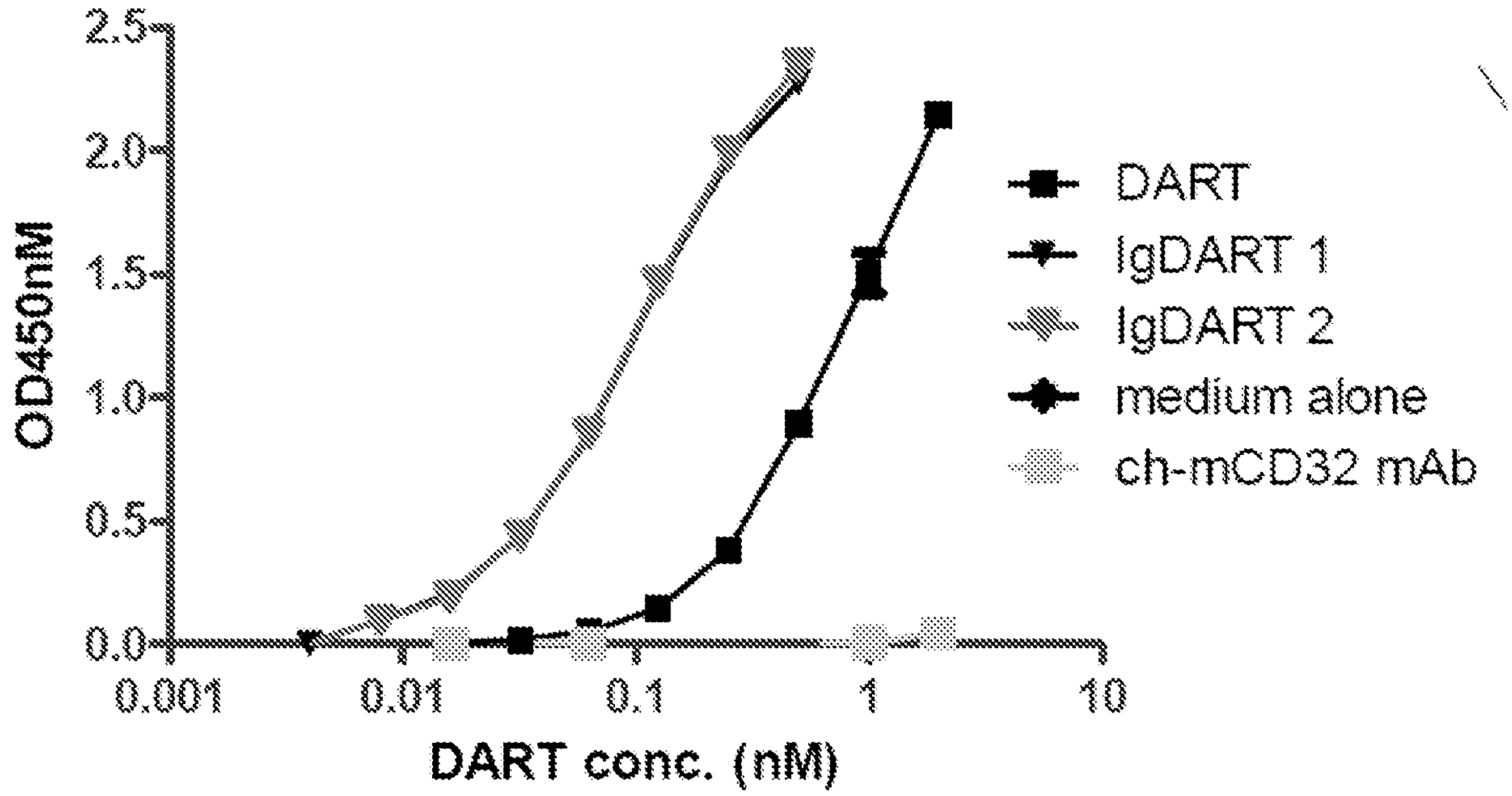


FIG. 34

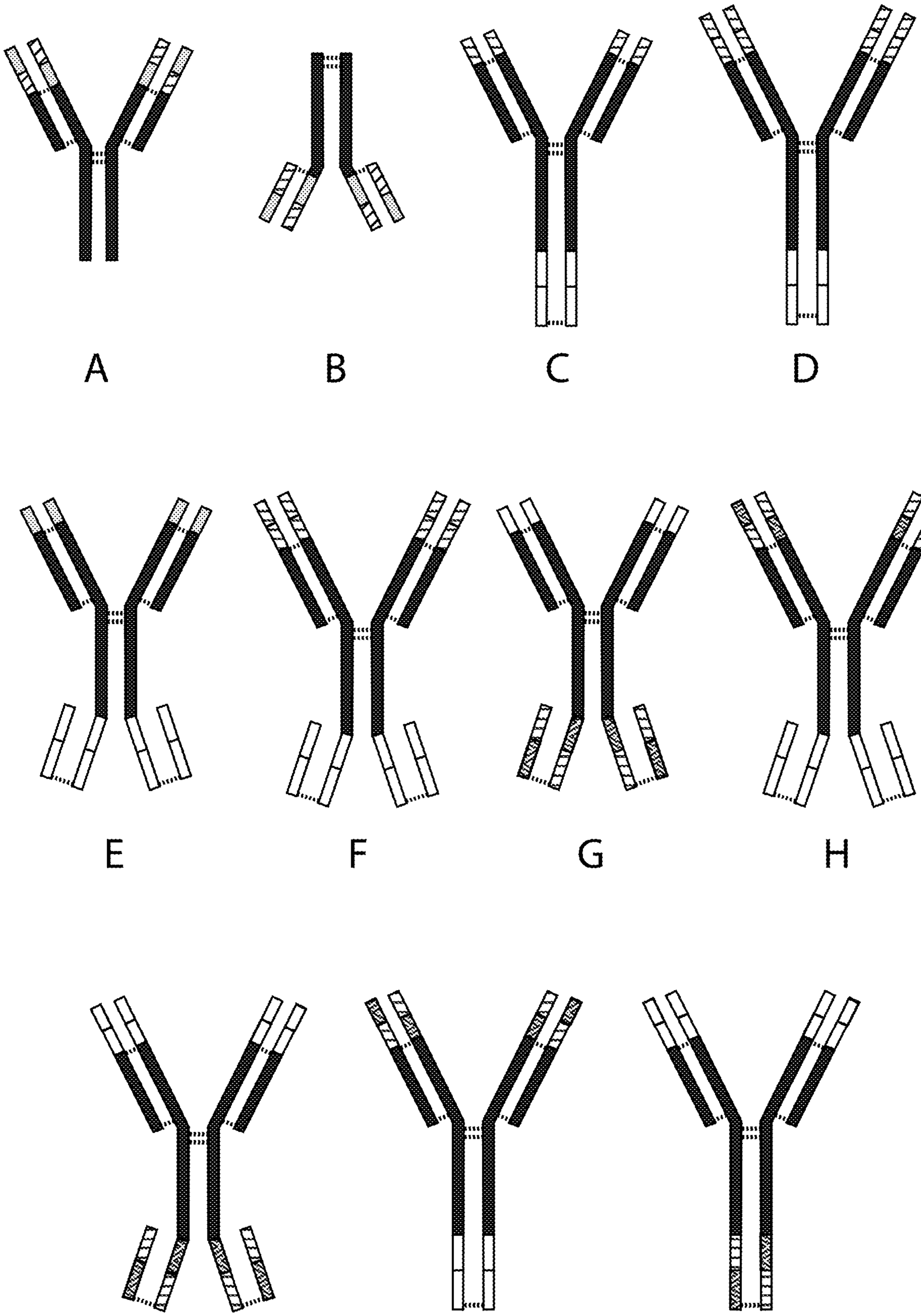


FIG. 35

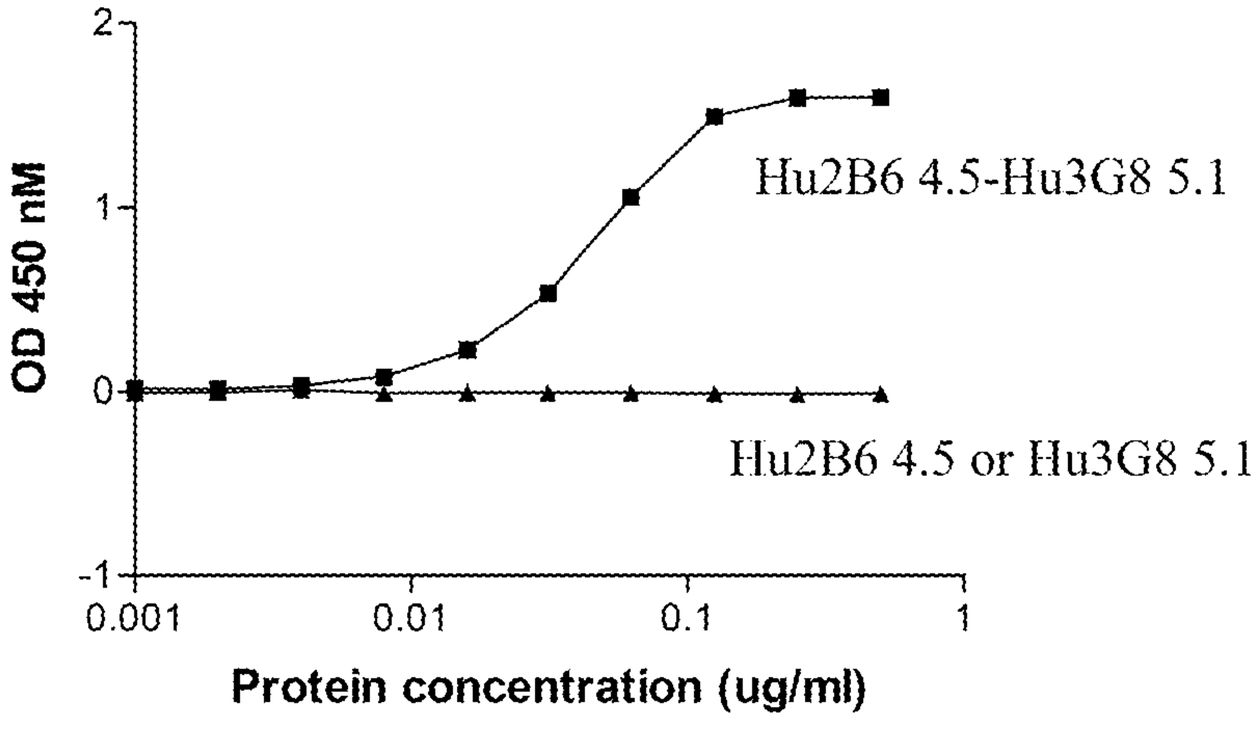

OD 450 nM
2
1
0
-1
0.001
0.01
0.1
1
Protein concentration (ug/ml)
Hu2B6 4.5-Hu3G8 5.1
Hu2B6 4.5 or Hu3G8 5.1

FIG. 36

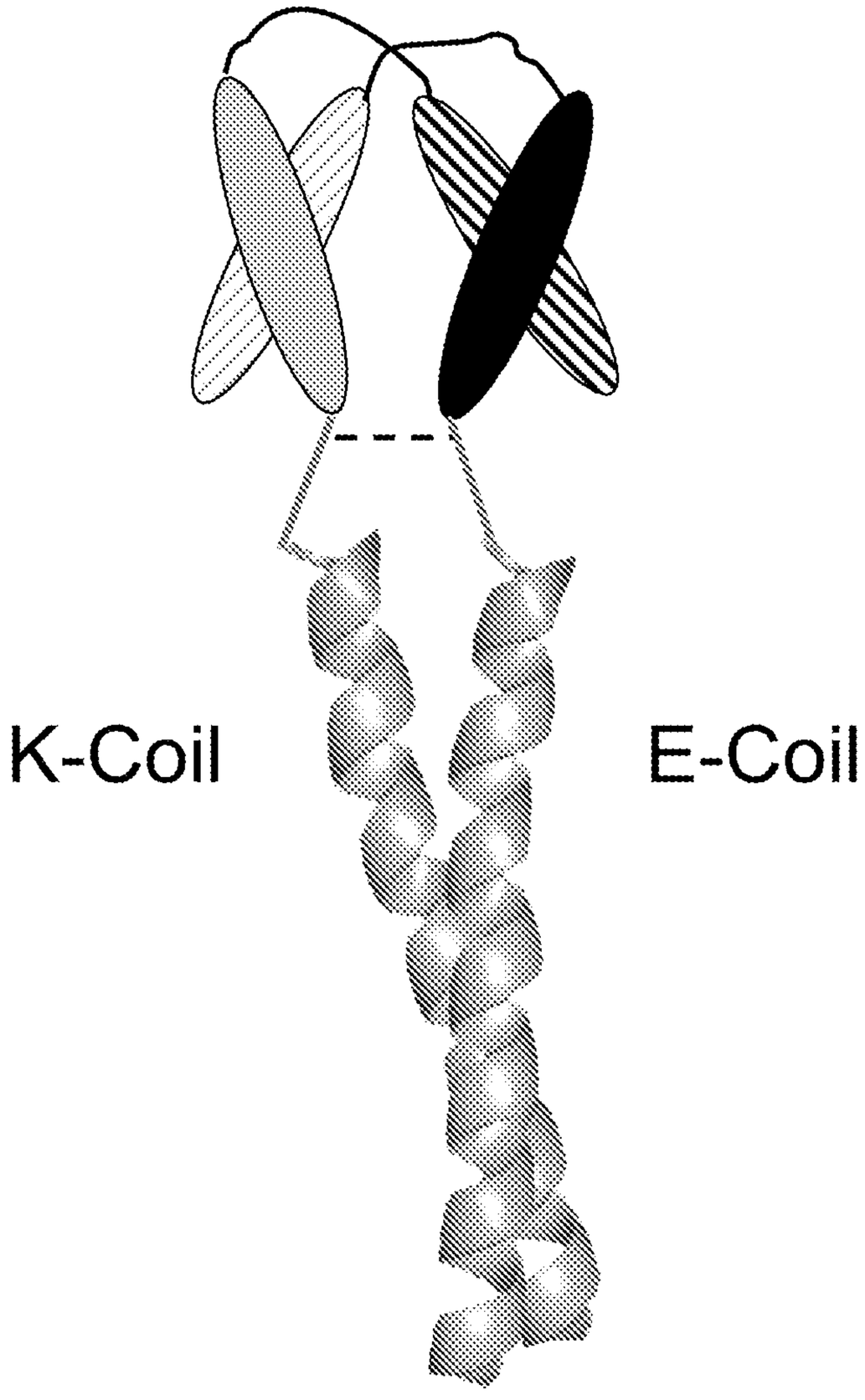


FIG. 37

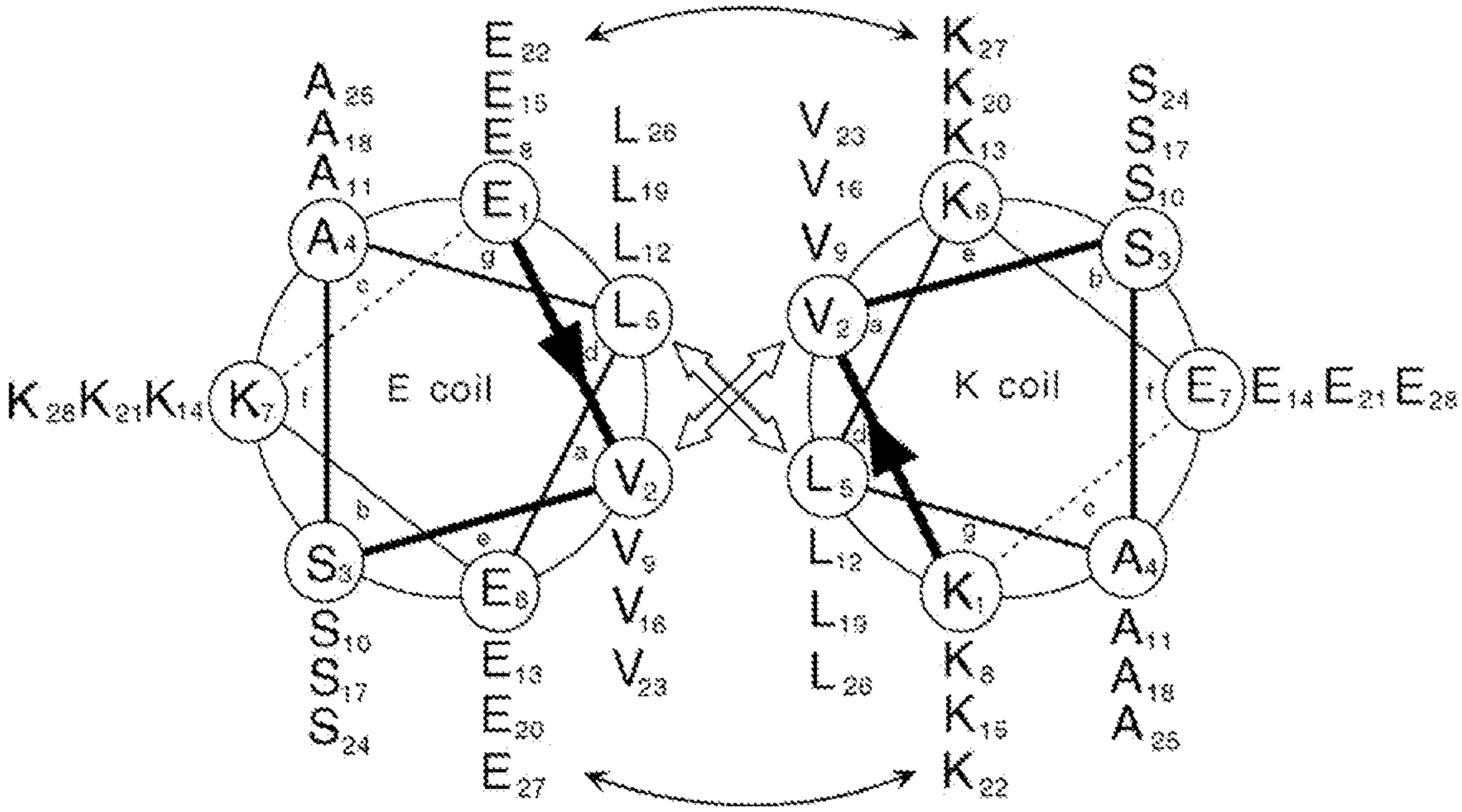


FIG. 38

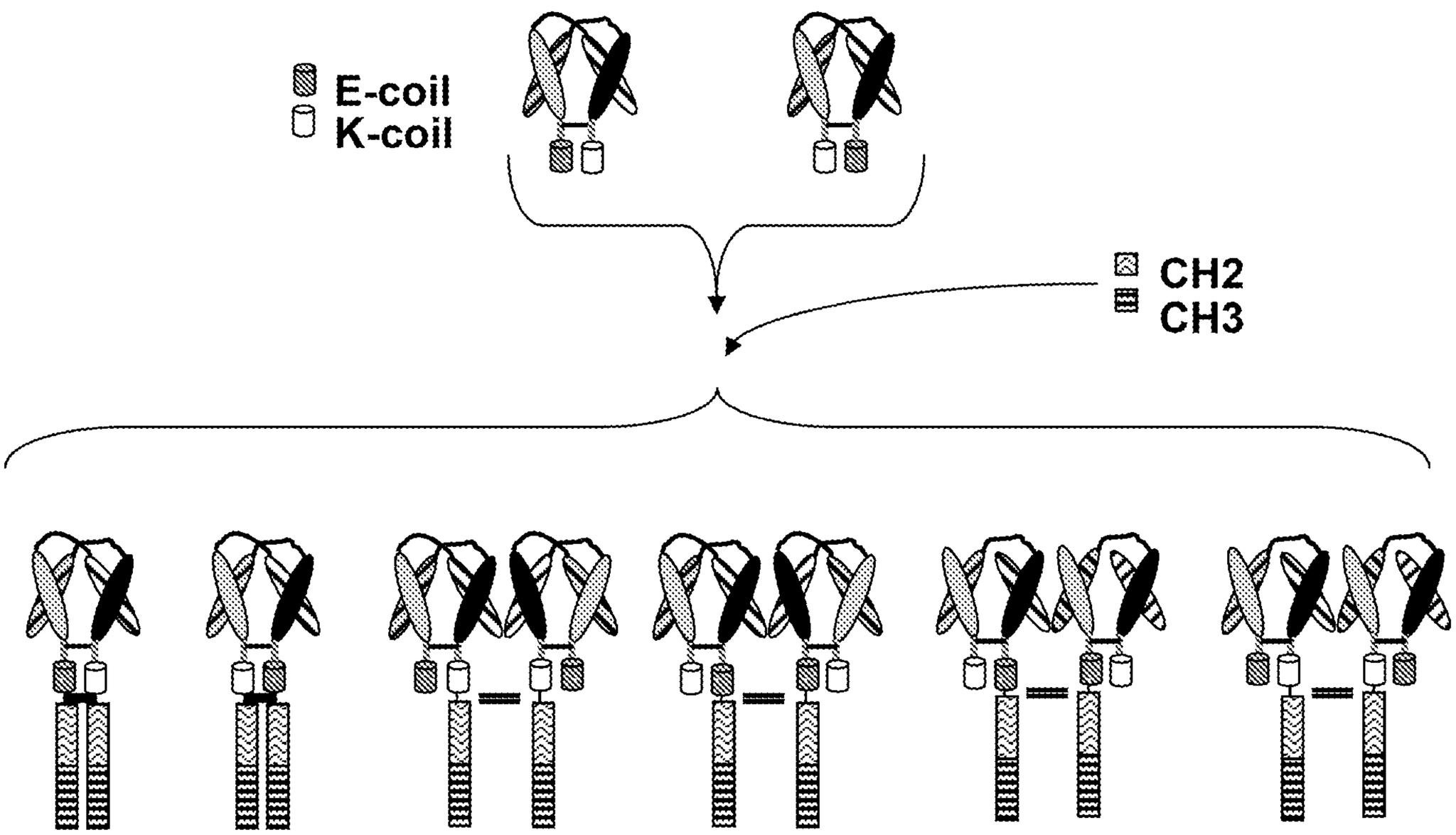


Figure 39

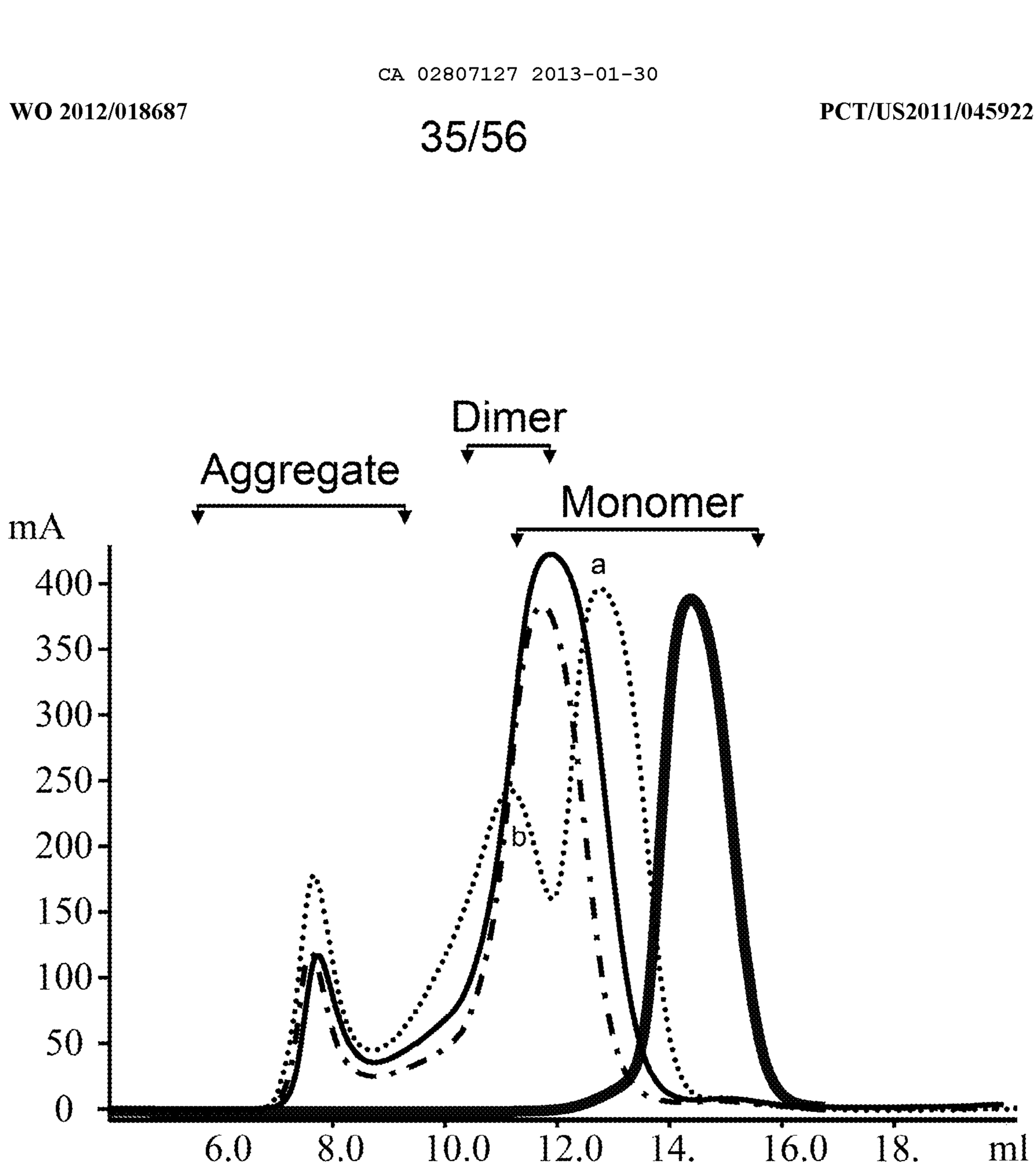

Dimer
Aggregate
Monomer
mA
400
350
300
250
200
150
100
50
0
a
b
6.0
8.0
10.0
12.0
14.
16.0
18.
ml
EK
EFc/K
EFc/KFc
E/KFc

Figure 40

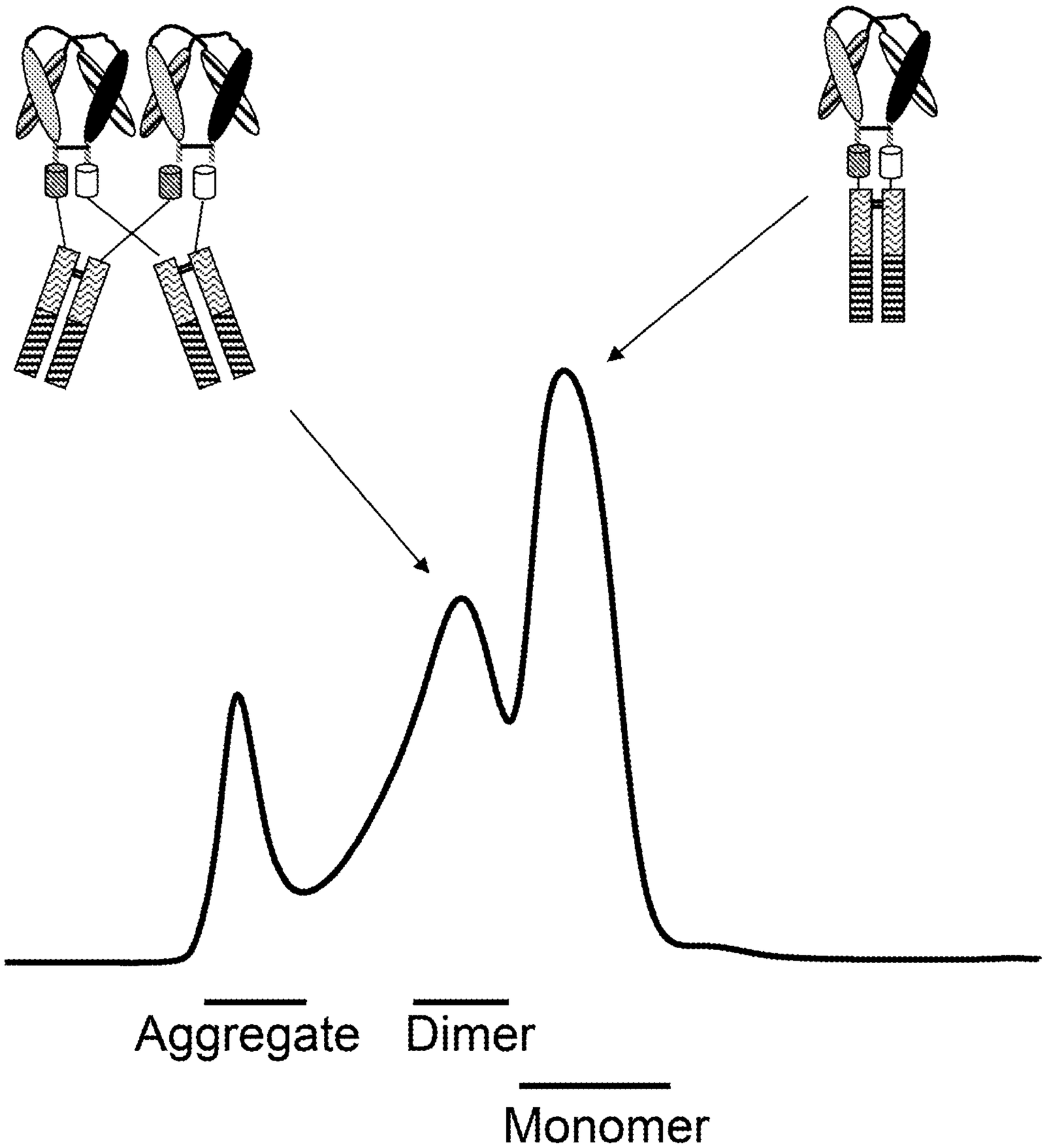


Figure 41

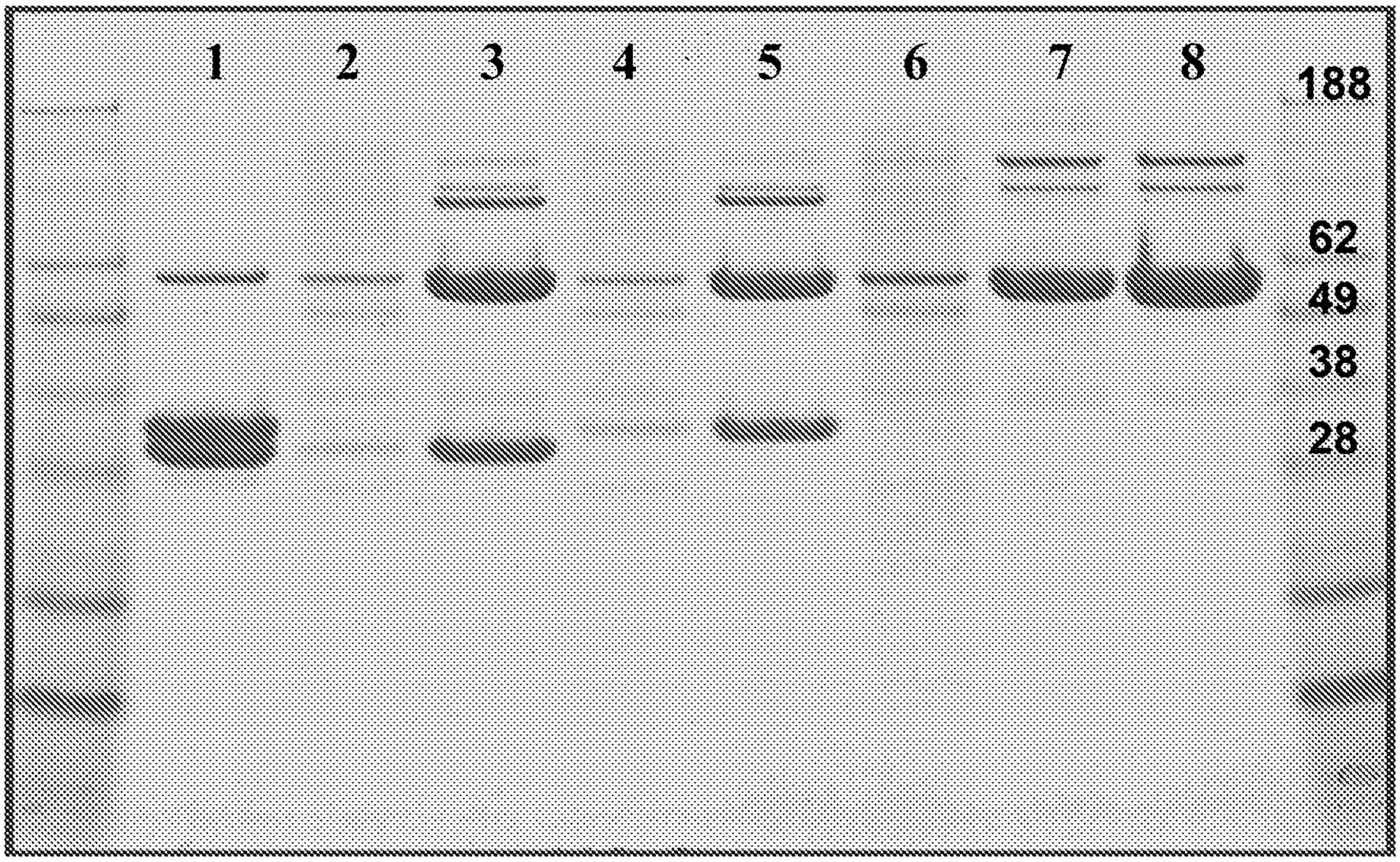


Figure 42

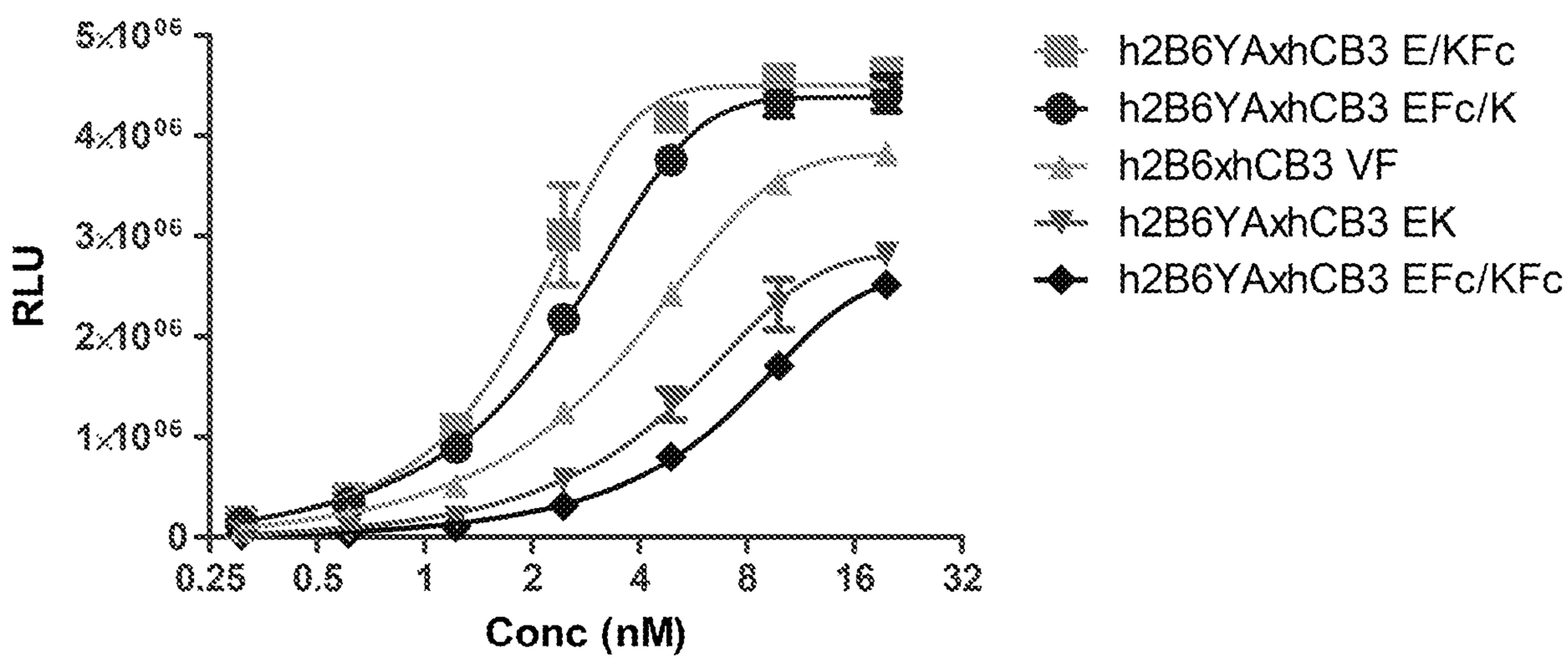

RLU
5x10^06
4x10^06
3x10^06
2x10^06
1x10^06
0
0.25
0.5
1
2
4
8
16
32
Conc (nM)
h2B6YAxhCB3 E/KFc
h2B6YAxhCB3 EFc/K
h2B6xhCB3 VF
h2B6YAxhCB3 EK
h2B6YAxhCB3 EFc/KFc

Figure 43

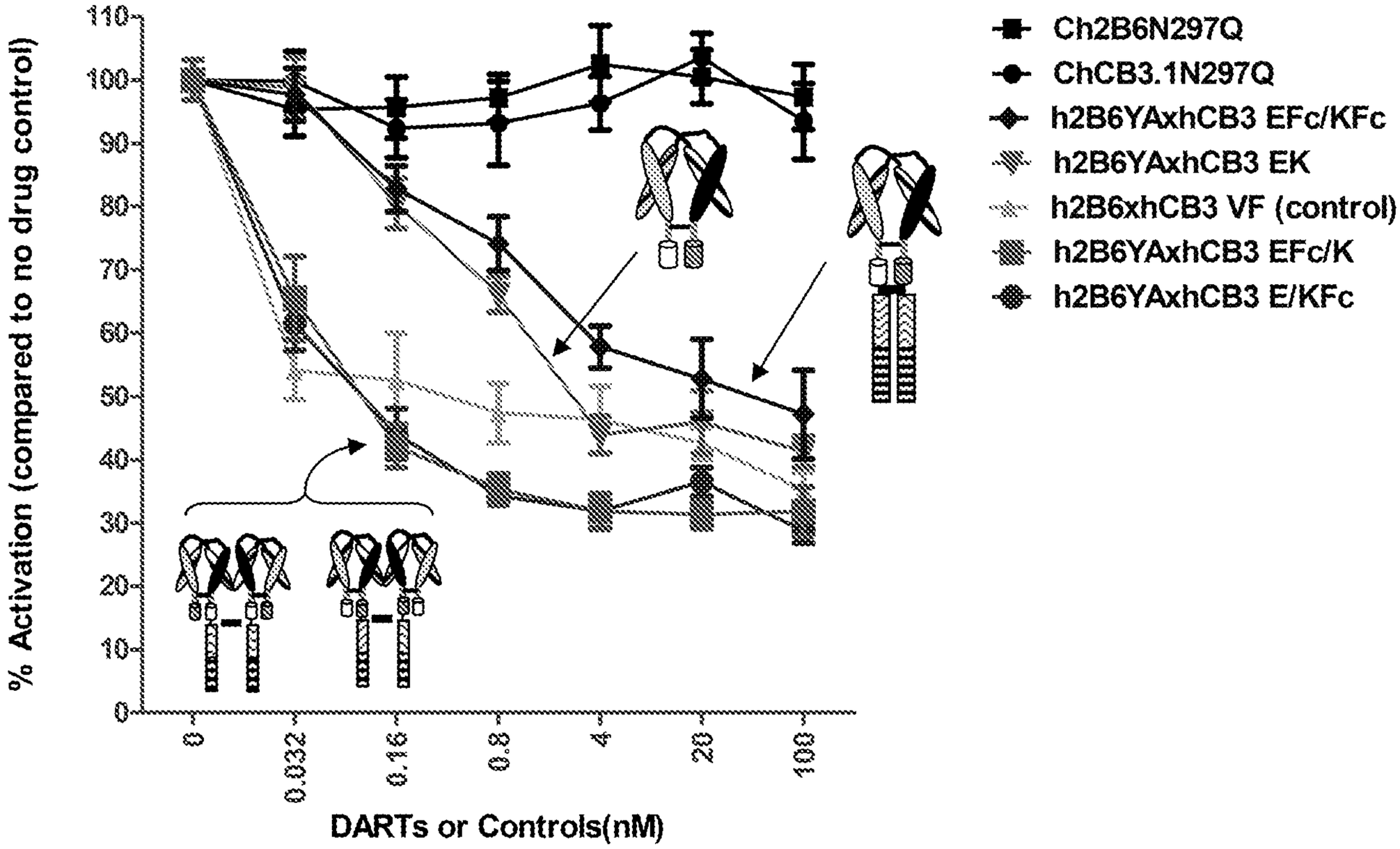

% Activation (compared to no drug control)
110
100
90
80
70
60
50
40
30
20
10
0
Ch2B6N297Q
ChCB3.1N297Q
h2B6YAxhCB3 EFc/KFc
h2B6YAxhCB3 EK
h2B6xhCB3 VF (control)
h2B6YAxhCB3 EFc/K
h2B6YAxhCB3 E/KFc
0
0.032
0.16
0.8
4
20
100
DARTs or Controls(nM)

Figure 44

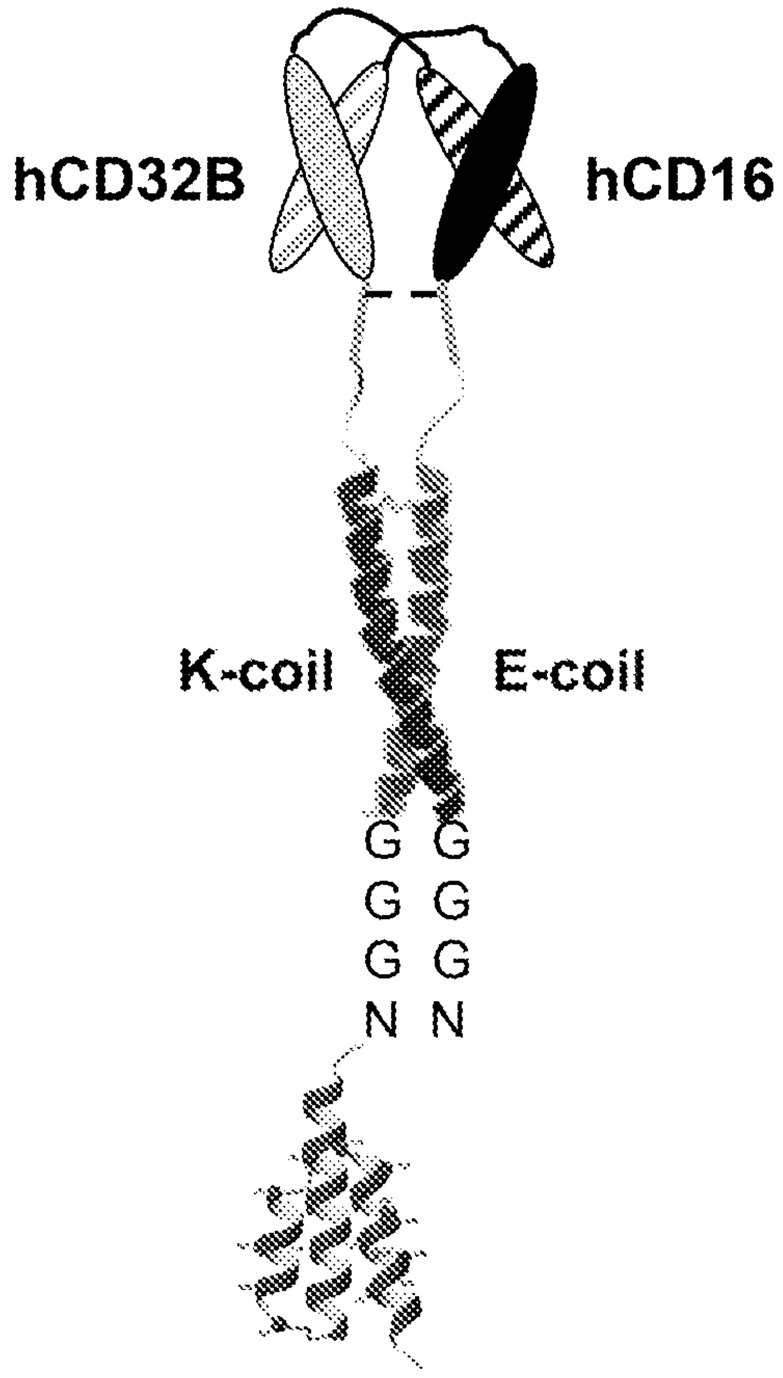


FIG. 45

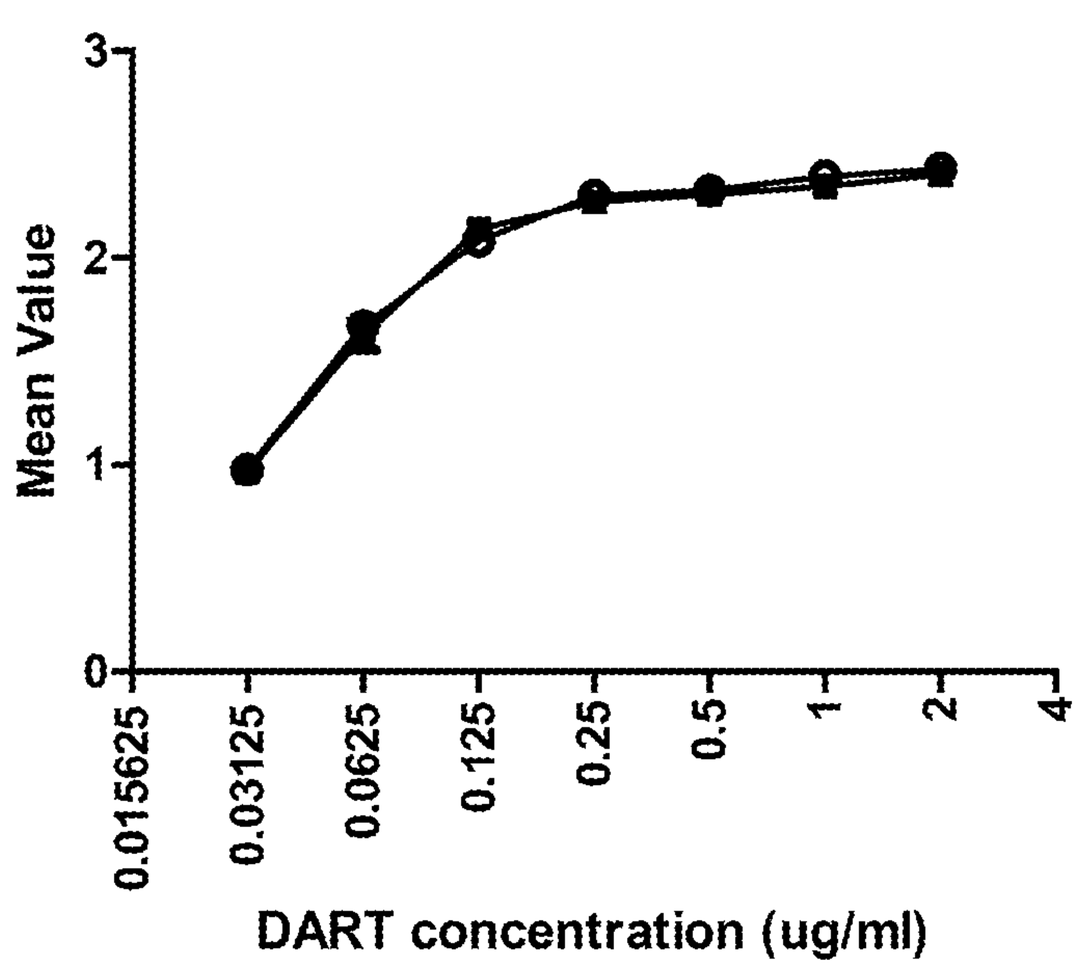


FIG. 46A

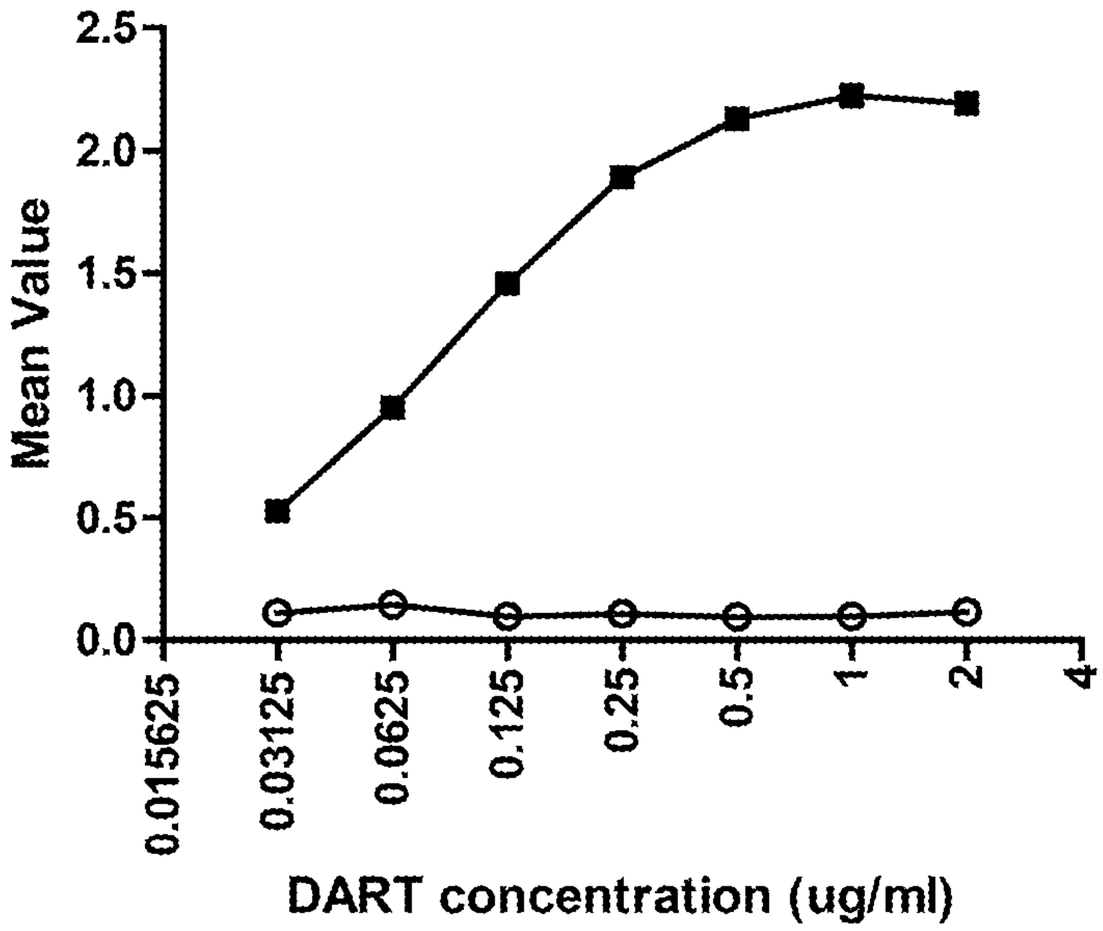


FIG. 46B

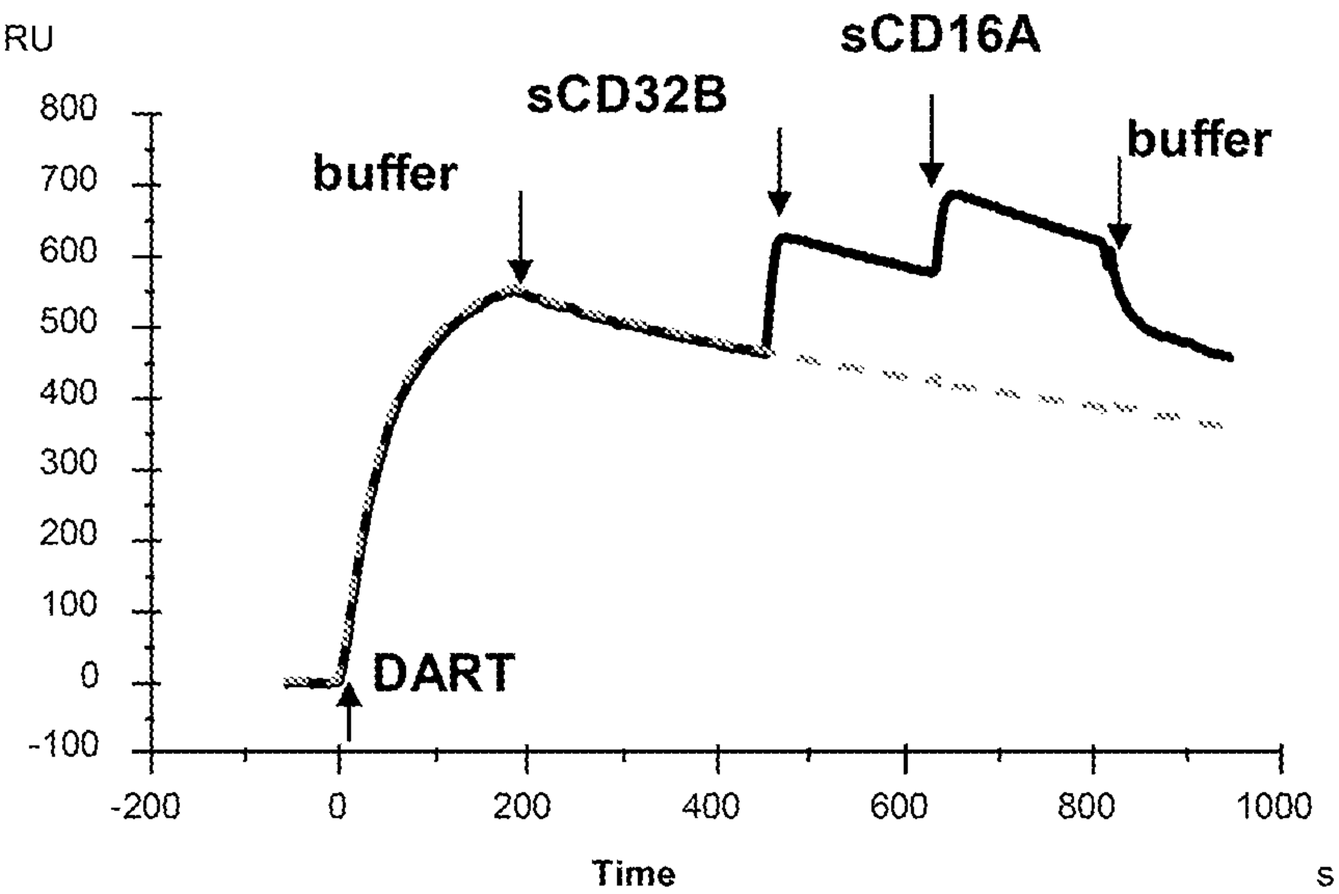
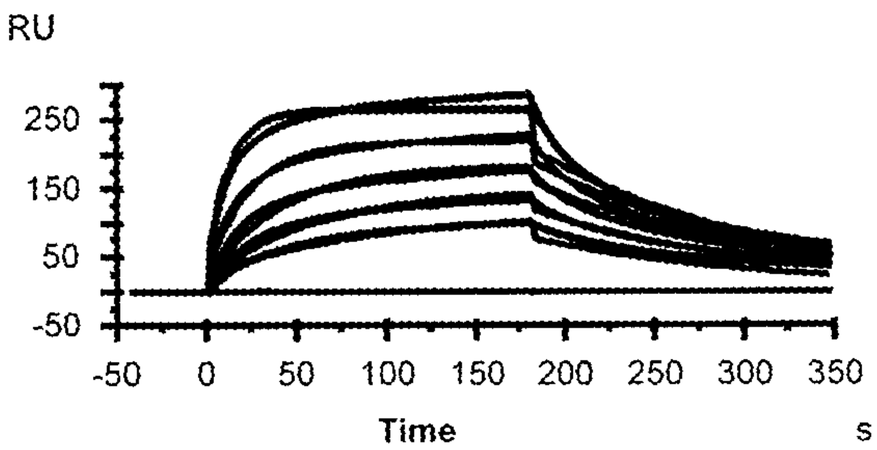

RU
800
700
600
500
400
300
200
100
0
-100
-200
0
200
400
600
800
1000
Time
s
buffer
sCD32B
sCD16A
buffer
DART

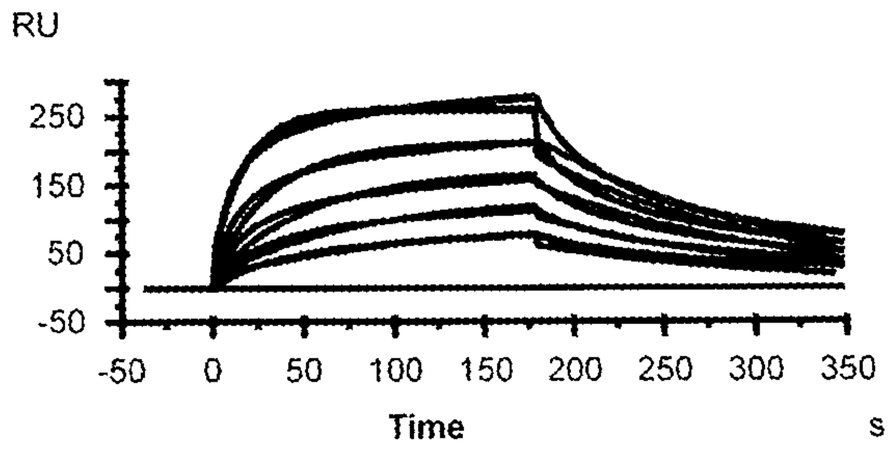
FIG. 46C

FIG. 46D
DART



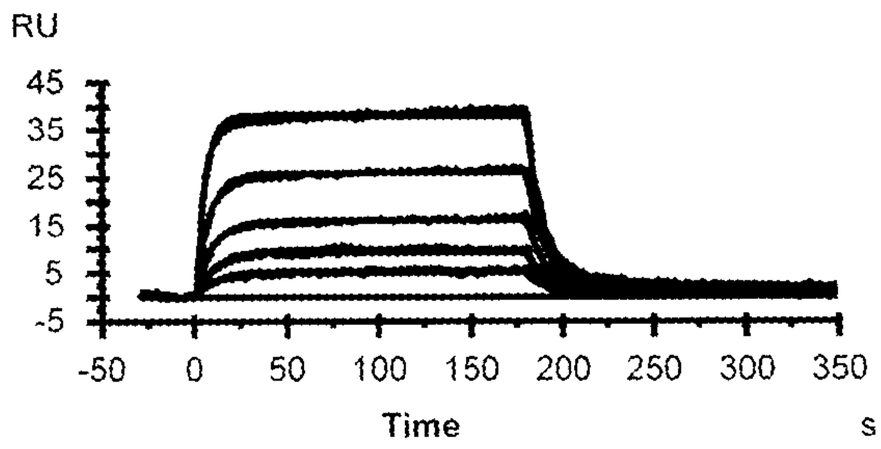
sCD32B
ka1/Ms 3.7e5 kd,1/s 7.4e-3
Kd,nM=20

FIG. 46E
ABD-DART



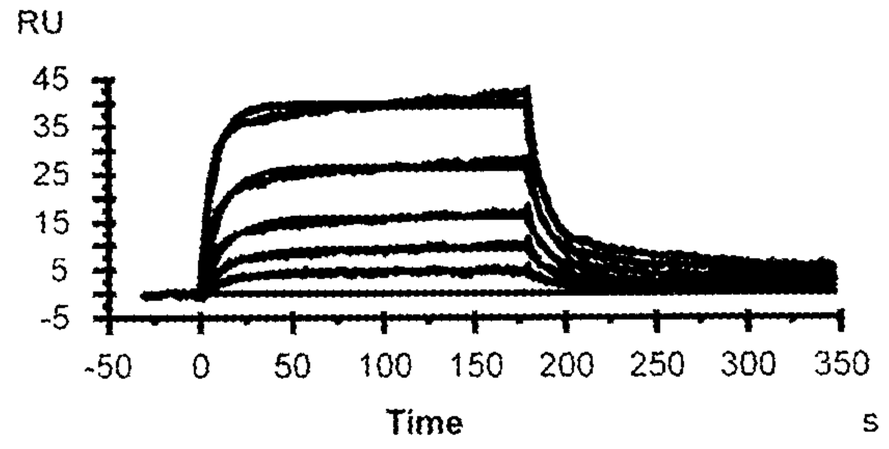
sCD32B
ka1/Ms 2.4e5 kd,1/s 7.2e-3
Kd,nM=30

FIG. 46F
DART



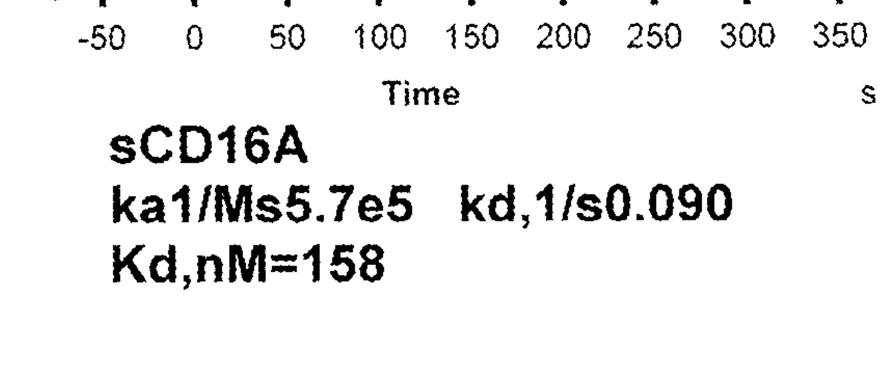
sCD16A
ka1/Ms5.7e5 kd,1/s0.090
Kd,nM=158

FIG. 46G
ABD-DART



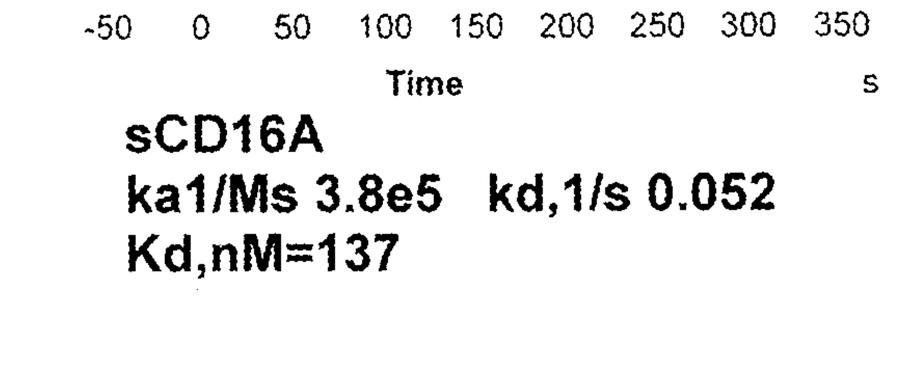
sCD16A
ka1/Ms 3.8e5 kd,1/s 0.052
Kd,nM=137

FIG. 46H
DART



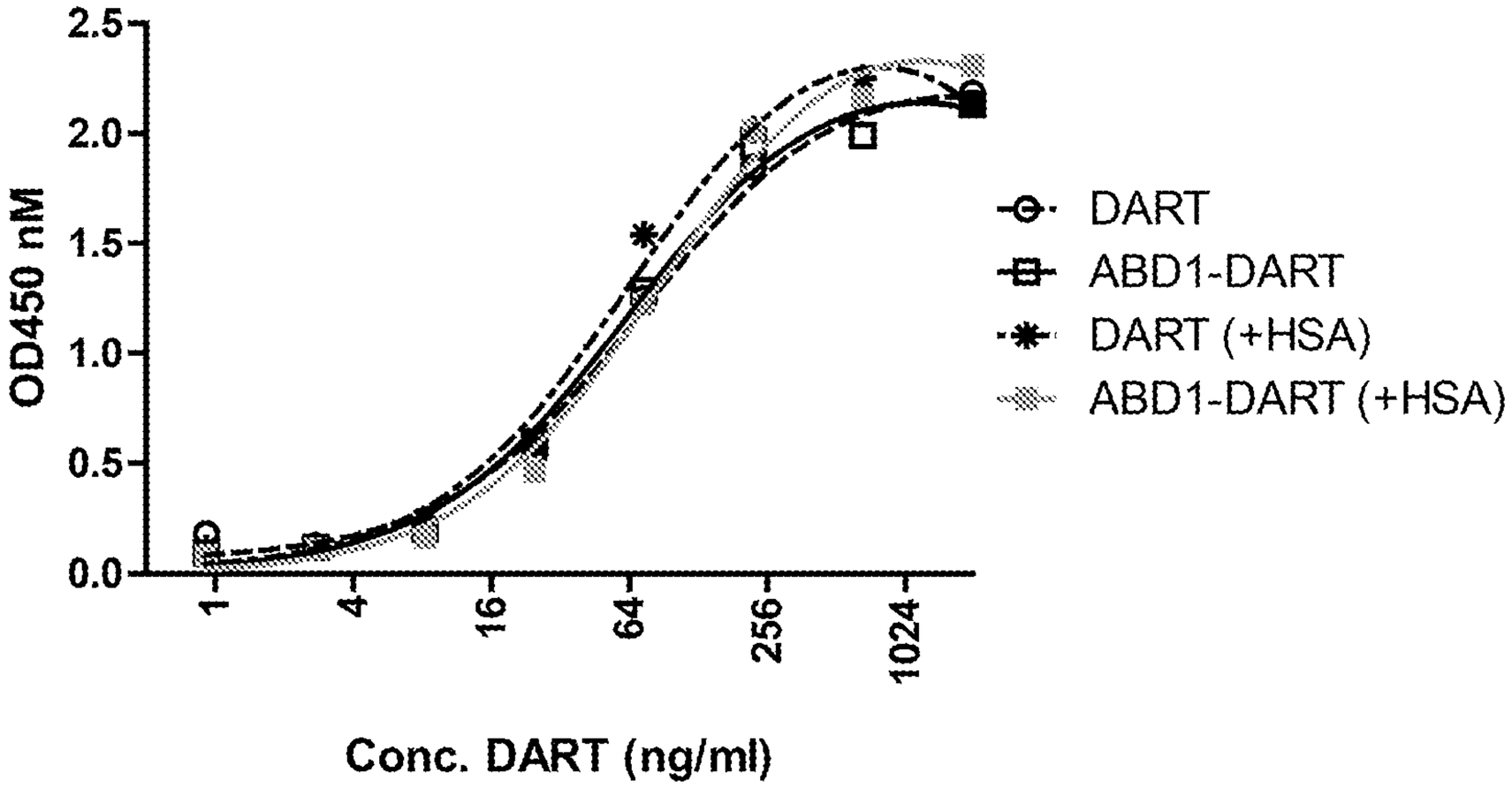
HSA

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)

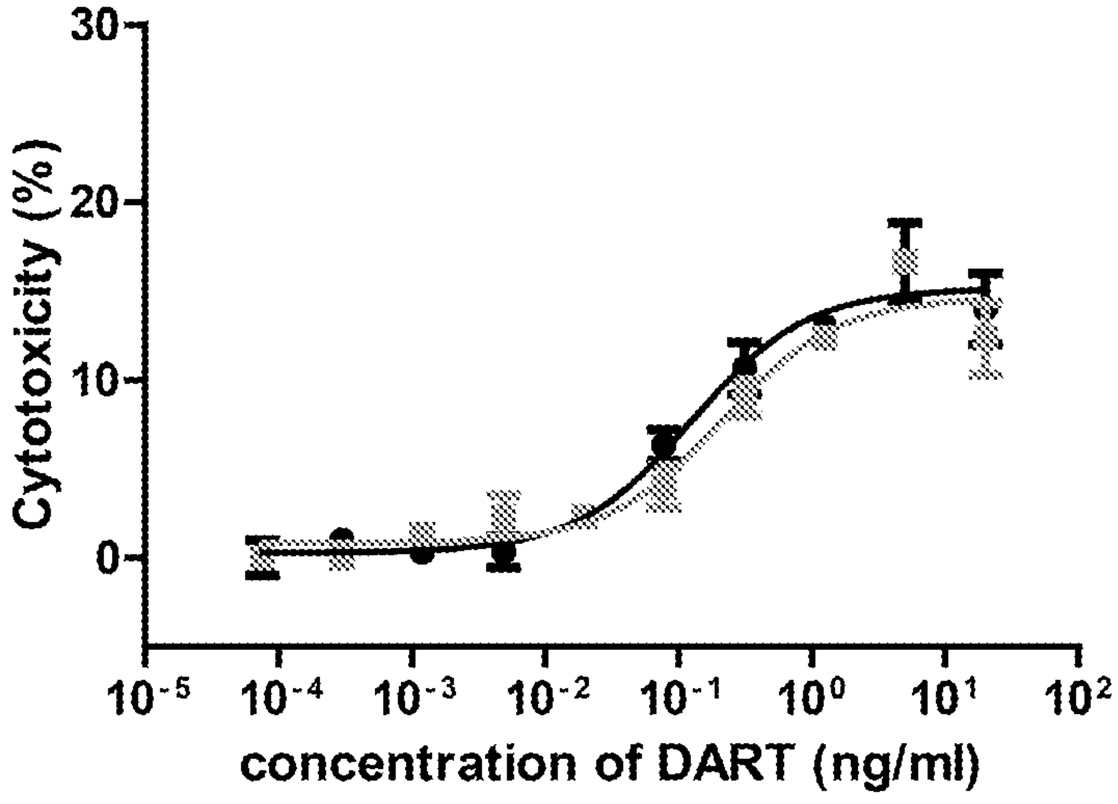


FIG. 47A

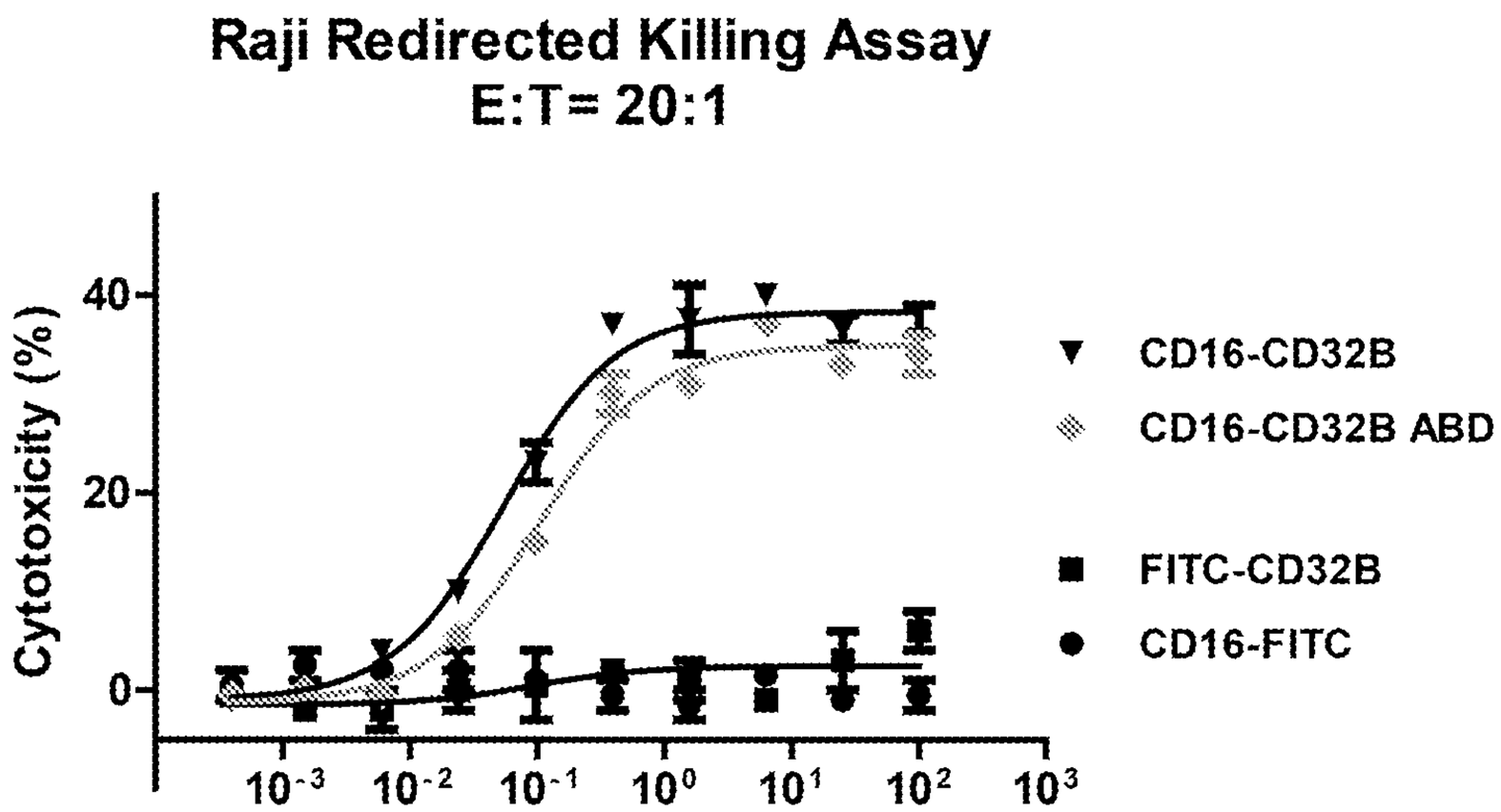


FIG. 47B

FIG. 48

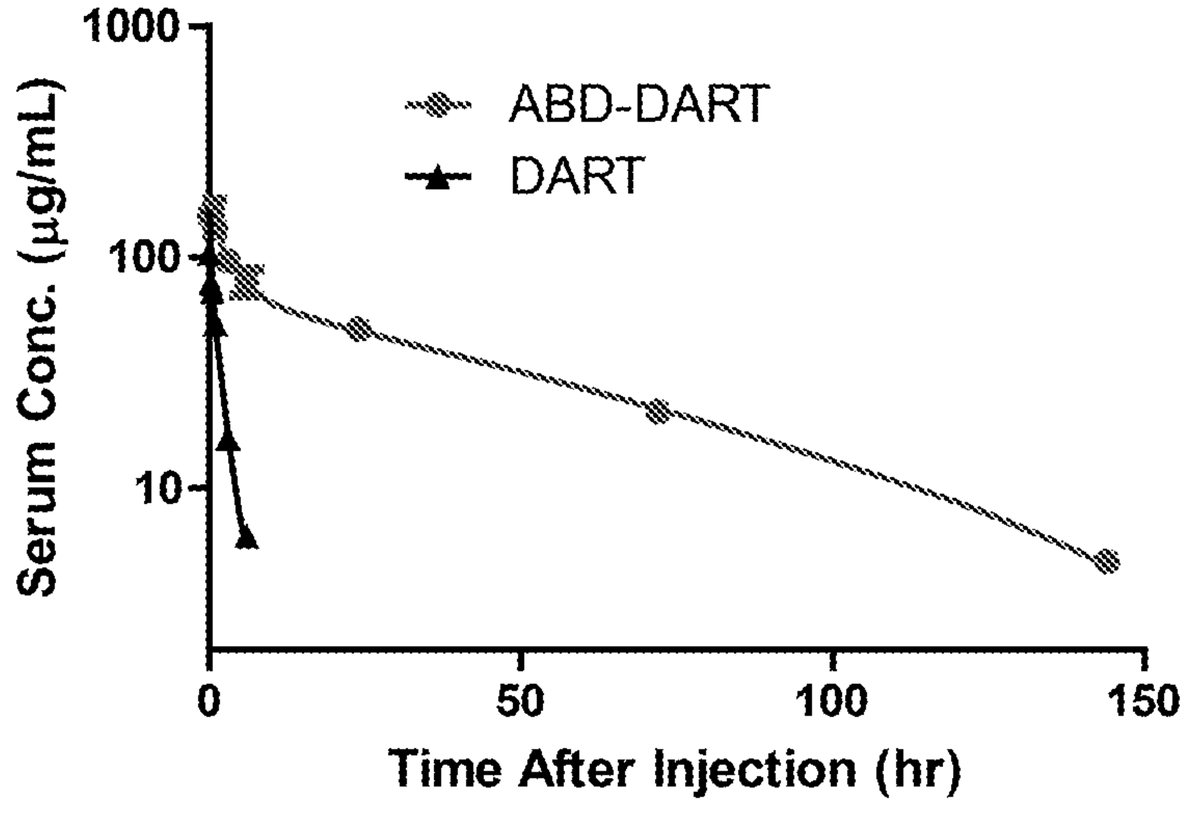


FIG. 49A

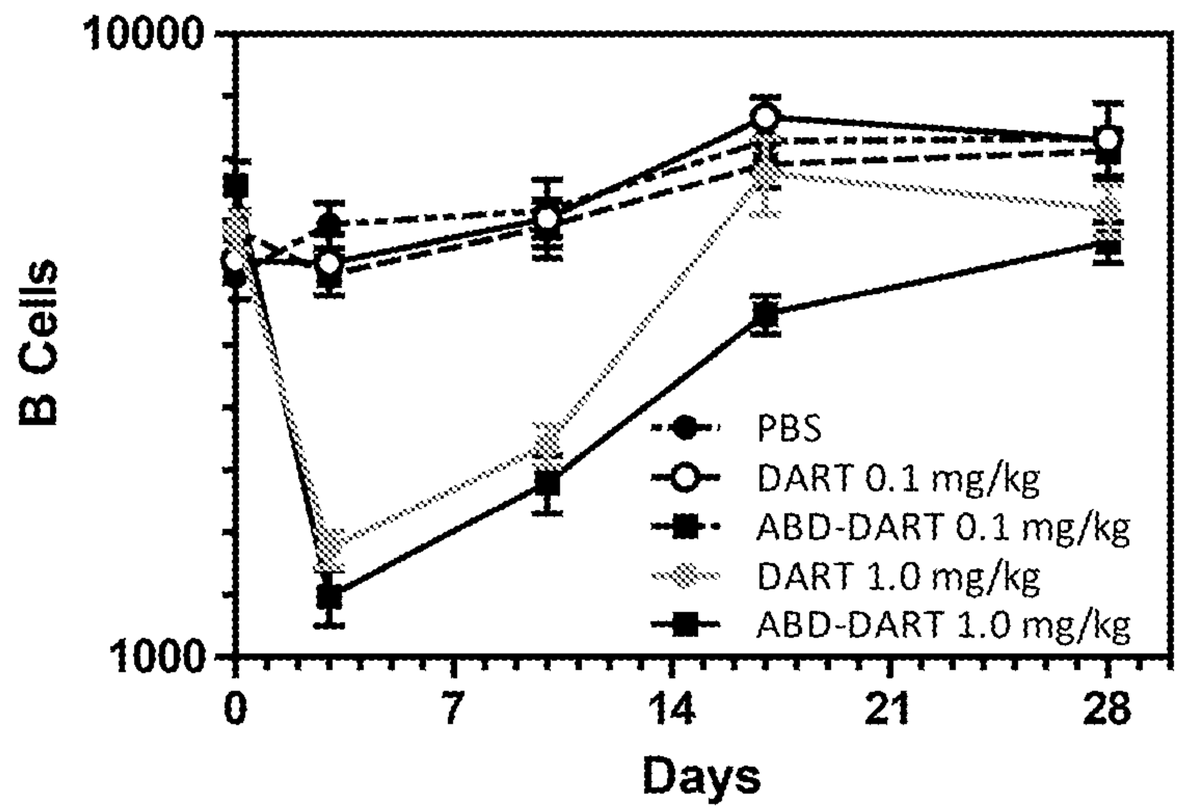


FIG. 49B

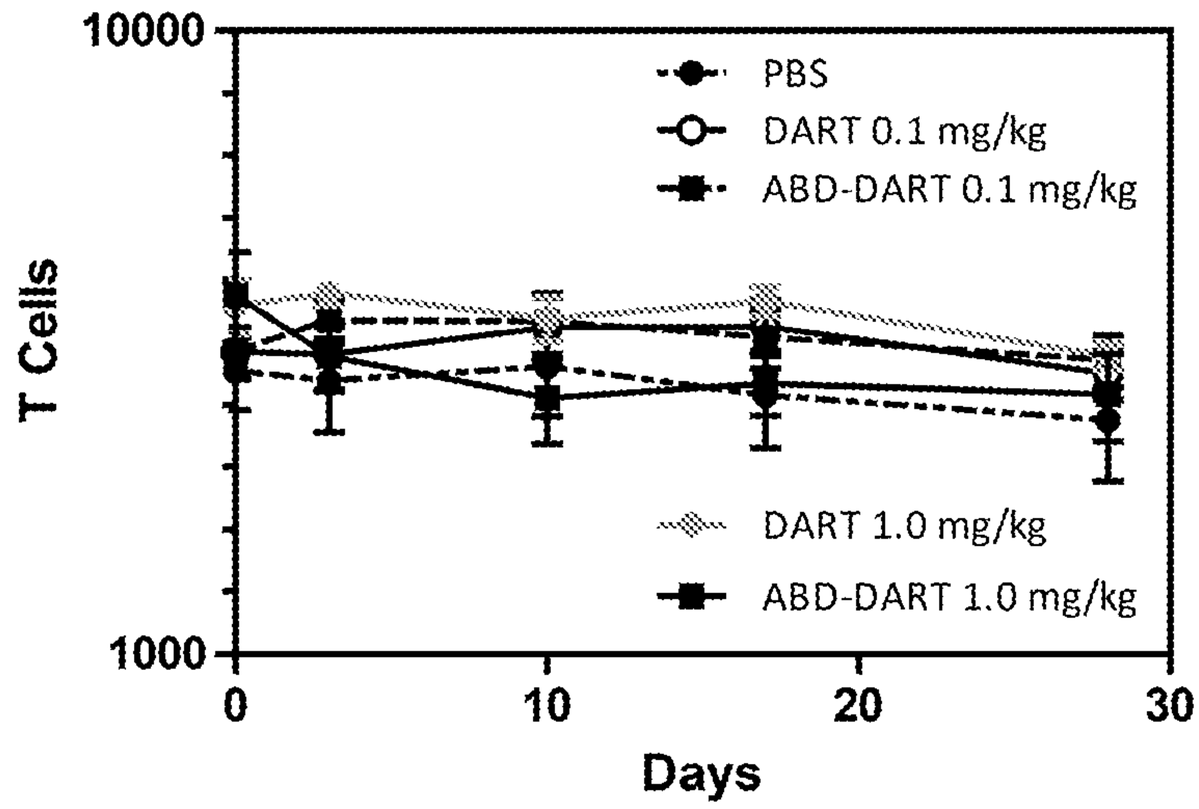


FIG. 49C

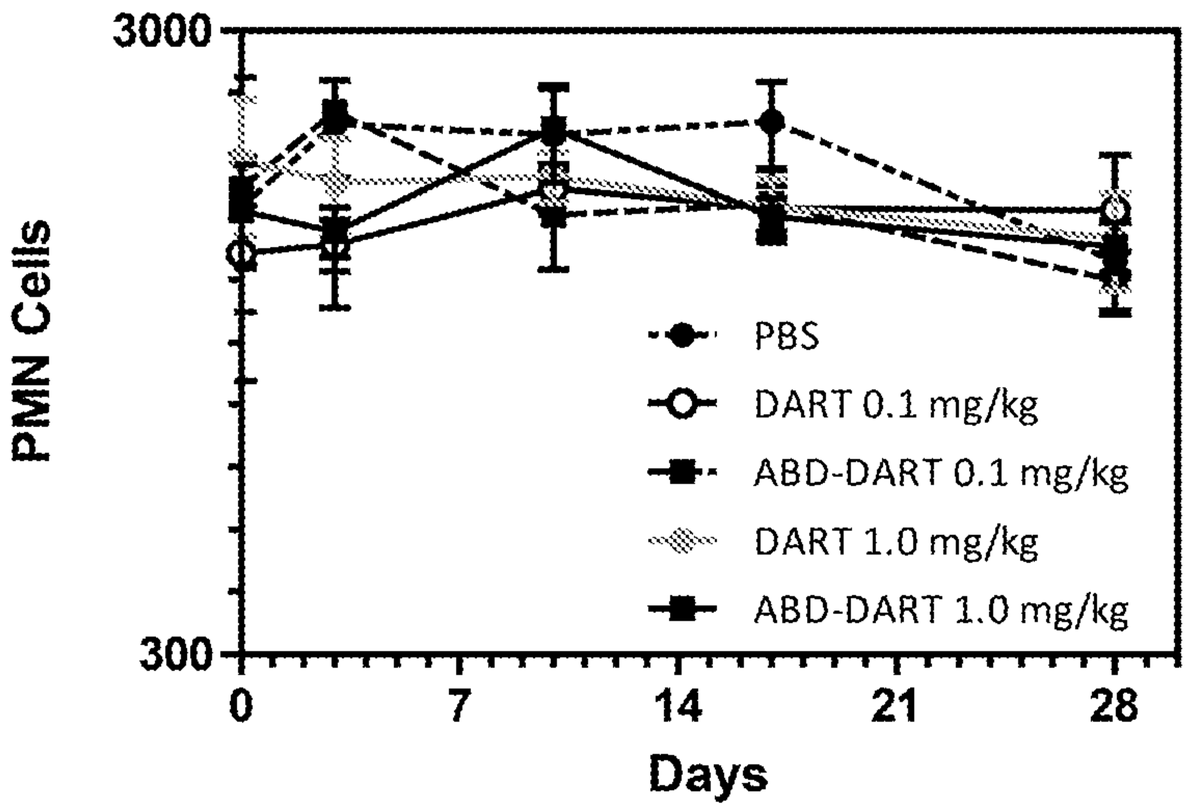


FIG. 50A

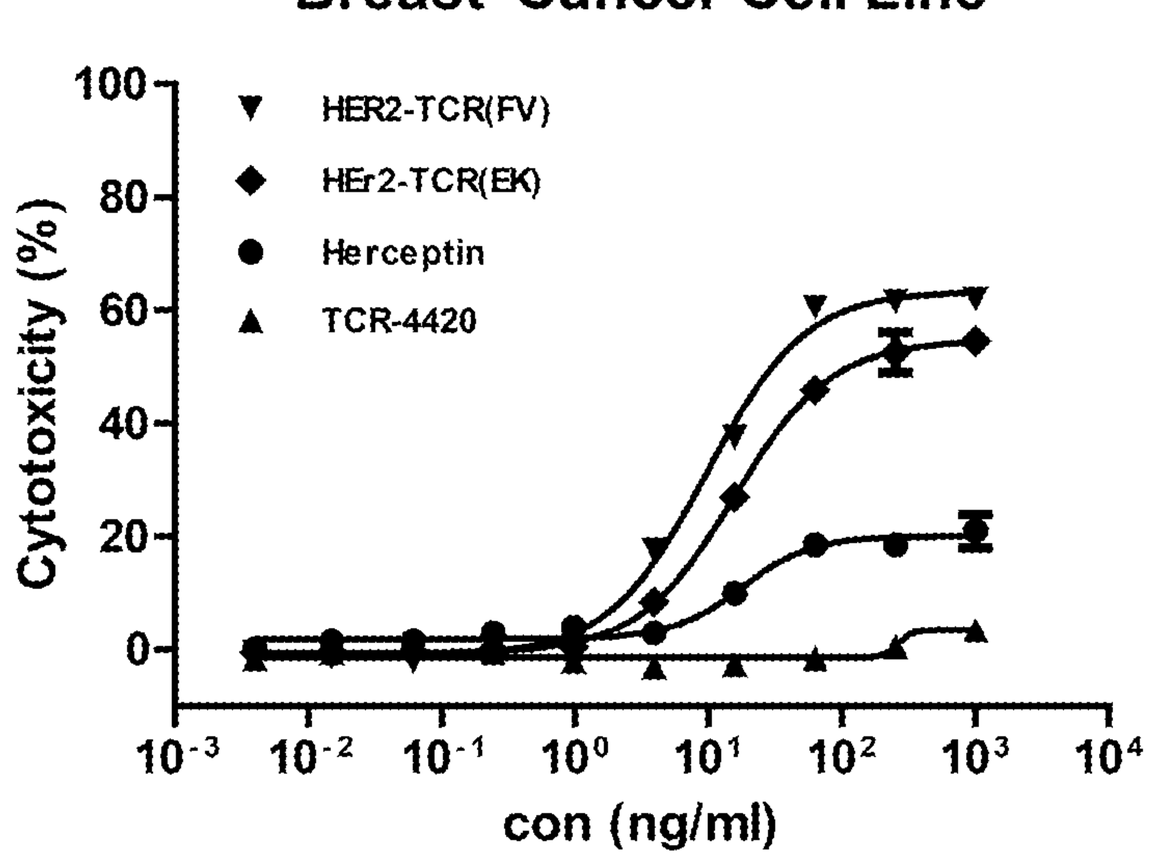


FIG. 50B

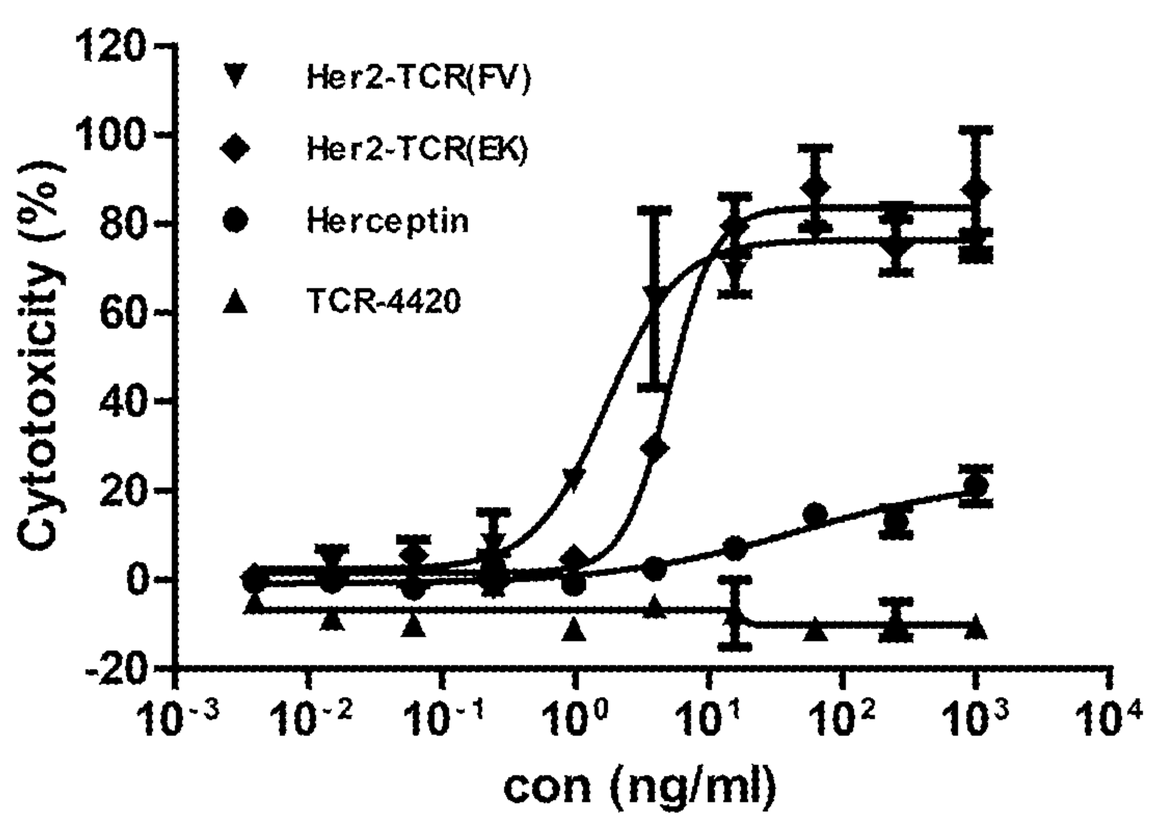


FIG. 50C

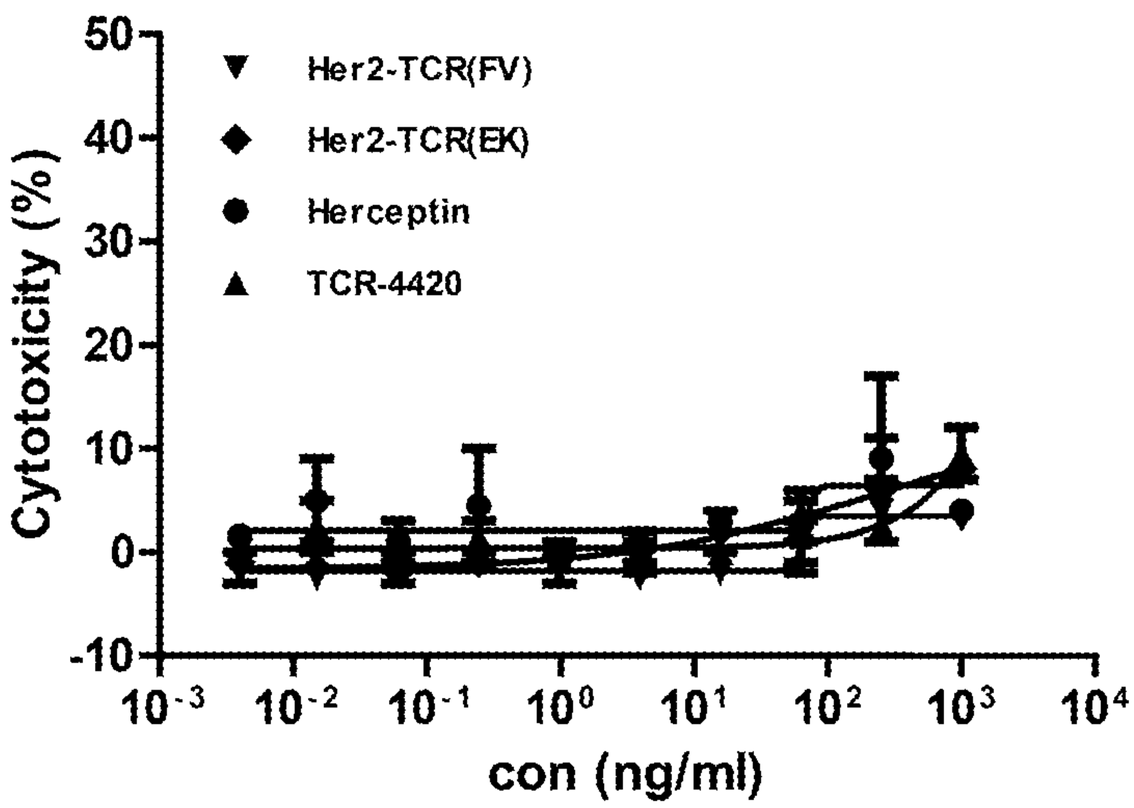


FIG. 50D

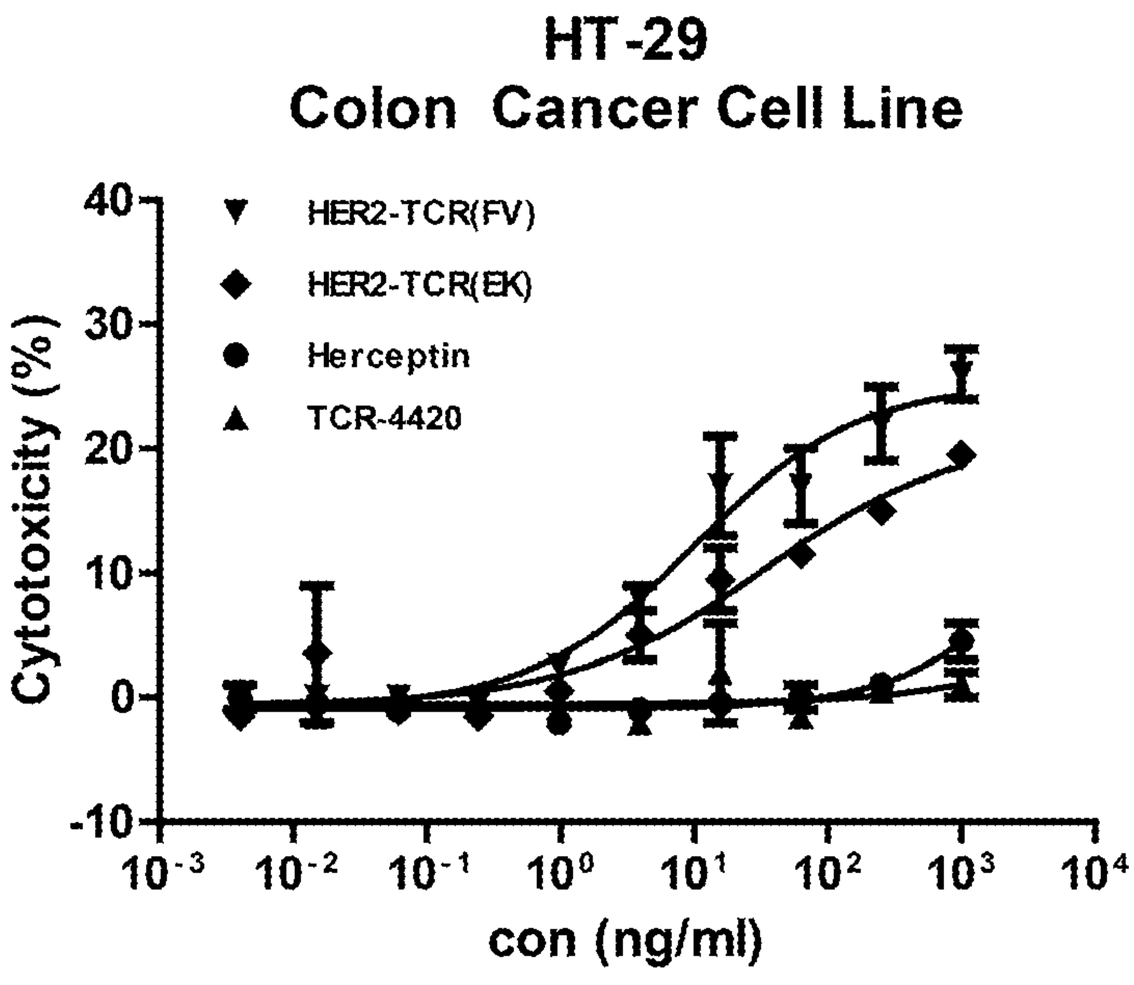


FIG. 50E

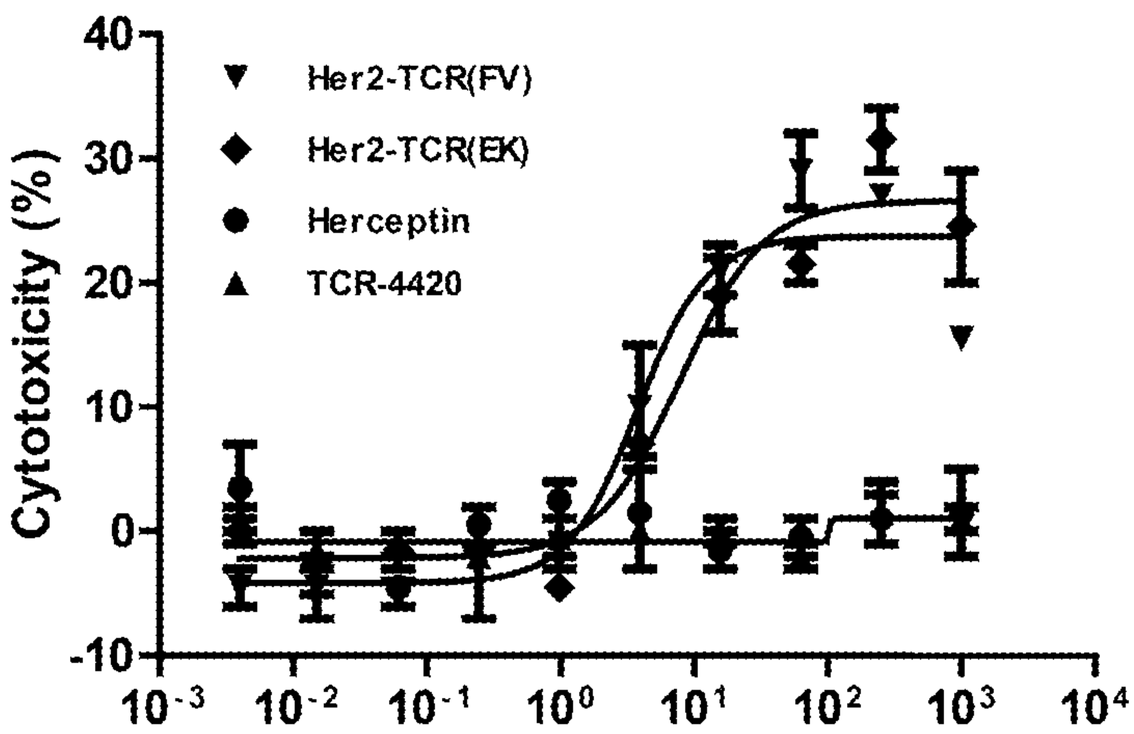


FIG. 51A

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

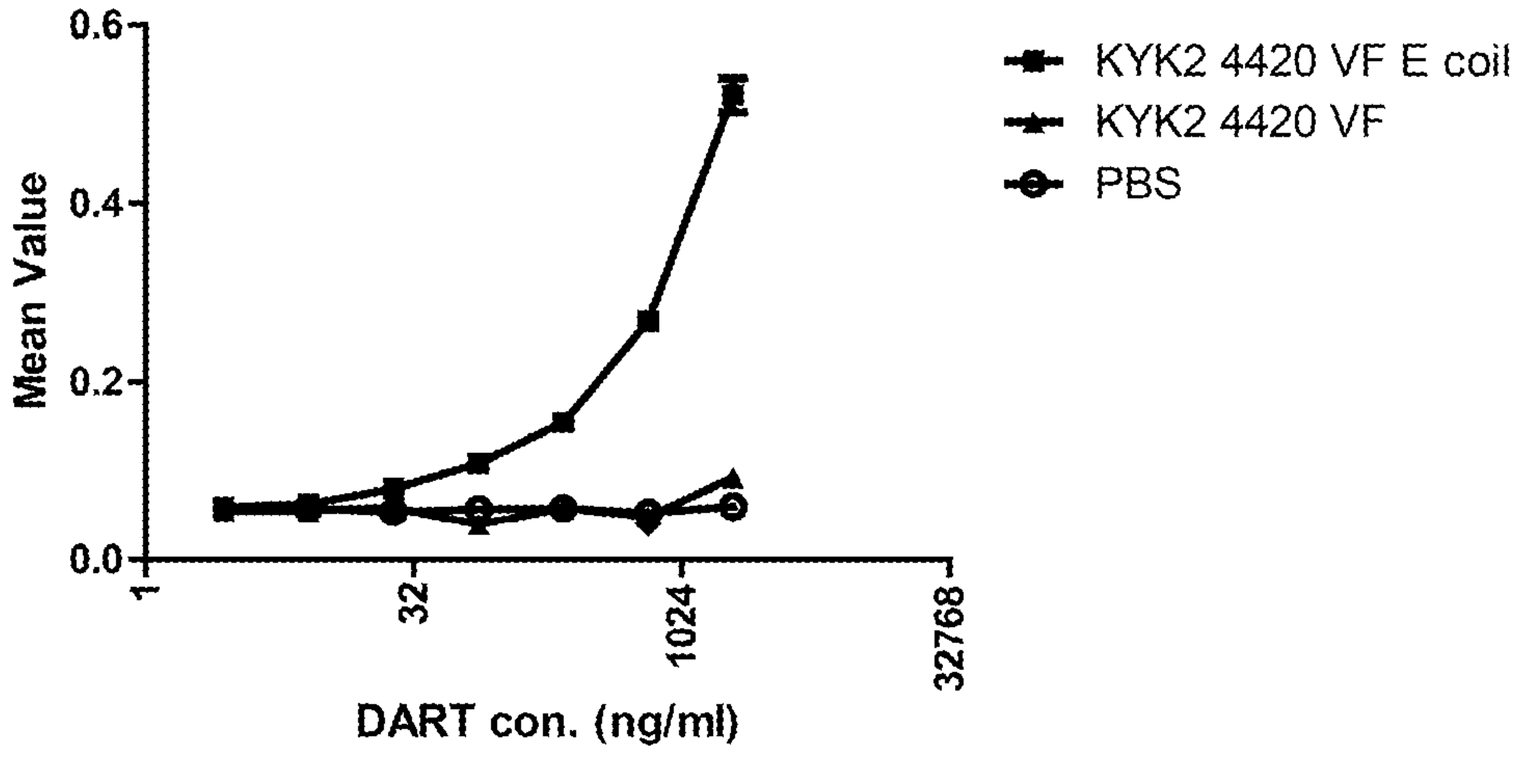


FIG 51B

KYK2-4420 VF E coil P312-009, CHO Trans. 0.45mg/L MSA Purified

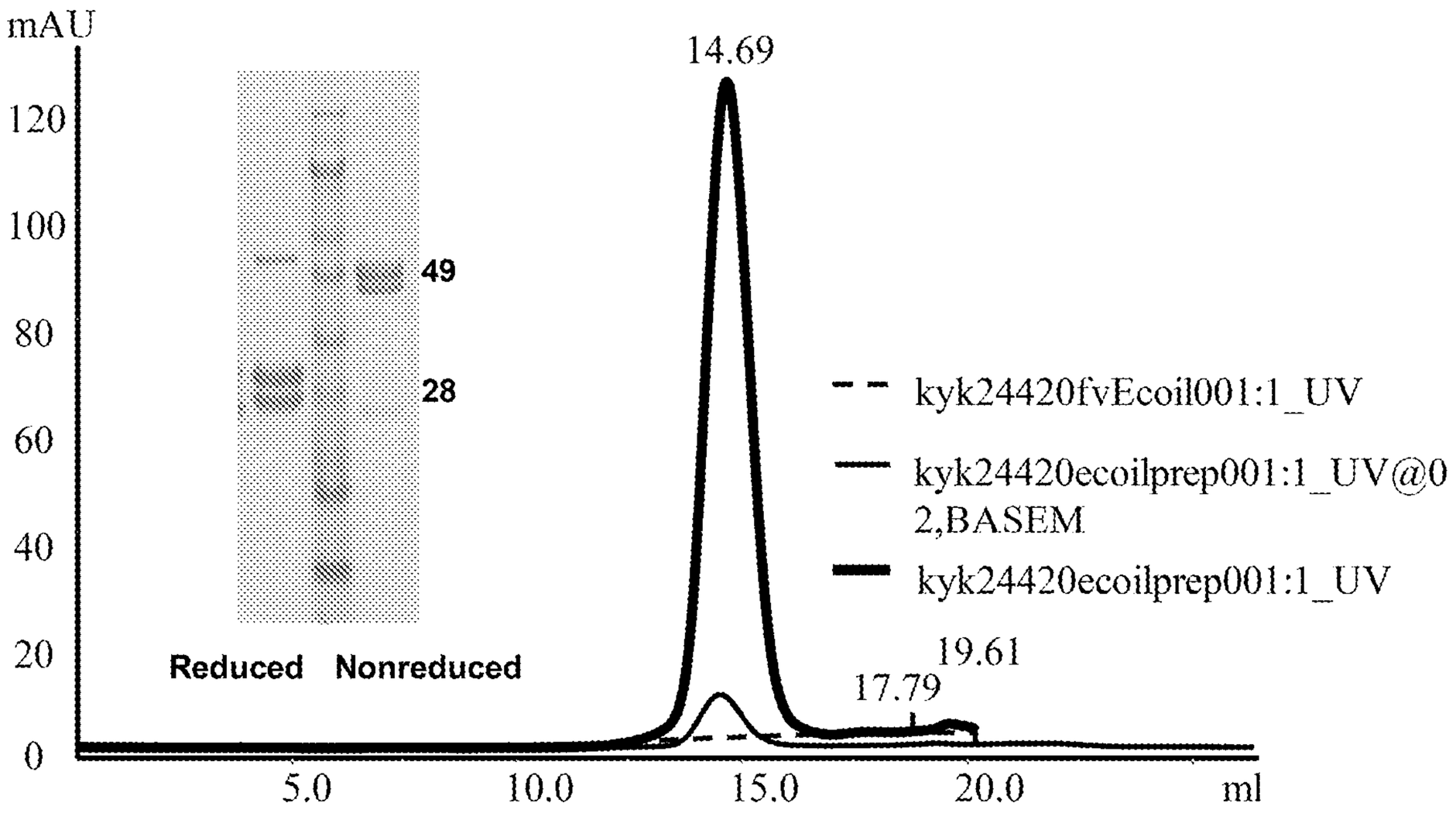


FIG. 52

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

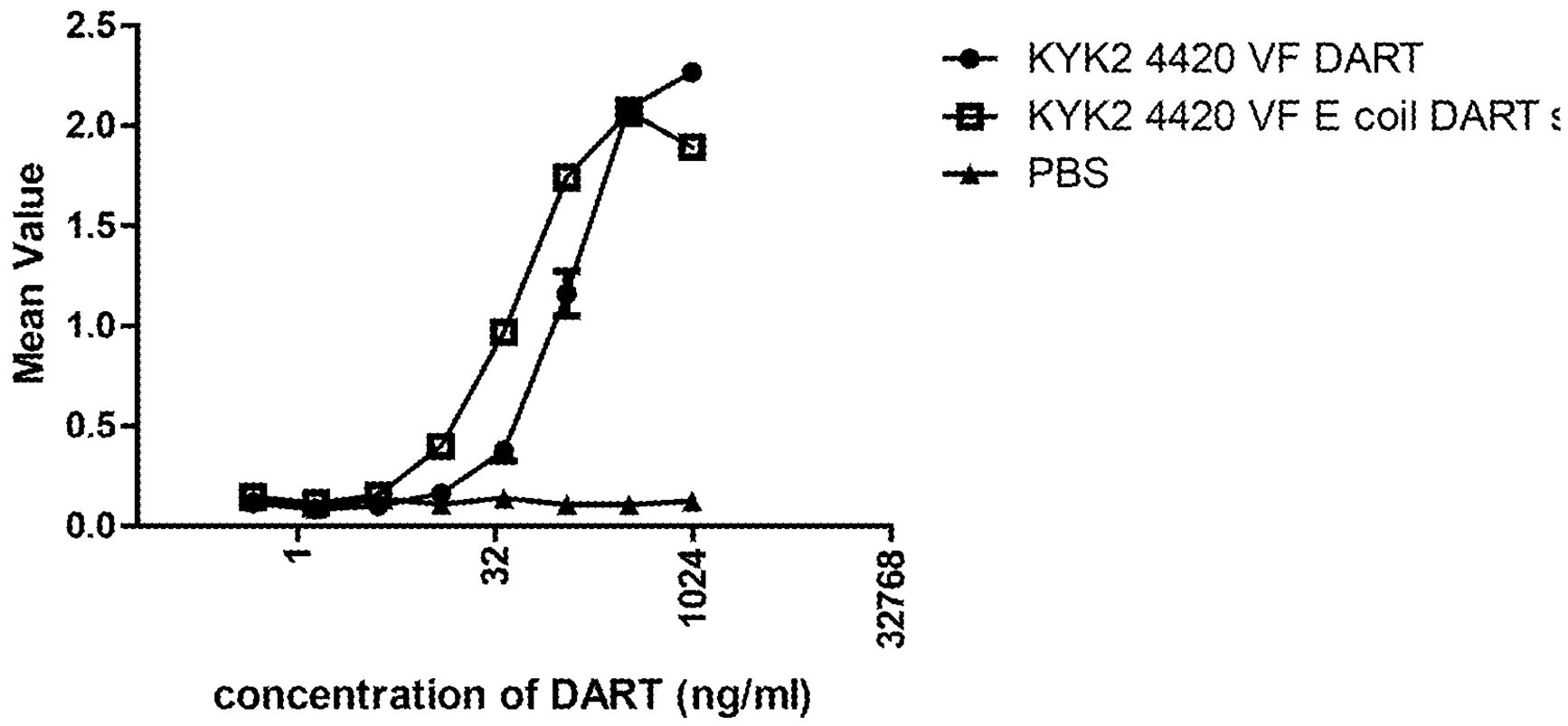
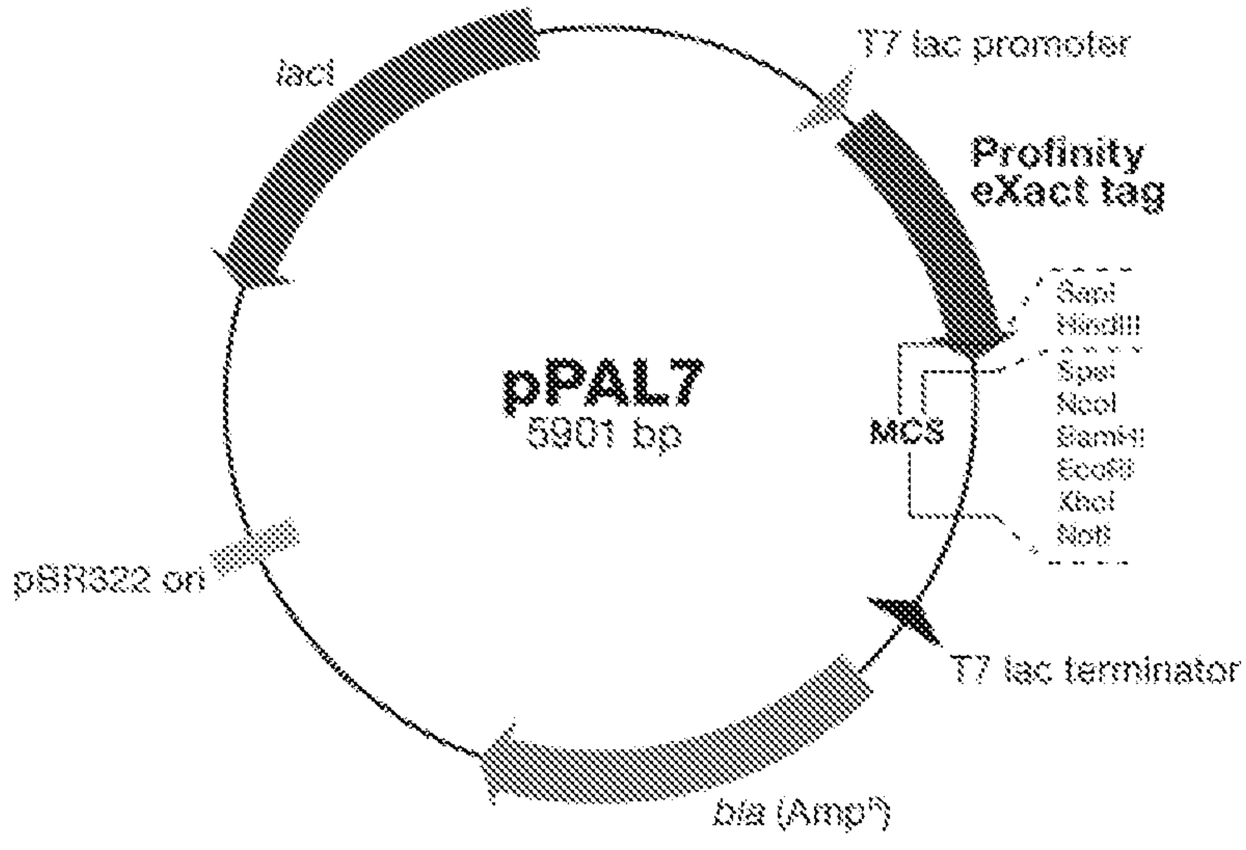
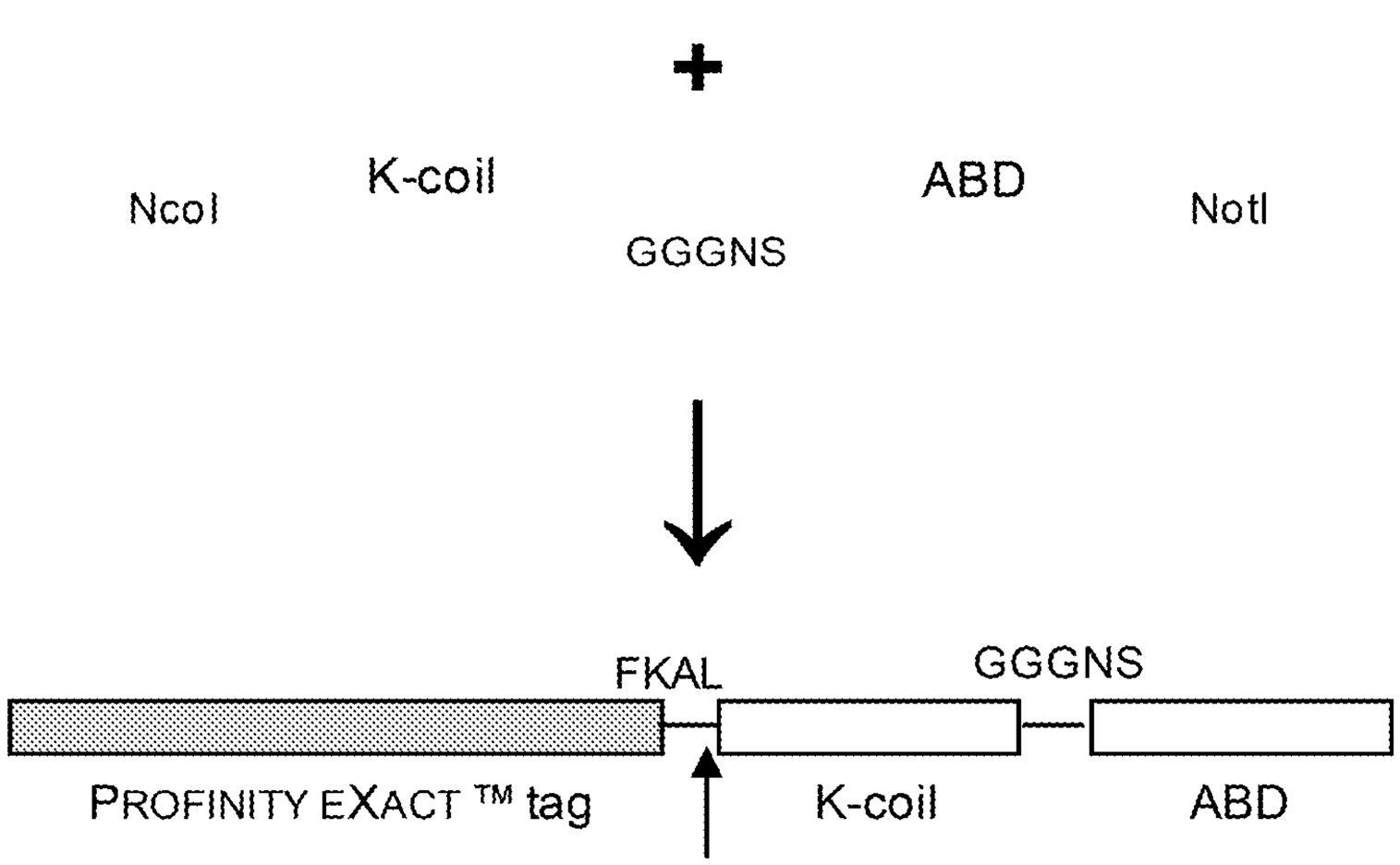


FIG. 53





pPAL7 Kcoil-ABD

FIG. 54

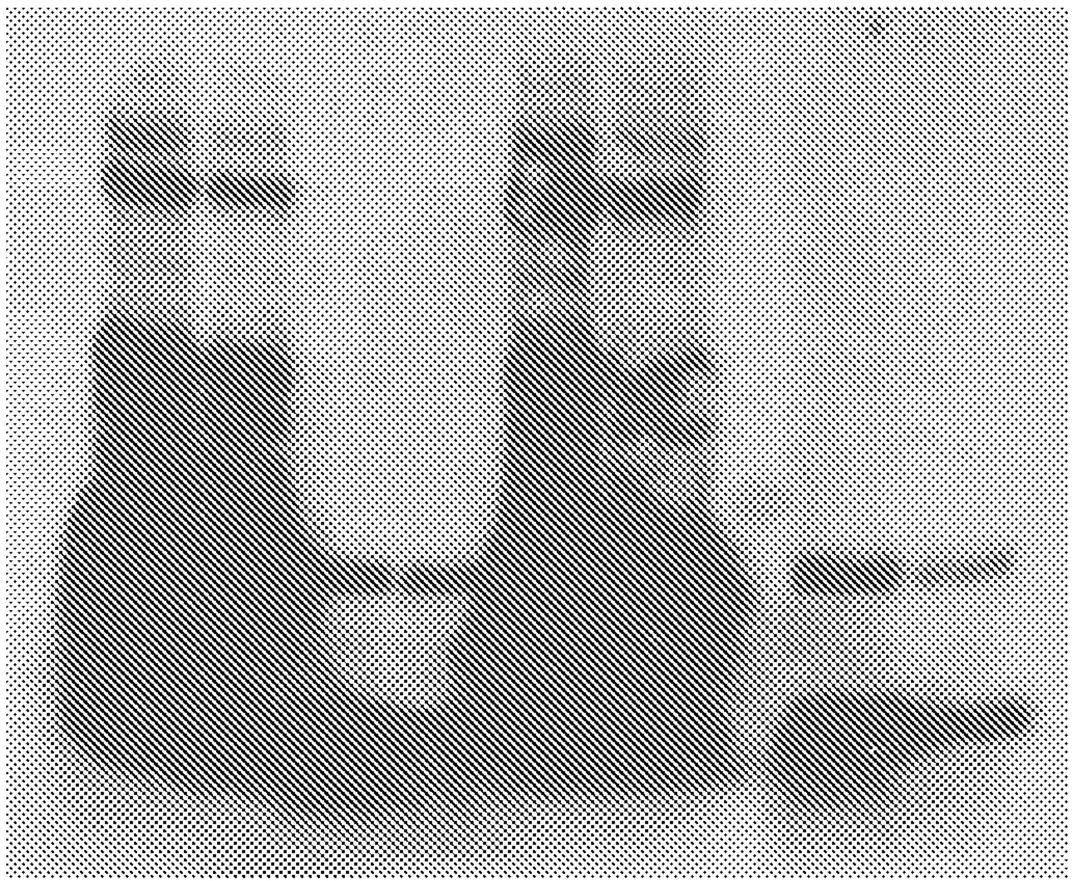

1 2 3 4 5 6M 8 9

FIG. 55

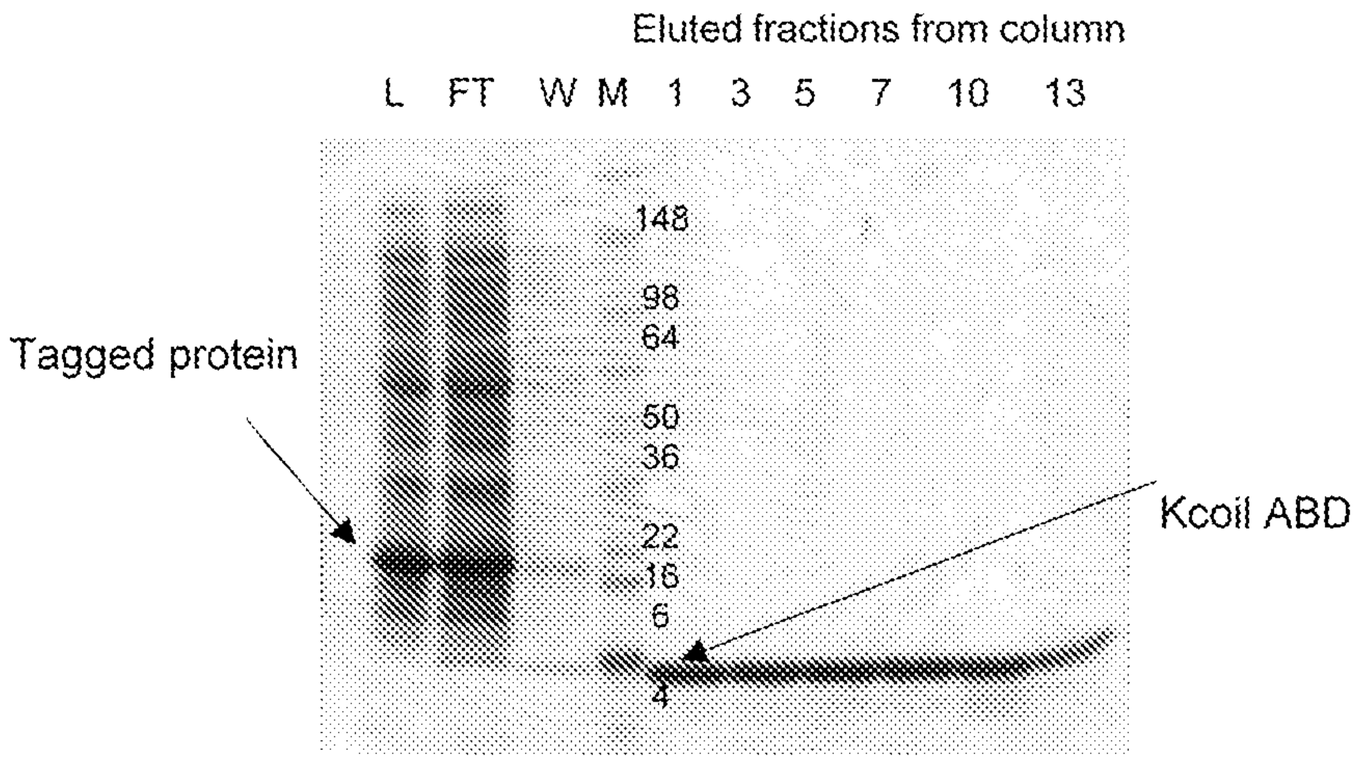

Eluted fractions from column
L FT W M 1 3 5 7 10 13
148
98
64
50
36
22
16
6
4
Tagged protein
Kcoil ABD

FIG. 56A

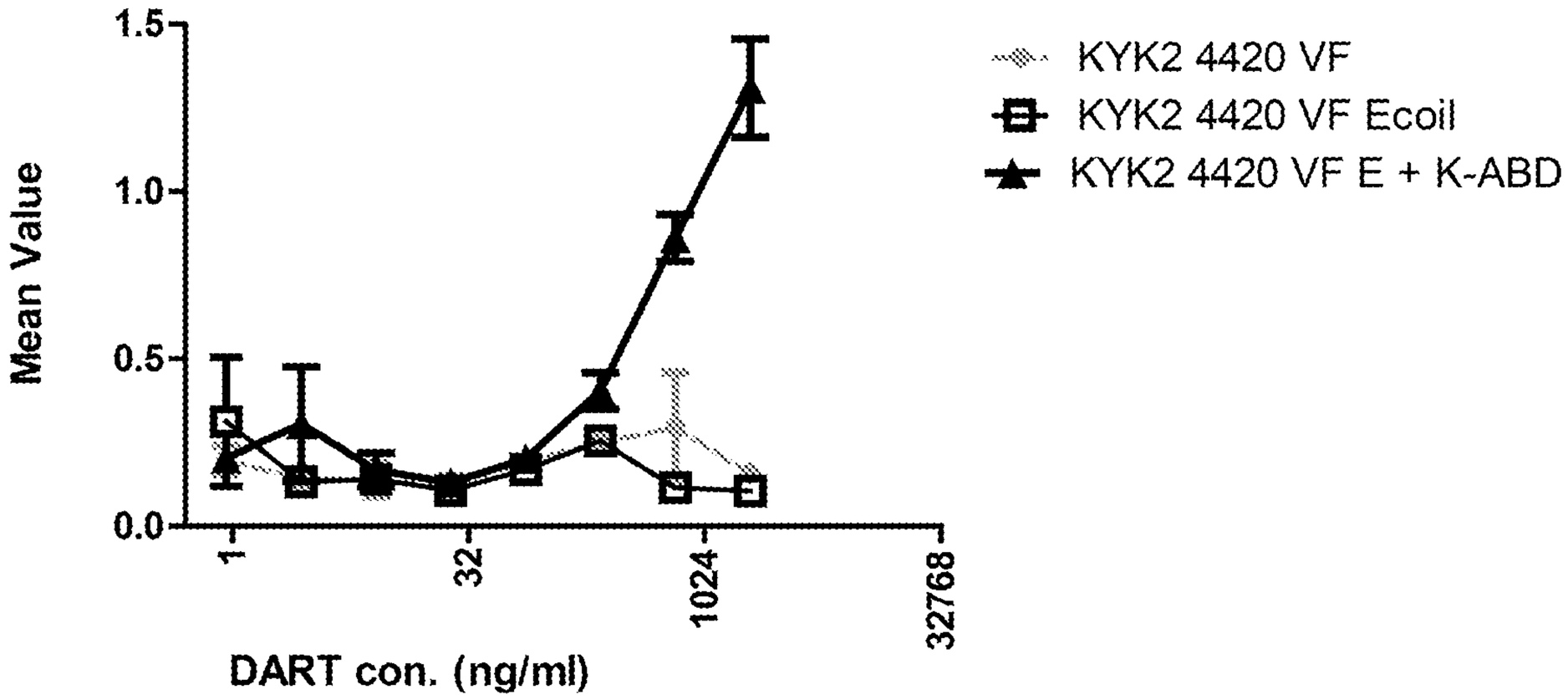


FIG. 56B

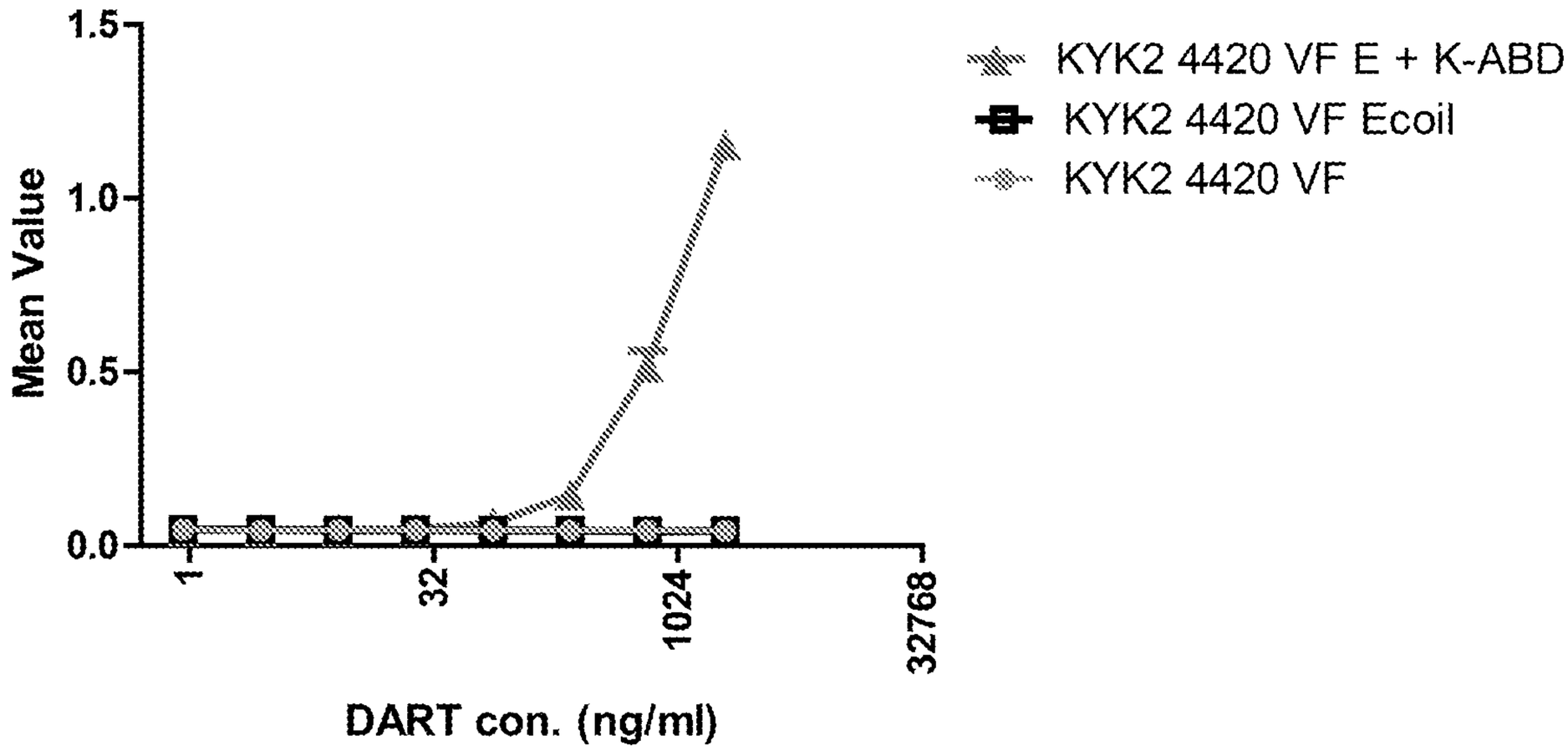
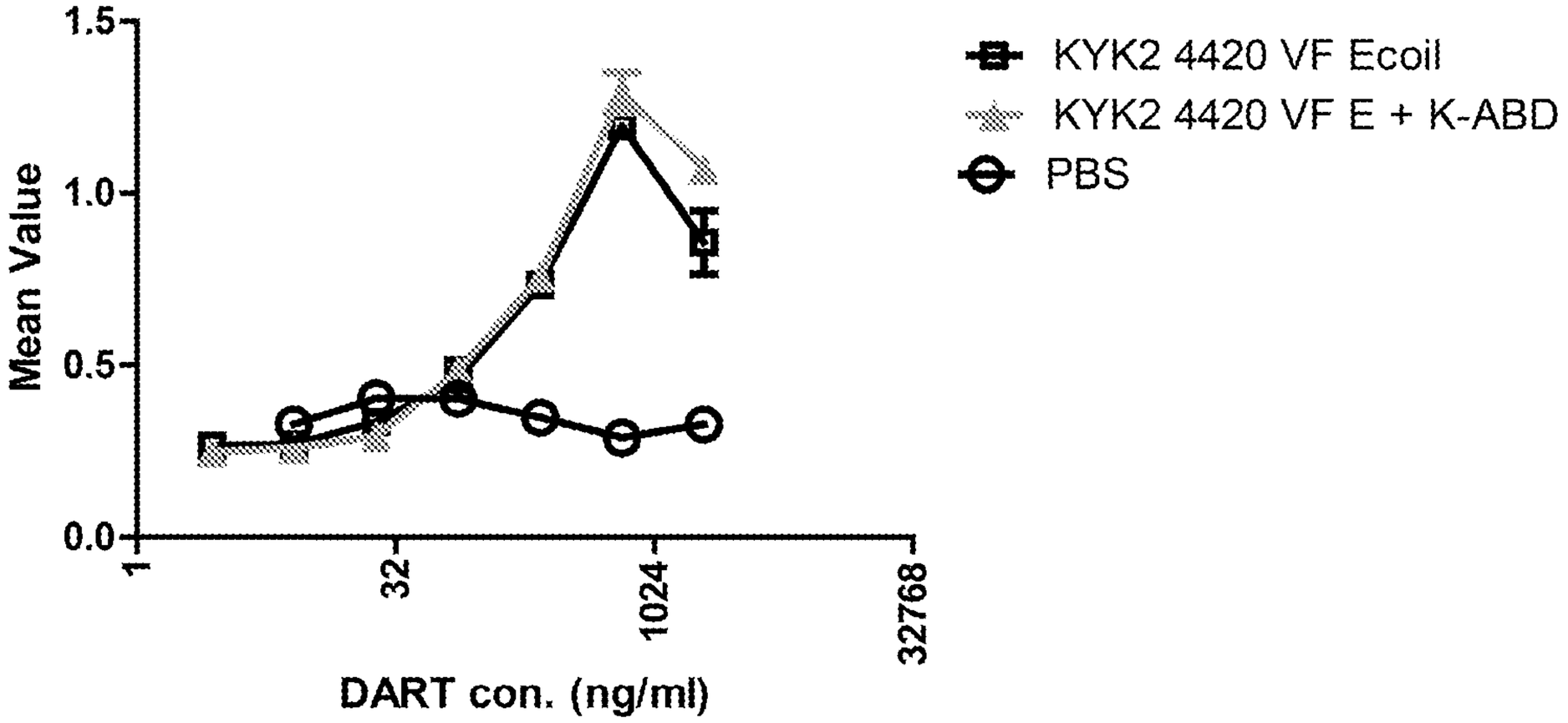
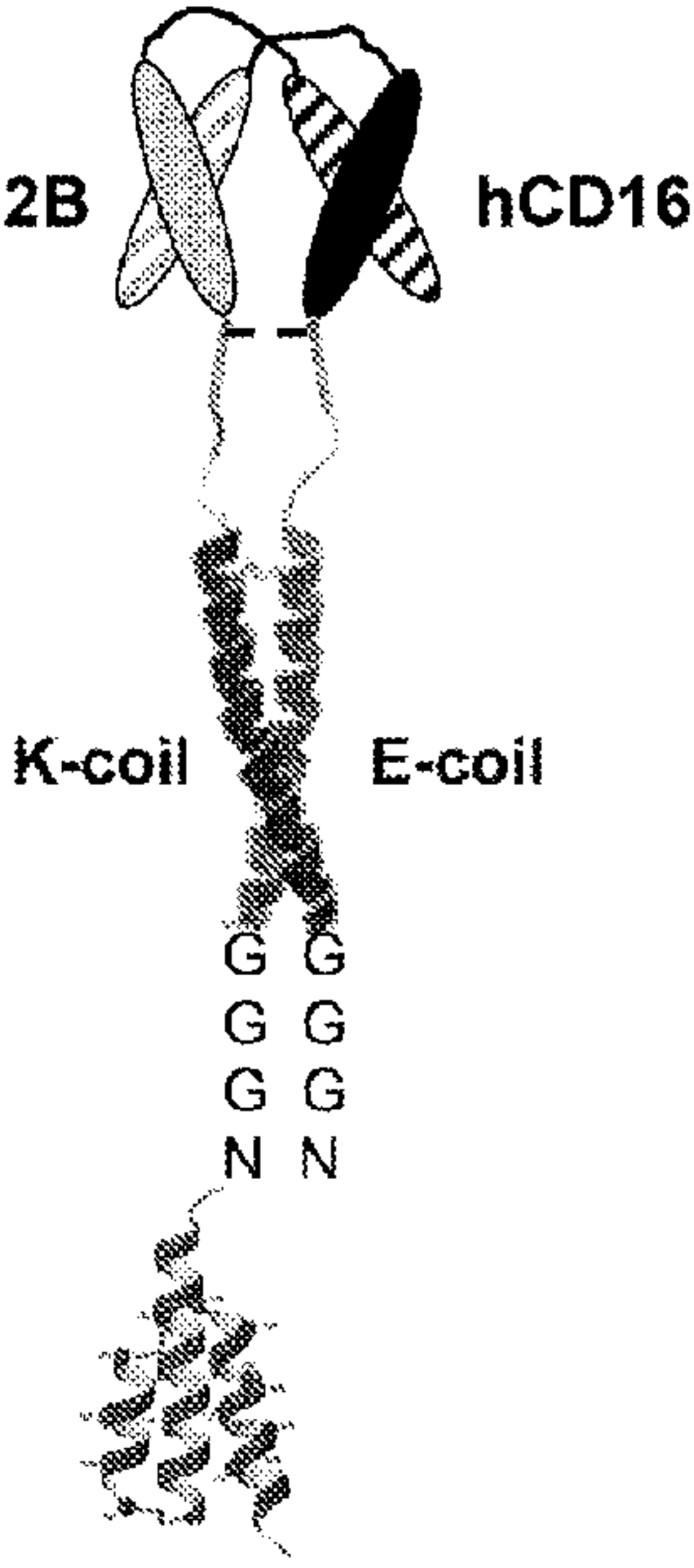


FIG. 56C


1.5
1.0
0.5
0.0
Mean Value
1
32
1024
32768
DART con. (ng/ml)
KYK2 4420 VF Ecoil
KYK2 4420 VF E + K-ABD
PBS


hCD32B
hCD16
K-coil
E-coil
G G
G G
G G
N N