b22 hinge region:

[A]

- ACT CAC ACA TGC CCA CCG TGC CCA —— CH2 — CH3 SEQ ID NO:1
  T H T C P P C P

↓

[B]

=BamHI=

- ACT CAC ACA TGC CCA CCG TGA GGA TCC- SEQ ID NO:2
  T H T C P P *

↓

[C]

=XhoI= =NotI= =BamHI=

- ACT CAC ACA TGC TCG AGC CTT CAC GGC GGC CGC TGA GGA TCC SEQ ID NO:3
  T H T C S S L H G G R *

FIGURE 1

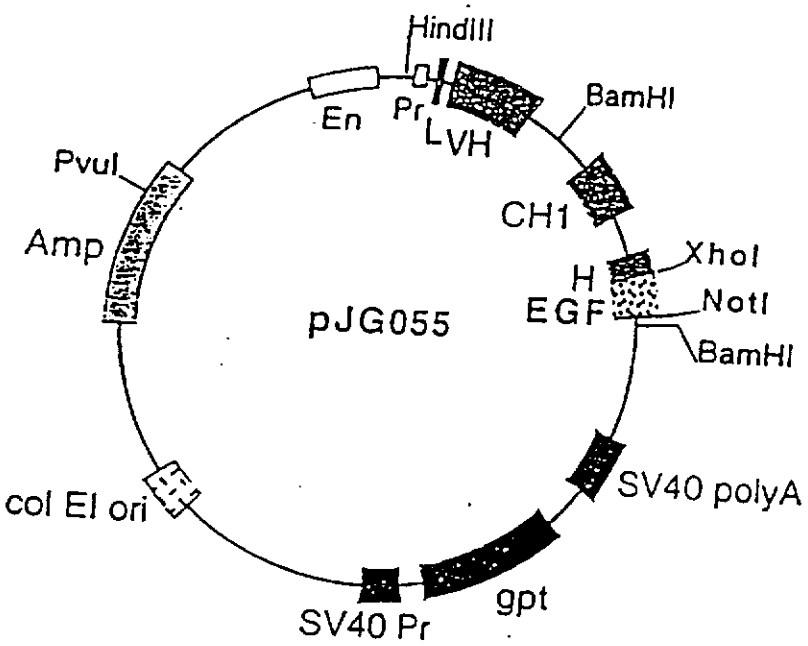

HindIII
En
Pr
LVH
BamHI
CH1
XhoI
H
EGF
NotI
BamHI
pJG055
SV40 polyA
gpt
SV40 Pr
col El ori
Amp
PvuI

FIGURE 2

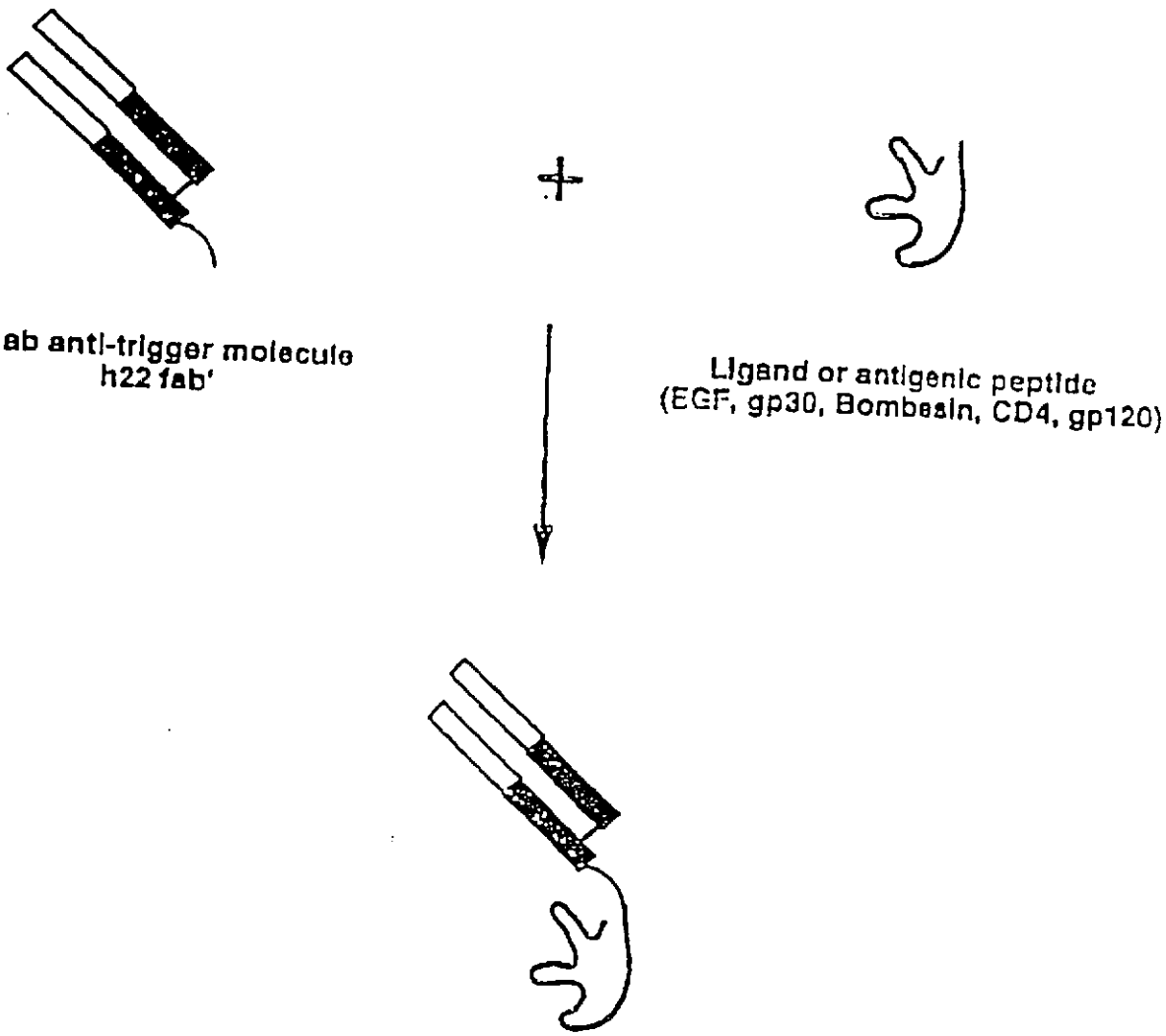

Mab anti-trigger molecule
h22 fab'
Ligand or antigenic peptide
(EGF, gp30, Bombesin, CD4, gp120)
Fab-ligand Fusions

FIGURE 3

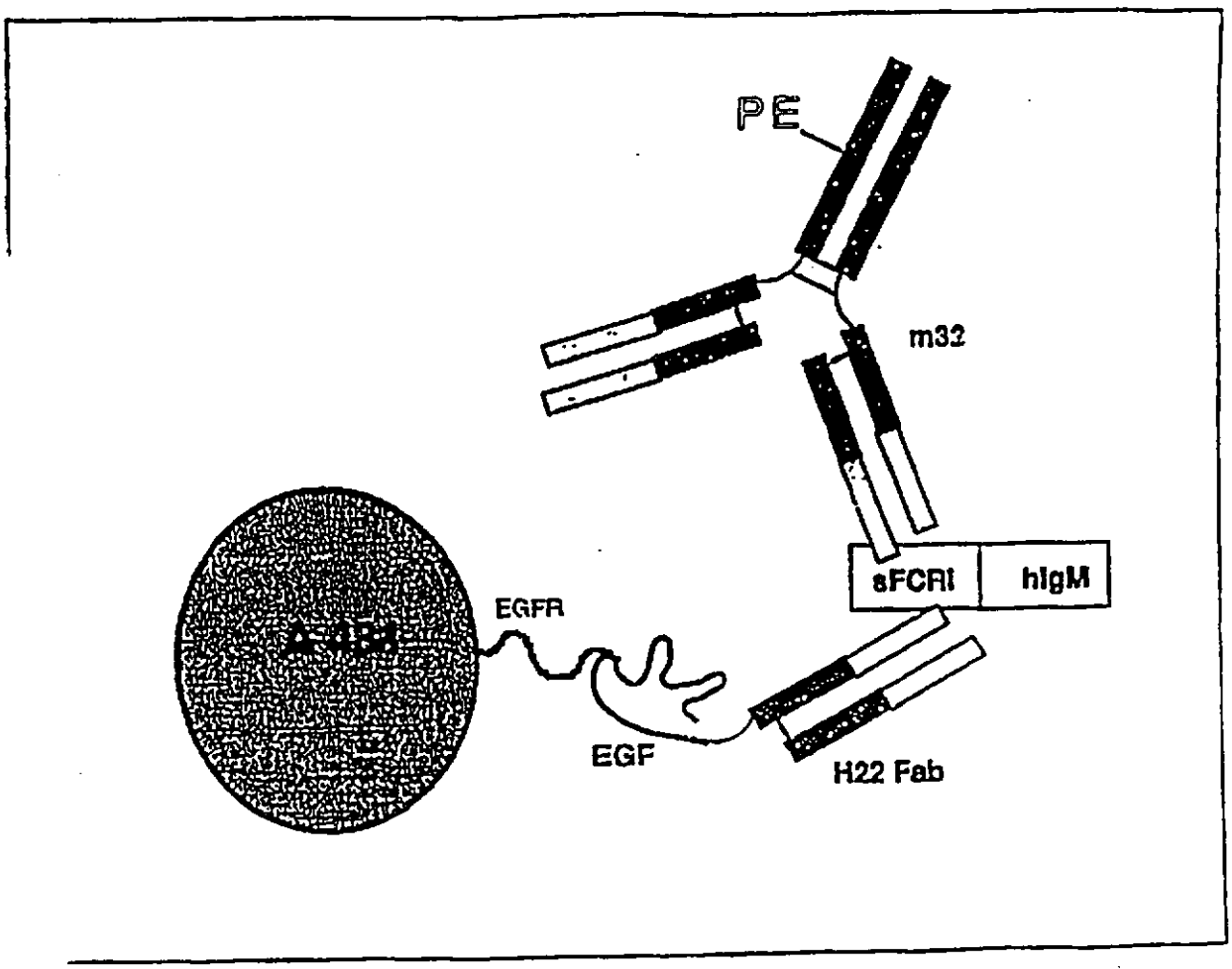


FIGURE 4

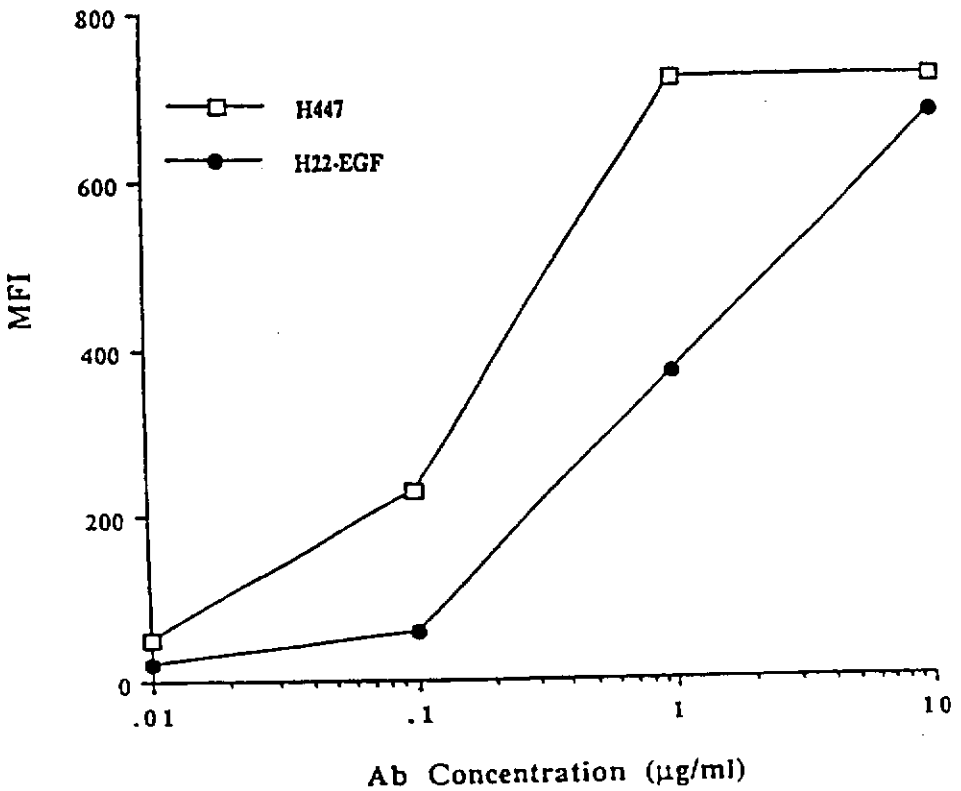

800
600
400
200
0
MFI
H447
H22-EGF
.01
.1
1
10
Ab Concentration (µg/ml)

FIGURE 5

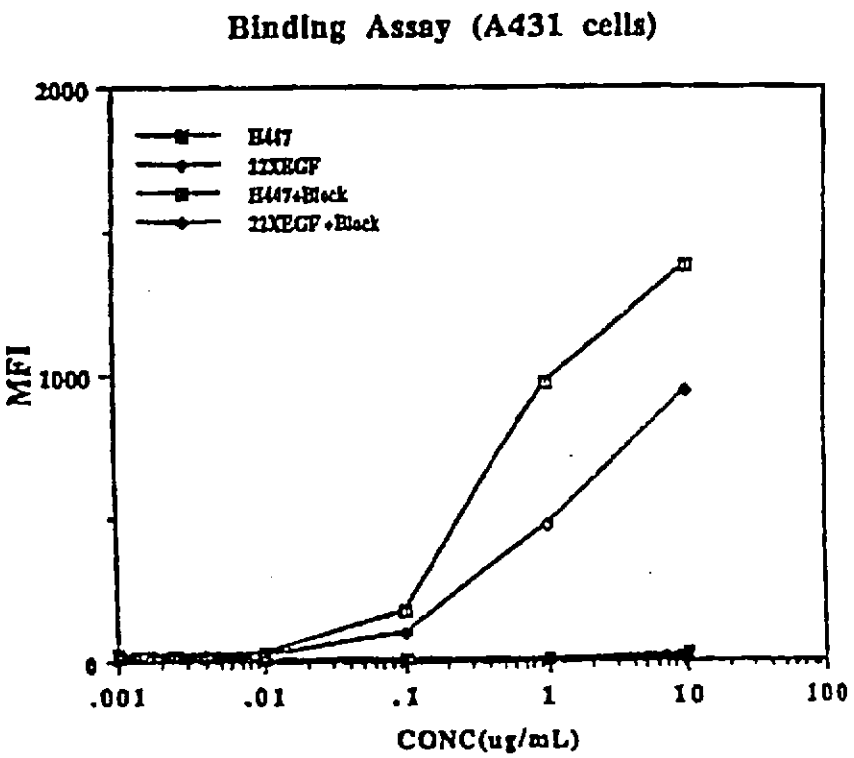

Binding Assay (A431 cells)
2000
1000
0
MFI
H447
12XEGF
H447+Block
12XEGF+Block
.001
.01
.1
1
10
100
CONC(ug/mL)

FIGURE 6

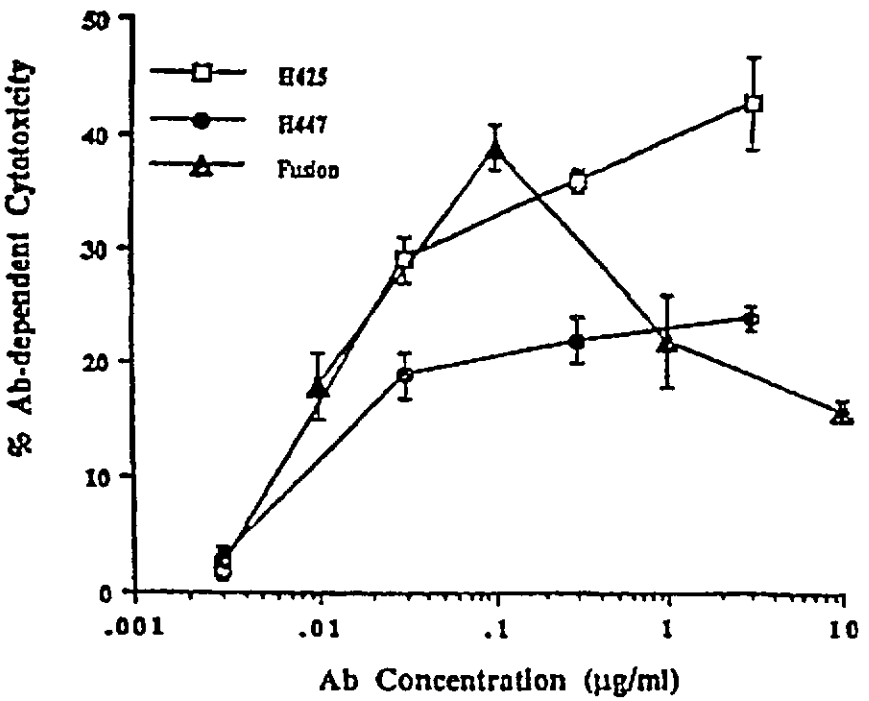

% Ab-dependent Cytotoxicity
50
40
30
20
10
0
H425
H447
Fusion
.001
.01
.1
1
10
Ab Concentration (µg/ml)

FIGURE 7

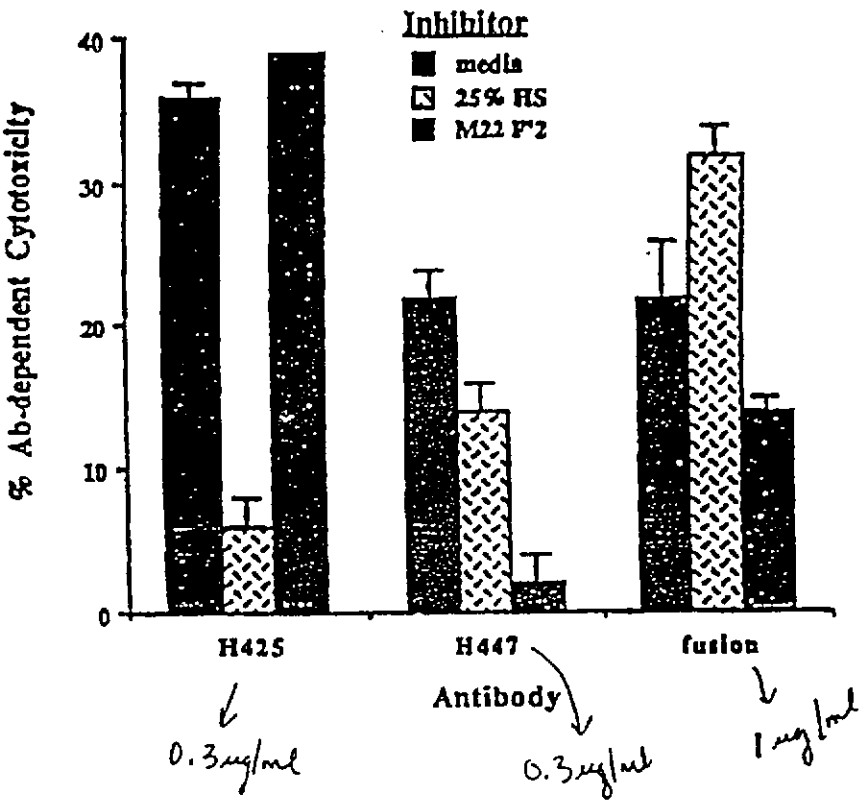

Inhibitor
media
25% HS
M22 F'2
% Ab-dependent Cytotoxicity
40
30
20
10
0
H425
H447
fusion
Antibody
0.3ug/ml
0.3ug/ml
1 ug/ml

FIGURE 8

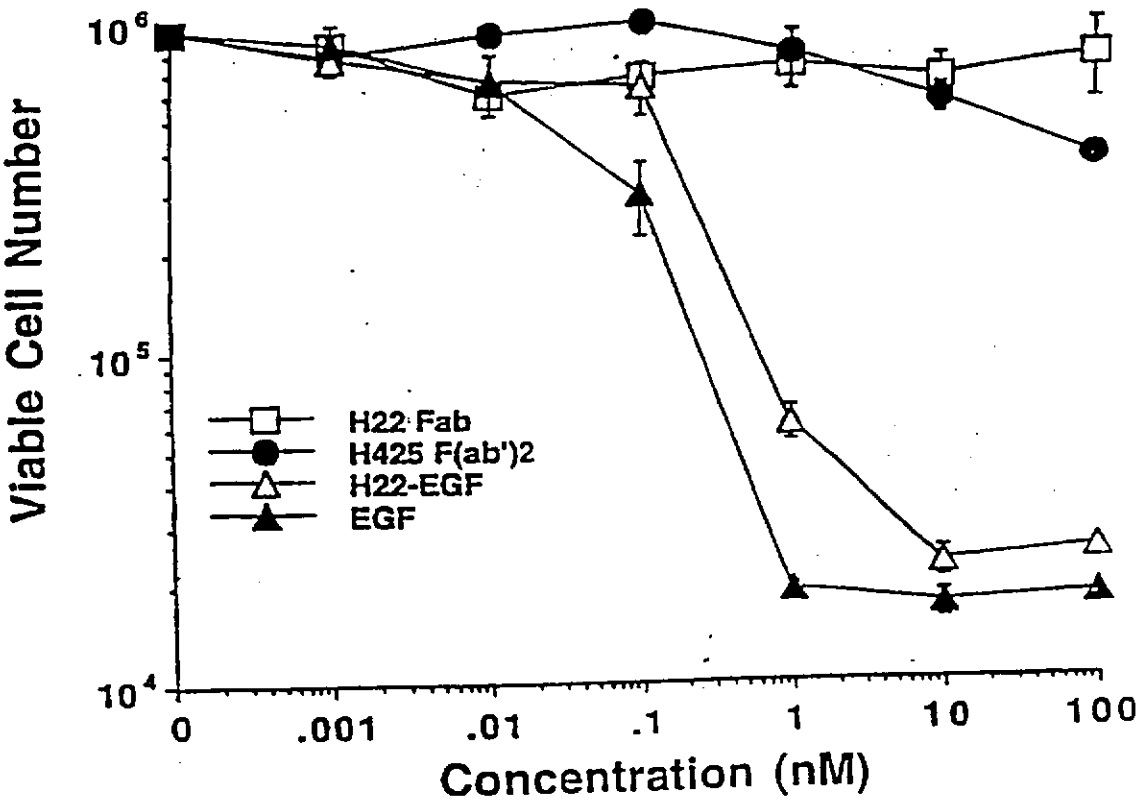

Viable Cell Number
10^6
10^5
10^4
H22 Fab
H425 F(ab')2
H22-EGF
EGF
0
.001
.01
.1
1
10
100
Concentration (nM)

FIGURE 9

```
        10         20         30         40         50
 *   *   *   *   *   *   *   *   *   *   *   *   *   *
EVQLVESGGG VVQPGRSLRL SCSSSGFIFS DNYMYWVRQA PGKGLEWVAT

        60         70         80         90        100
 *   *   *   *   *   *   *   *   *   *   *   *   *   *
ISDGGSYTYY PDSVKGRFTI SRDNSKNTLF LQMDSLRPED TGVYFCARGY

       110        120        130        140        150
 *   *   *   *   *   *   *   *   *   *   *   *   *   *
YRYEGAMDYW GQGTPVTVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK

       160        170        180        190        200
 *   *   *   *   *   *   *   *   *   *   *   *   *   *
DYFPERVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT

       210        220        230        240        250
 *   *   *   *   *   *   *   *   *   *   *   *   *   *
YICNVNHKPS NTKVDKKVEP KSCDKTHTCS TTSTTGTSHL VKCAEKEKTF
                                 Hz2Fd  Heregulin β2 EGF domain
       260        270        280        290        300
 *   *   *   *   *   *   *   *   *   *   *   *   *   *
CVNGGECFMV KDLSNPSRYL CKCPNEFTGD RCQNYVMASF YKAEELYQKR
```

SEQ ID NO: 4

FIGURE 10

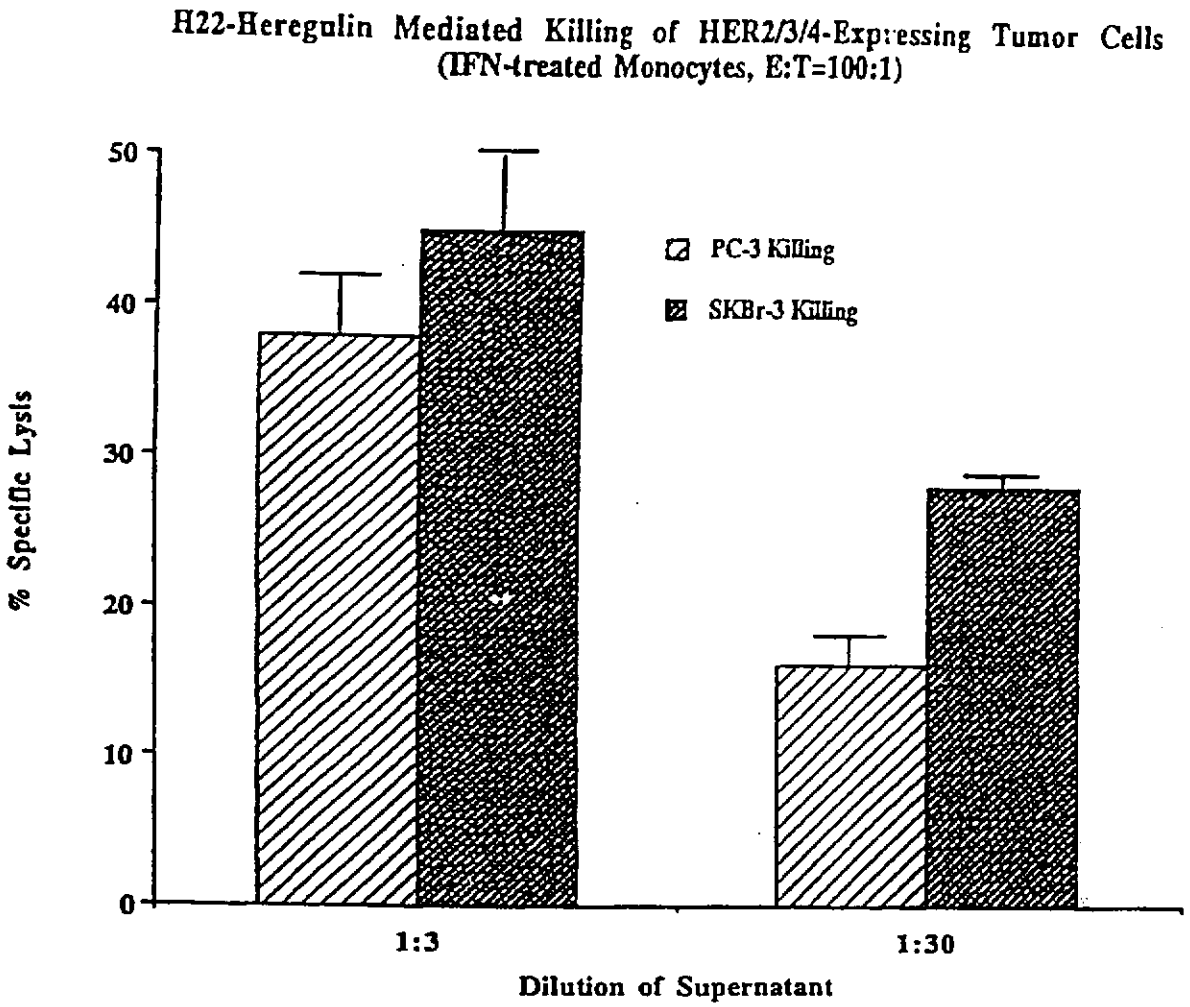

H22-Heregulin Mediated Killing of HER2/3/4-Expressing Tumor Cells
(IFN-treated Monocytes, E:T=100:1)
% Specific Lysis
50
40
30
20
10
0
PC-3 Killing
SKBr-3 Killing
1:3
1:30
Dilution of Supernatant

FIGURE 11

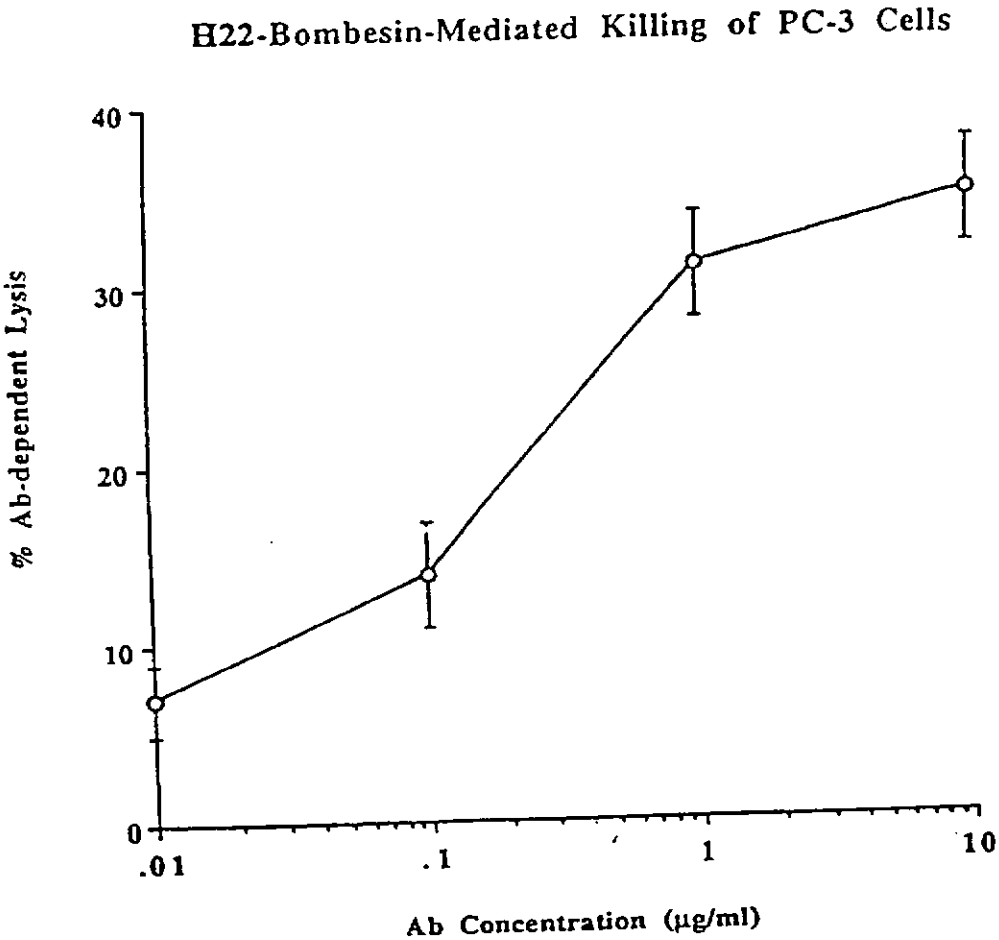

H22-Bombesin-Mediated Killing of PC-3 Cells
% Ab-dependent Lysis
40
30
20
10
0
.01
.1
1
10
Ab Concentration (µg/ml)

FIGURE 12

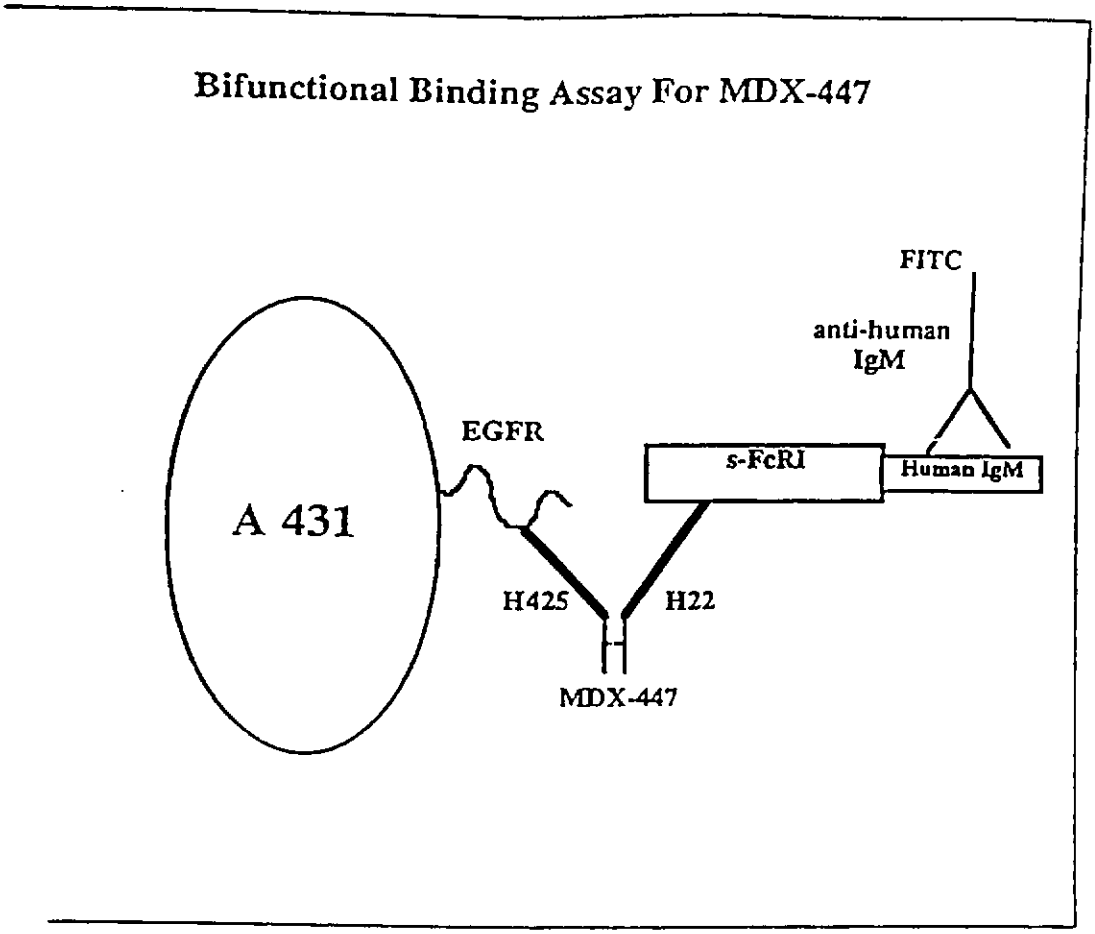

Bifunctional Binding Assay For MDX-447
FITC
anti-human IgM
EGFR
s-FcRI
Human IgM
A 431
H425
H22
MDX-447

FIGURE 13

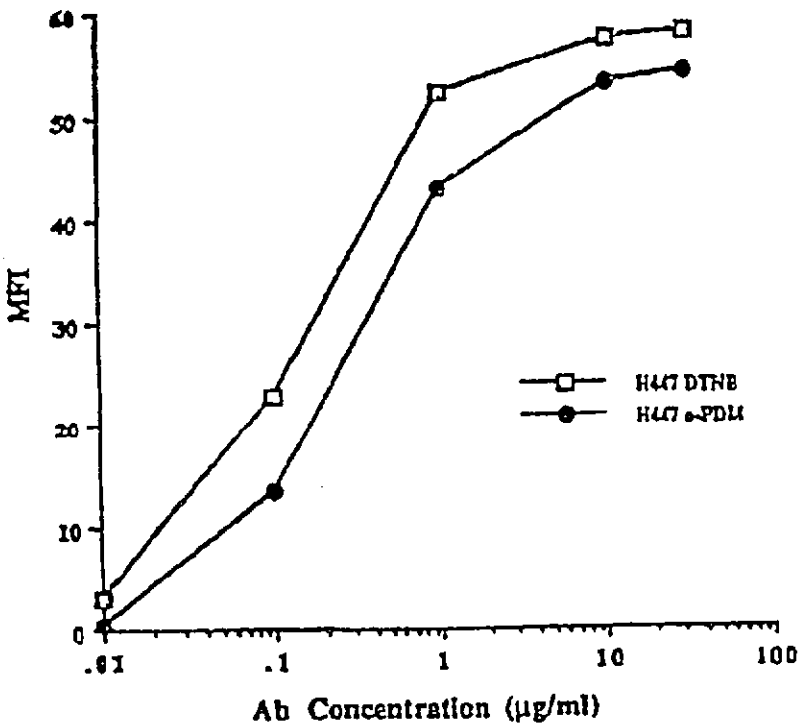

MFI
0
10
20
30
40
50
60
.01
.1
1
10
100
Ab Concentration (µg/ml)
H4.47 DTNB
H4.47 o-PDM

FIGURE 14

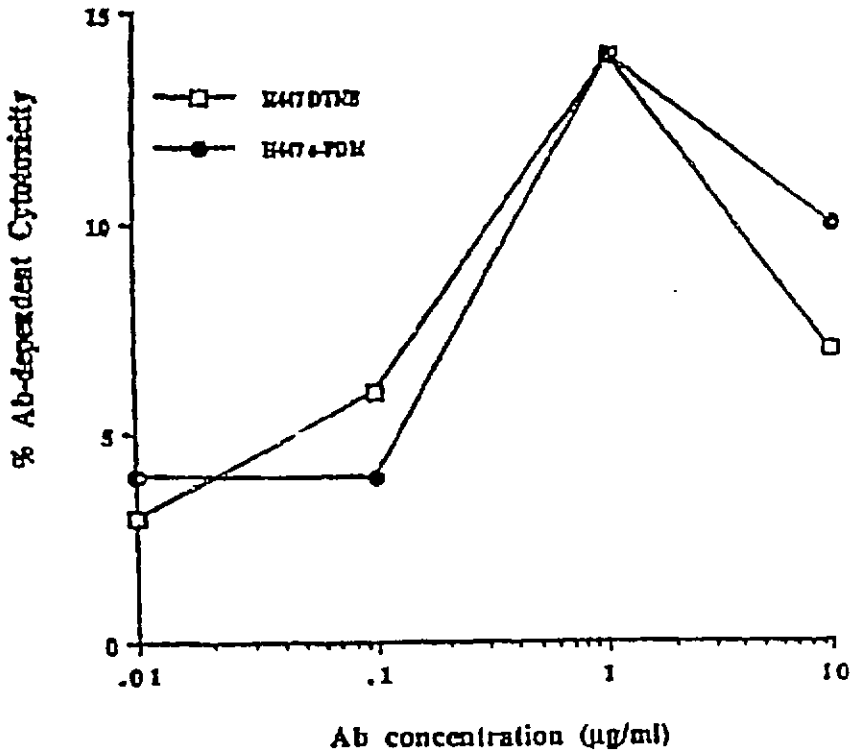

15
10
5
0
% Ab-dependent Cytotoxicity
.01
.1
1
10
Ab concentration (µg/ml)

FIGURE 15

Construction of Trispecific Antibodies

STAGE ONE: Coupling of M22 with H425

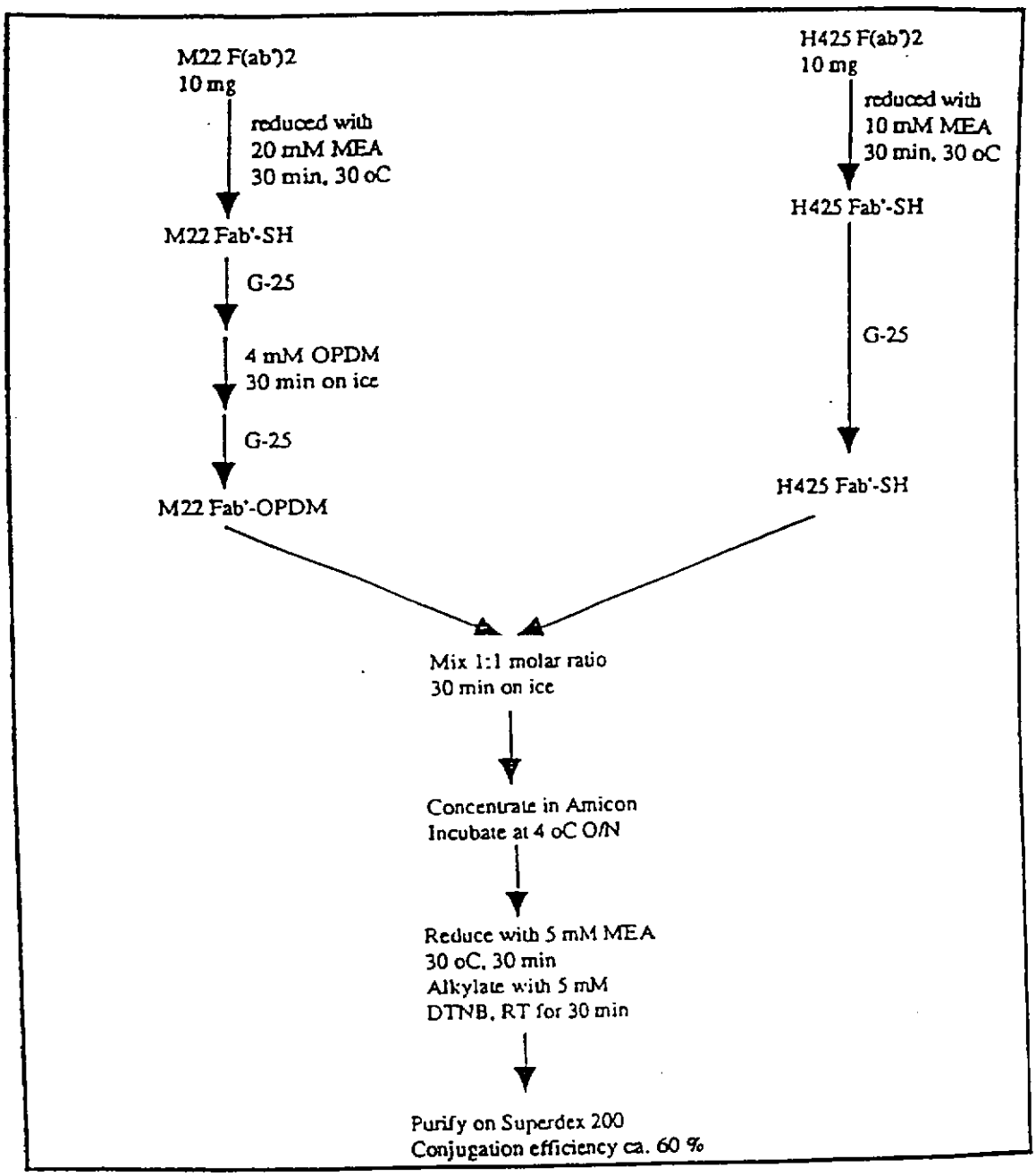


FIGURE 16A

Construction of Trispecific Antibodies (continued)

STAGE TWO: Coupling of 520C9 with bispecific M22xH425

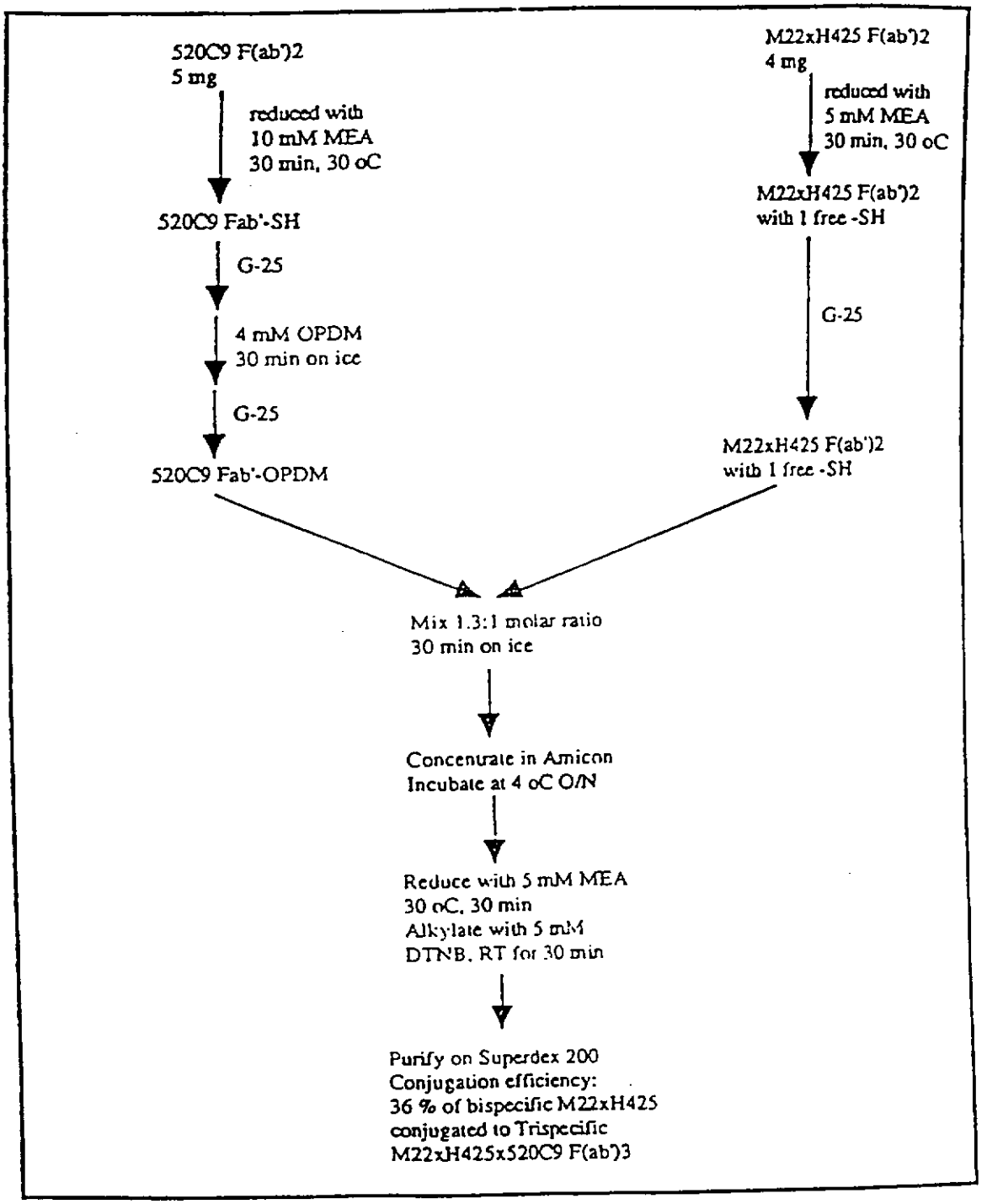


FIGURE 16B

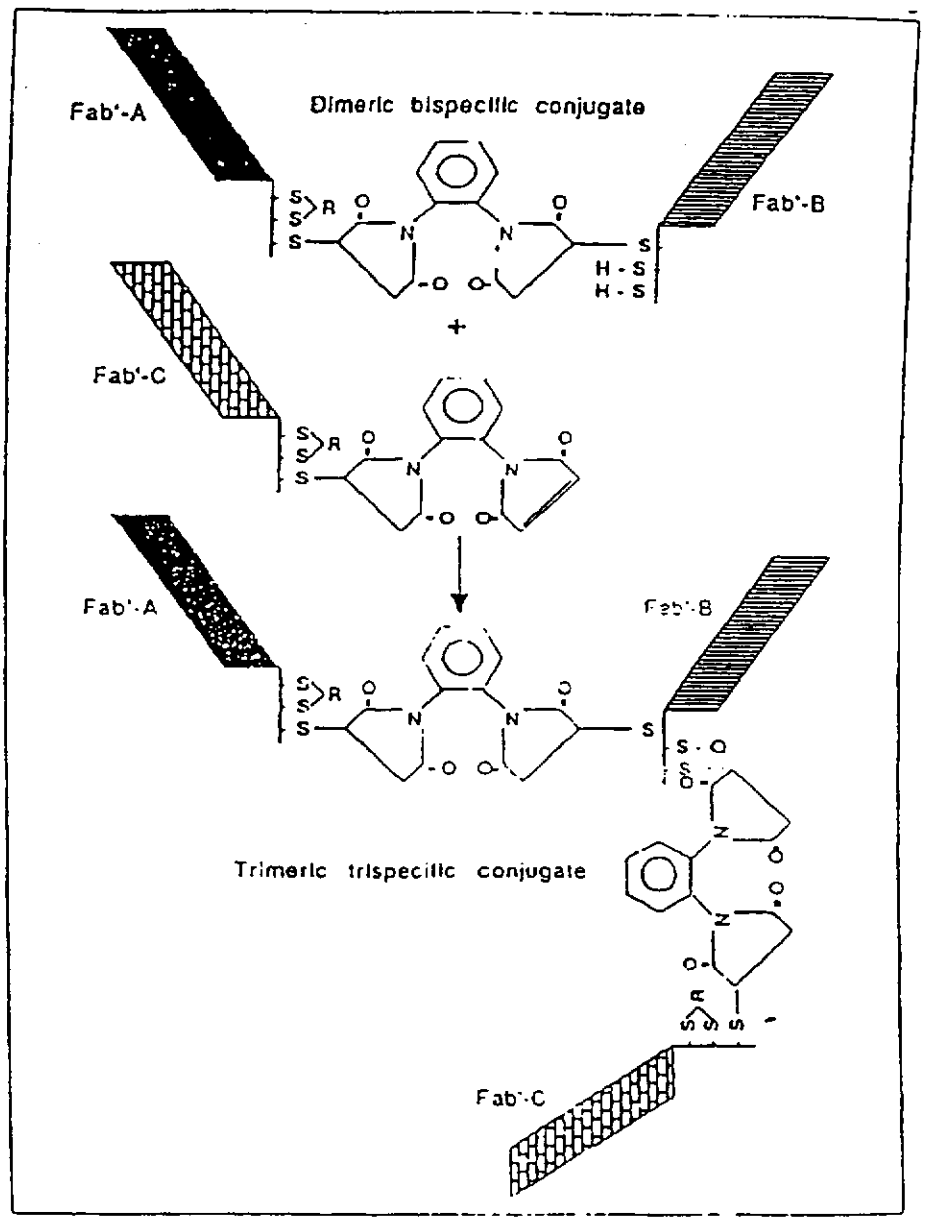

Fab'-A
Dimeric bispecific conjugate
Fab'-B
Fab'-C
Fab'-A
Fab'-B
Trimeric trispecific conjugate
Fab'-C

FIGURE 17

BIFUNCTIONAL FACS

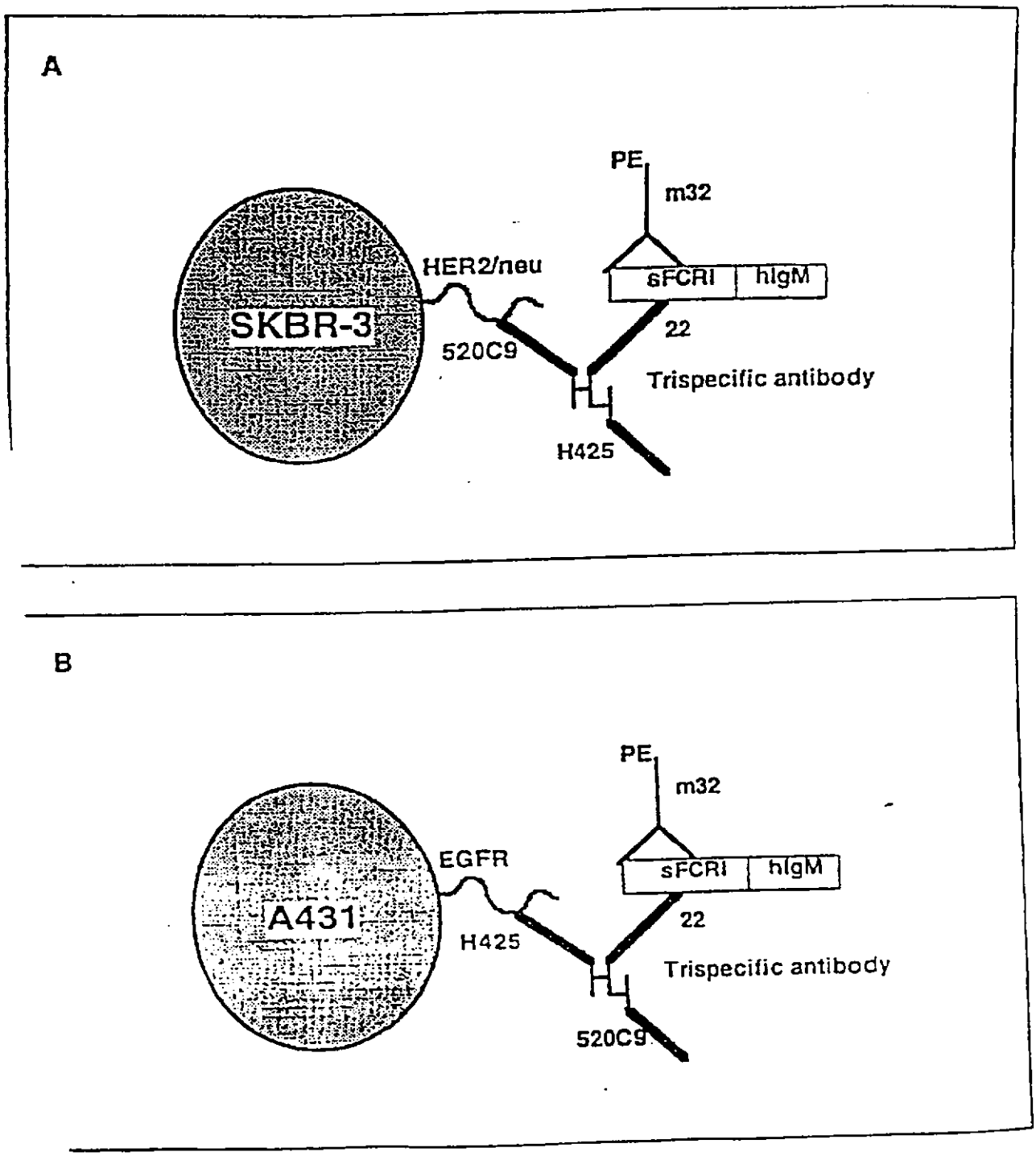


FIGURE 18

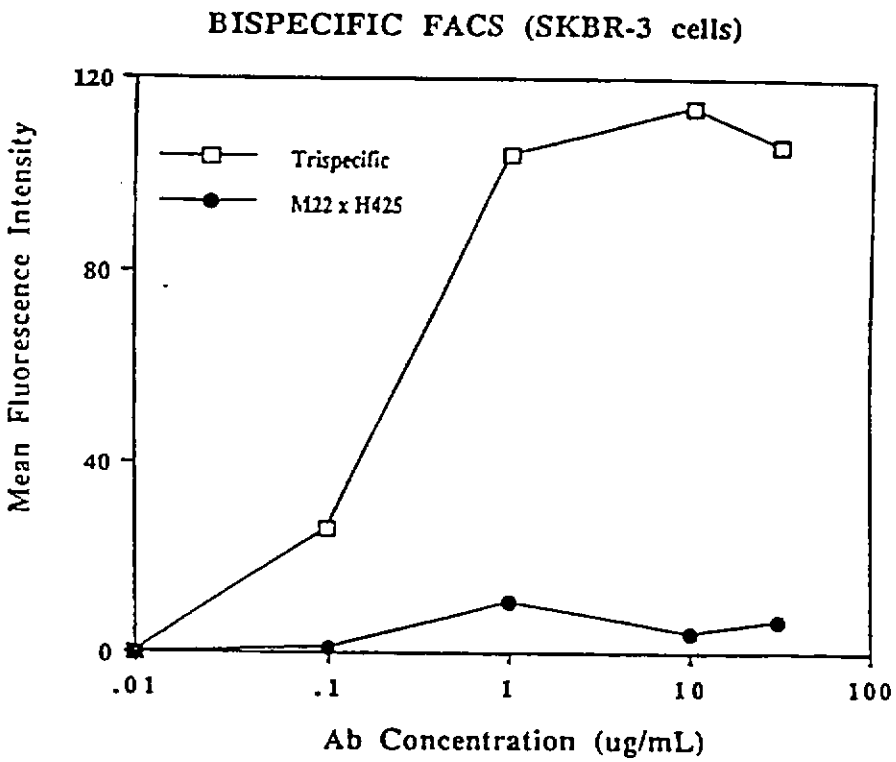

BISPECIFIC FACS (SKBR-3 cells)
Mean Fluorescence Intensity
120
80
40
0
Trispecific
M22 x H425
.01
.1
1
10
100
Ab Concentration (ug/mL)

FIGURE 19

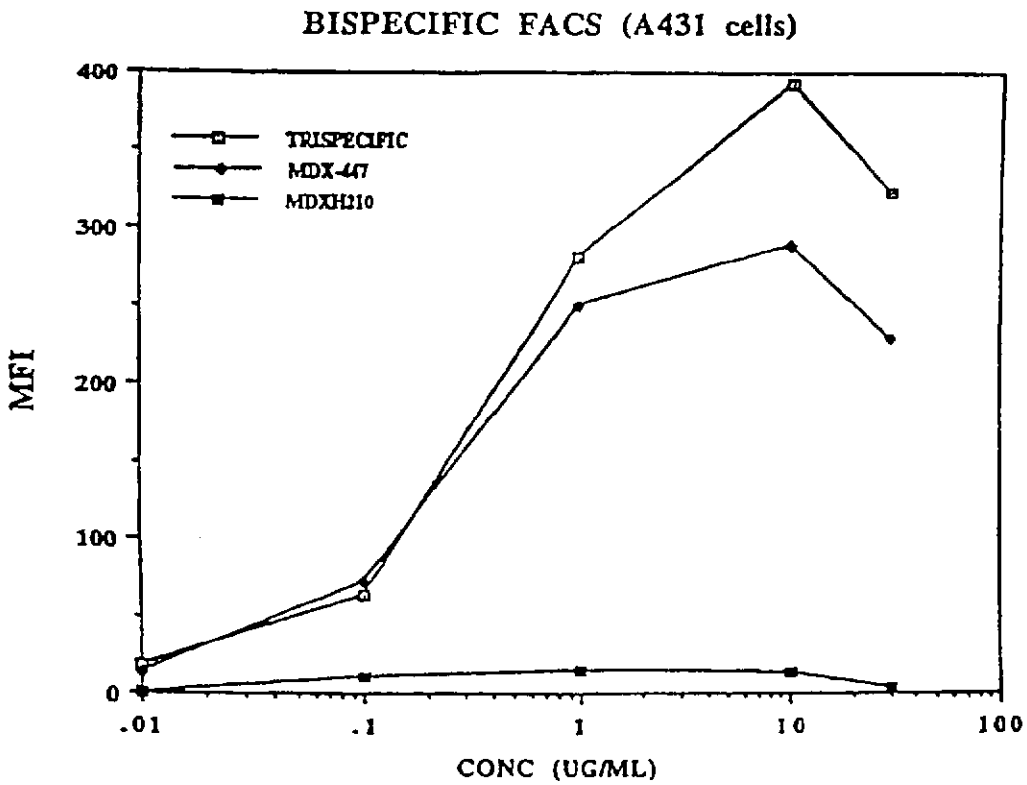

BISPECIFIC FACS (A431 cells)
400
300
200
100
0
MFI
TRISPECIFIC
MDX-447
MDXH210
.01
.1
1
10
100
CONC (UG/ML)

FIGURE 20

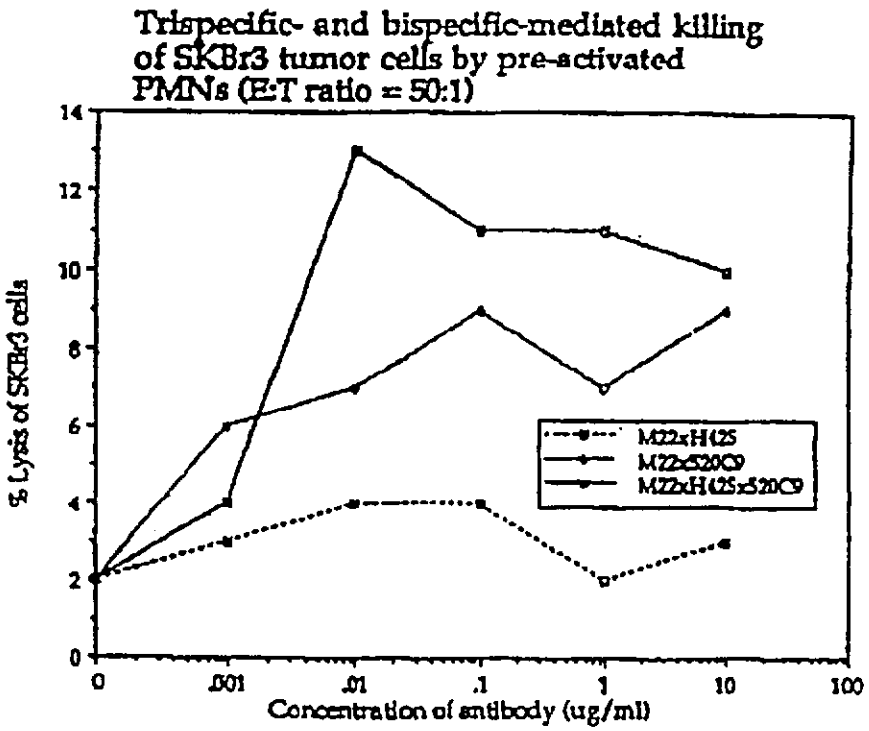

Trispecific- and bispecific-mediated killing of SKBr3 tumor cells by pre-activated PMNs (E:T ratio = 50:1)
% Lysis of SKBr3 cells
Concentration of antibody (ug/ml)
0
.001
.01
.1
1
10
100
2
4
6
8
10
12
14
M22xH125
M22x520C9
M22xH125x520C9

FIGURE 21

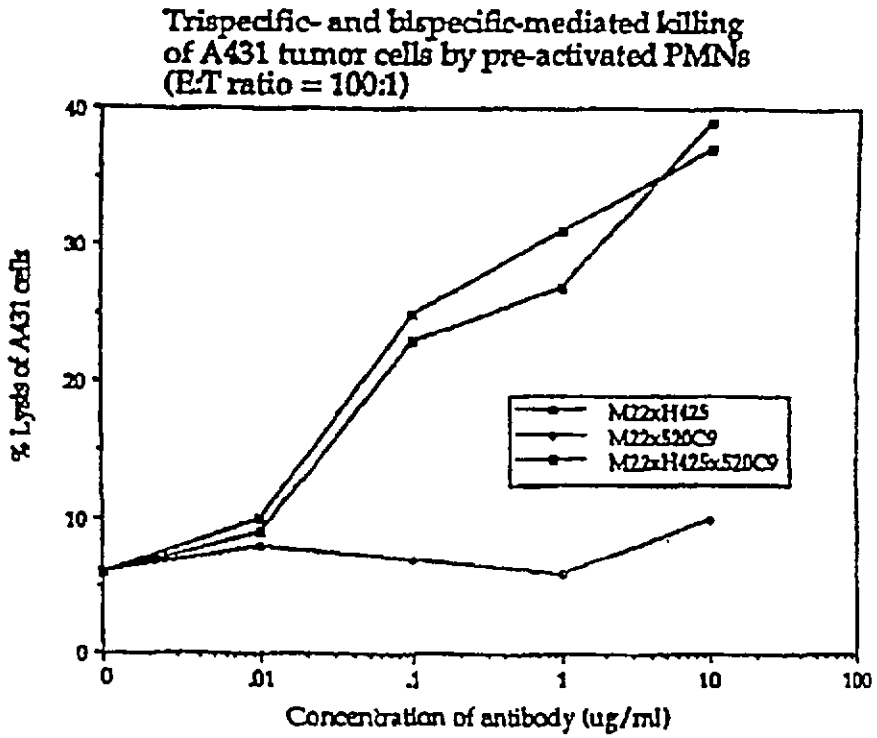

Trispecific- and bispecific-mediated killing
of A431 tumor cells by pre-activated PMNs
(E:T ratio = 100:1)
% Lysis of A431 cells
40
30
20
10
0
M22xH425
M22x520C9
M22xH425x520C9
0
.01
.1
1
10
100
Concentration of antibody (ug/ml)

FIGURE 22

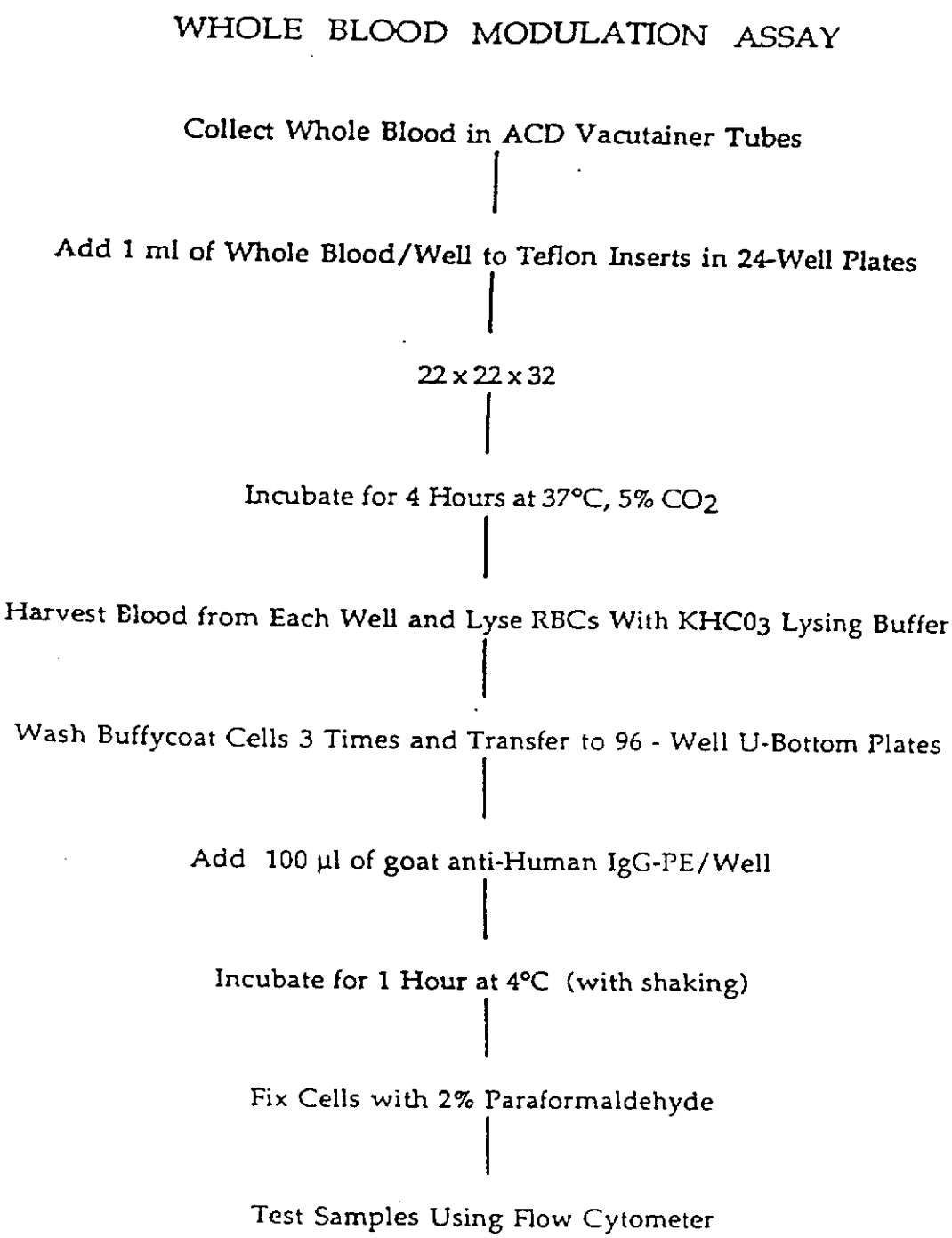

WHOLE BLOOD MODULATION ASSAY
Collect Whole Blood in ACD Vacutainer Tubes
Add 1 ml of Whole Blood/Well to Teflon Inserts in 24-Well Plates
22 x 22 x 32
Incubate for 4 Hours at 37°C, 5% CO2
Harvest Blood from Each Well and Lyse RBCs With KHC03 Lysing Buffer
Wash Buffycoat Cells 3 Times and Transfer to 96 - Well U-Bottom Plates
Add 100 µl of goat anti-Human IgG-PE/Well
Incubate for 1 Hour at 4°C (with shaking)
Fix Cells with 2% Paraformaldehyde
Test Samples Using Flow Cytometer

FIGURE 23A

MODULATION ASSAY USING m22xm22x32 TRISPECIFIC ANTIBOD

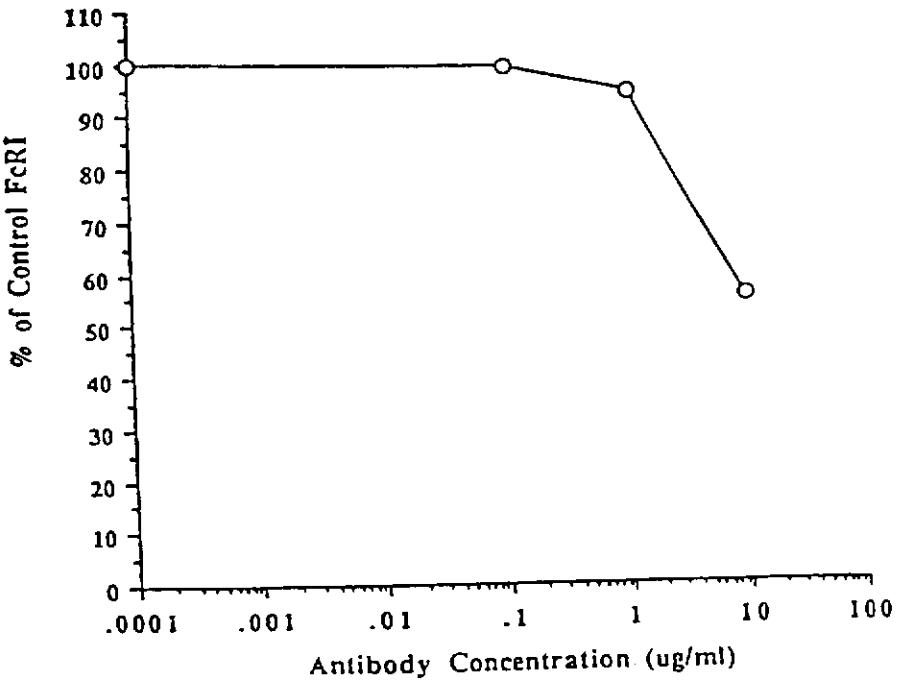


FIGURE 23B

A. Synthetic Oligonucleotides coding wildtype(TT830) and mutant (TT833S) TT peptides:

TT830: QYIKANSKFIGITEL (SEQ ID NO: 6)

5'-TCG AGC CAG TAC ATC AAG GCG AAT TCC AAG TTC ATC GGC ATC ACC GAG CTC TGA-3' (SEQ ID NO:7)

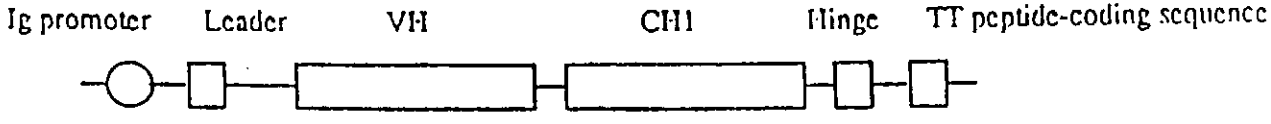
3'-CG GTC ATG TAG TTC CGC TTA AGG TTC AAG TAG CCG TAG TGG CTC GAG ACT CCG-5' (SEQ ID NO:8)

TT833S: QYISANSKFIGITEL (SEQ ID NO: 9)

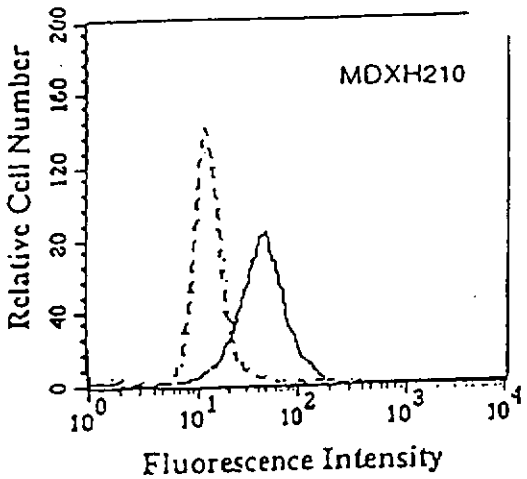
5'-TCG AG C CAG TAC ATC AGC GCG AAT TCC AAG TTC ATC GGC ATC ACC GAG CTC TGA-3' (SEQ ID NO:10)

3'-CG GTC ATG TAG TCG CGC TTA AGG TTC AAG TAG CCG TAG TGG CTC GAG ACT CCG-5' (SEQ ID NO:11)

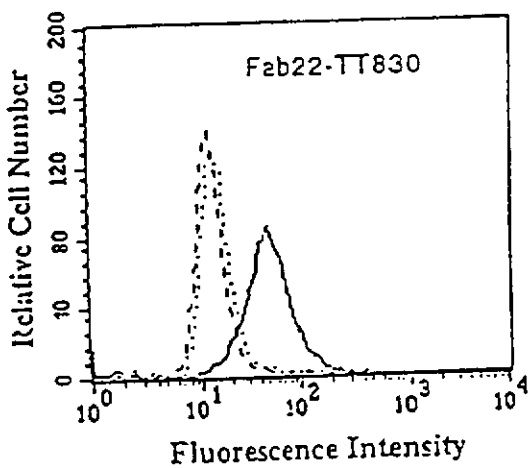
B. Final H22 chimeric constructs



A



B



C

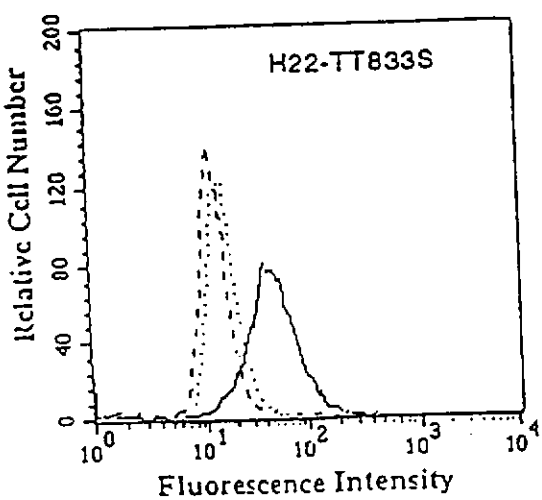


FIGURE 25

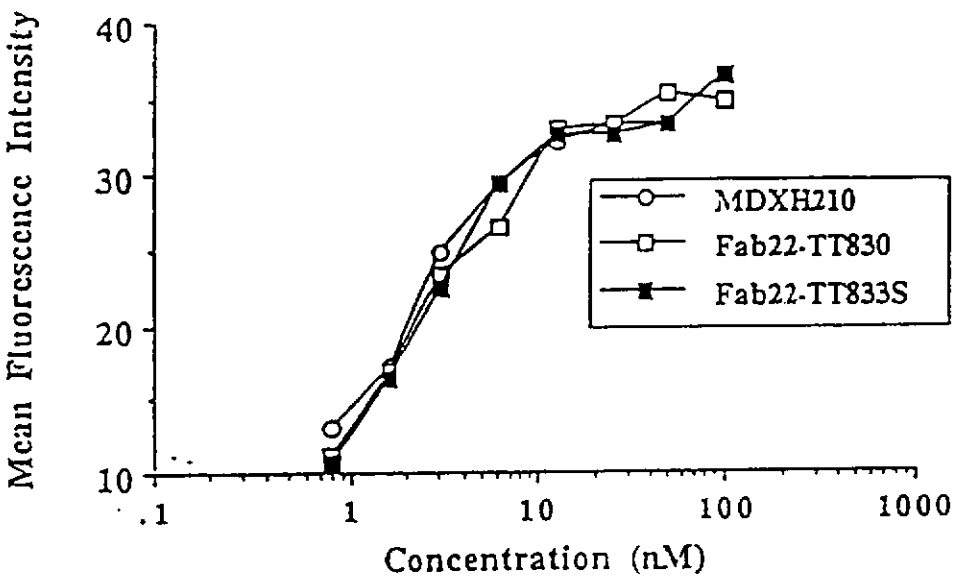

Mean Fluorescence Intensity
40
30
20
10
.1
1
10
100
1000
Concentration (nM)
MDXH210
Fab22-TT830
Fab22-TT833S

FIGURE 26

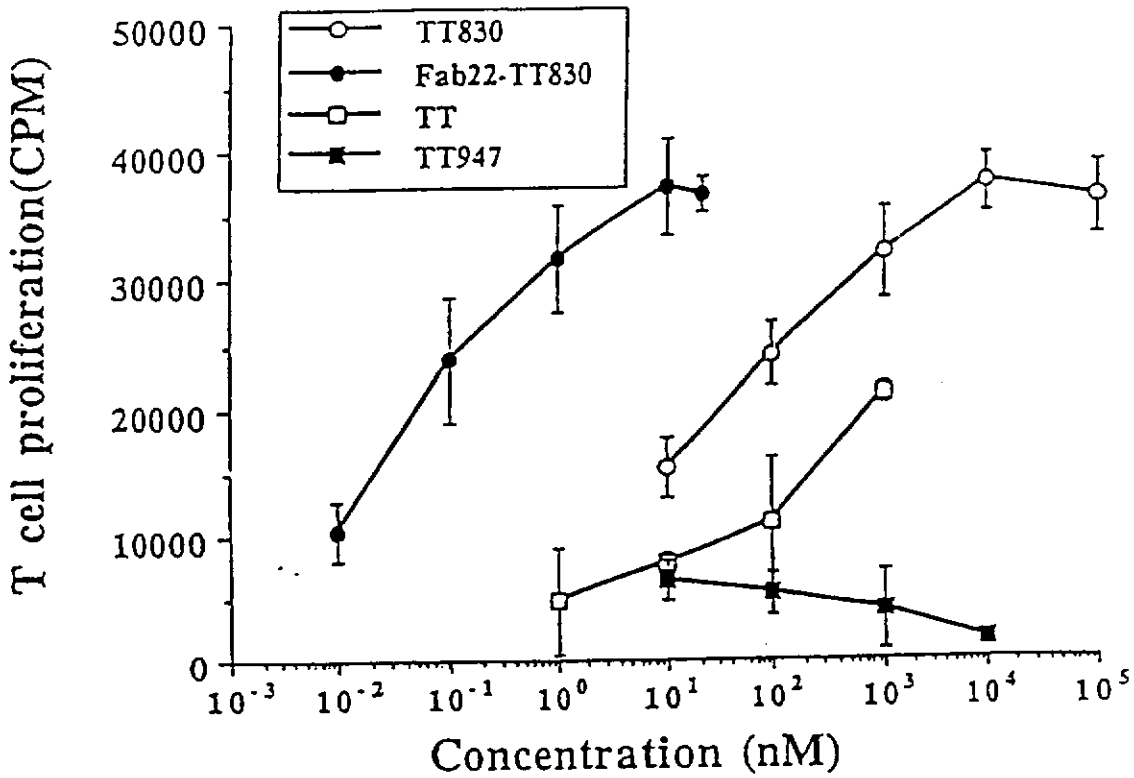

T cell proliferation(CPM)
50000
40000
30000
20000
10000
0
TT830
Fab22-TT830
TT
TT947
10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3} 10^{4} 10^{5}
Concentration (nM)

FIGURE 27

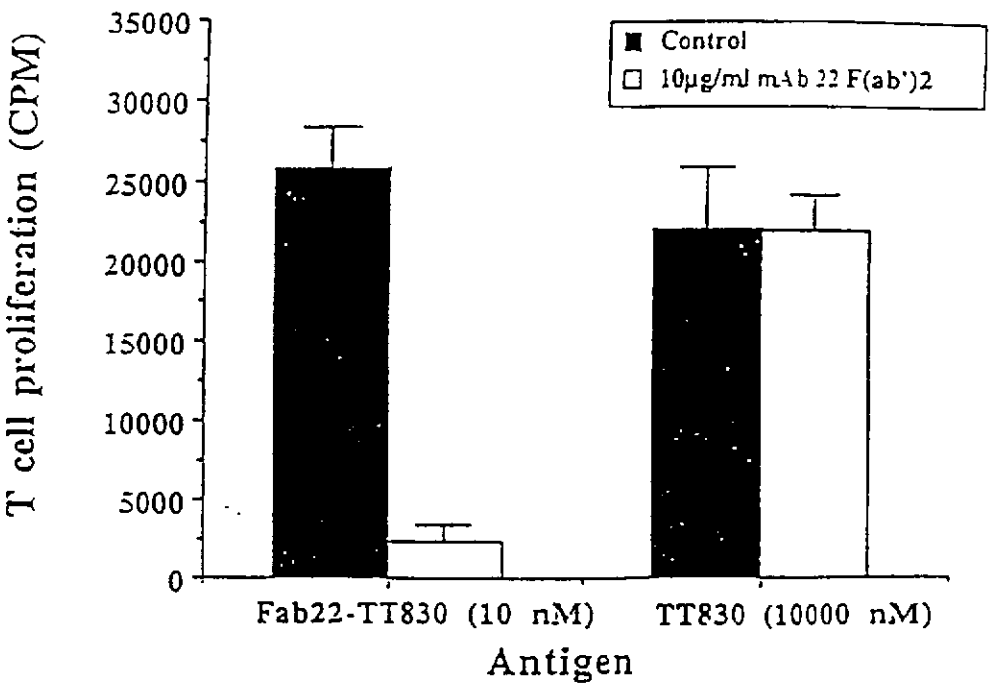

35000
30000
25000
20000
15000
10000
5000
0
T cell proliferation (CPM)
Control
10µg/ml mAb 22 F(ab')2
Fab22-TT830 (10 nM)
TT830 (10000 nM)
Antigen

FIGURE 28

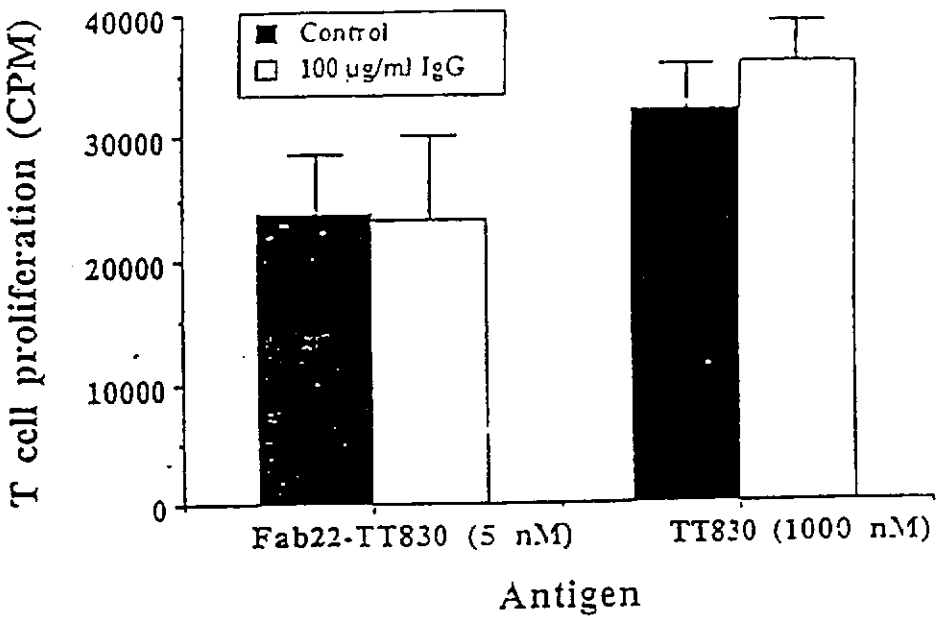

T cell proliferation (CPM)
40000
30000
20000
10000
0
Control
100 µg/ml IgG
Fab22-TT830 (5 nM)
TT830 (1000 nM)
Antigen

FIGURE 29

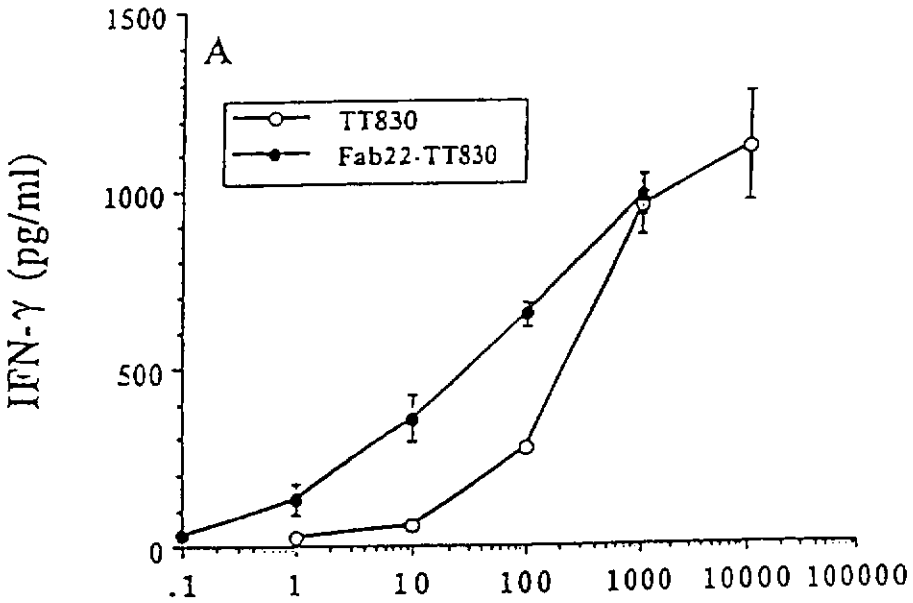


FIGURE 30A

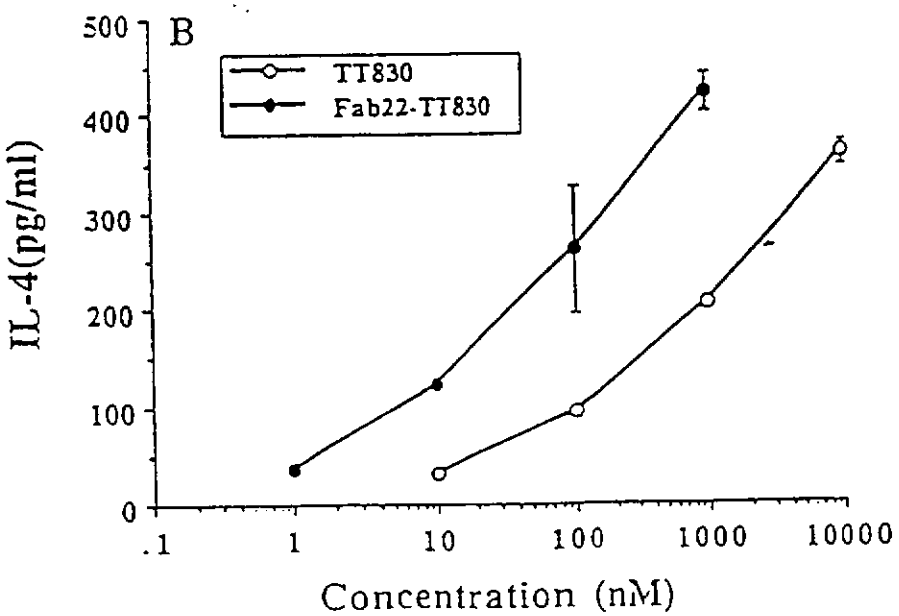


FIGURE 30B

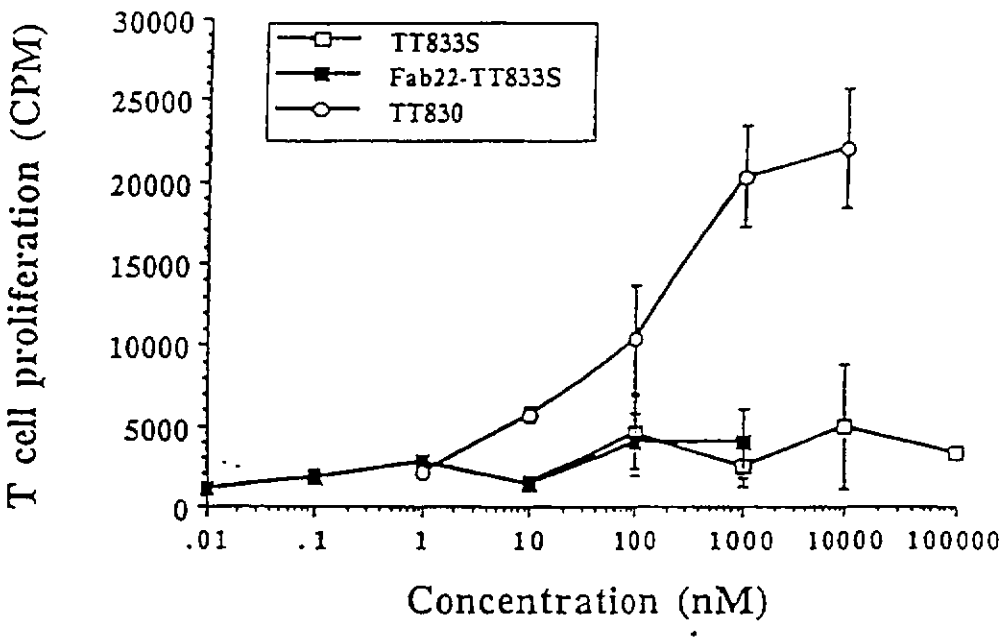

T cell proliferation (CPM)
30000
25000
20000
15000
10000
5000
0
TT833S
Fab22-TT833S
TT830
.01
.1
1
10
100
1000
10000
100000
Concentration (nM)

FIGURE 31

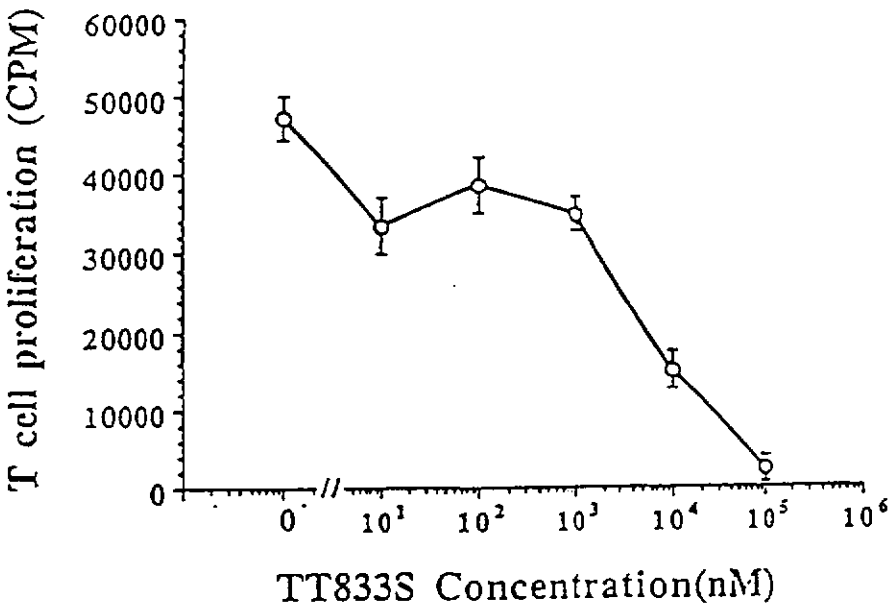

T cell proliferation (CPM)
60000
50000
40000
30000
20000
10000
0
0
10¹
10²
10³
10⁴
10⁵
10⁶
TT833S Concentration(nM)

FIGURE 32

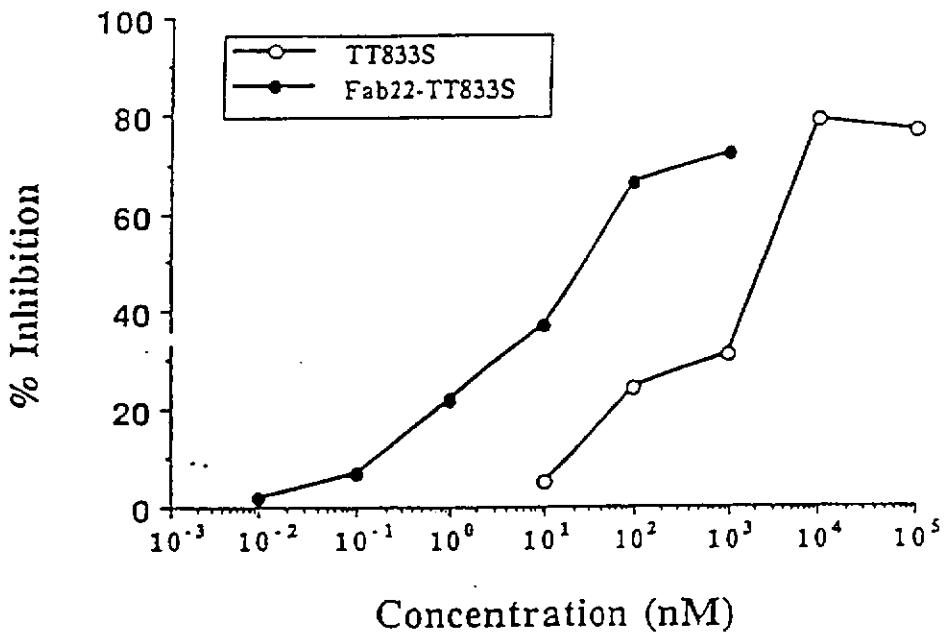

100
80
60
40
20
0
% Inhibition
TT833S
Fab22-TT833S
10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3} 10^{4} 10^{5}
Concentration (nM)

FIGURE 33

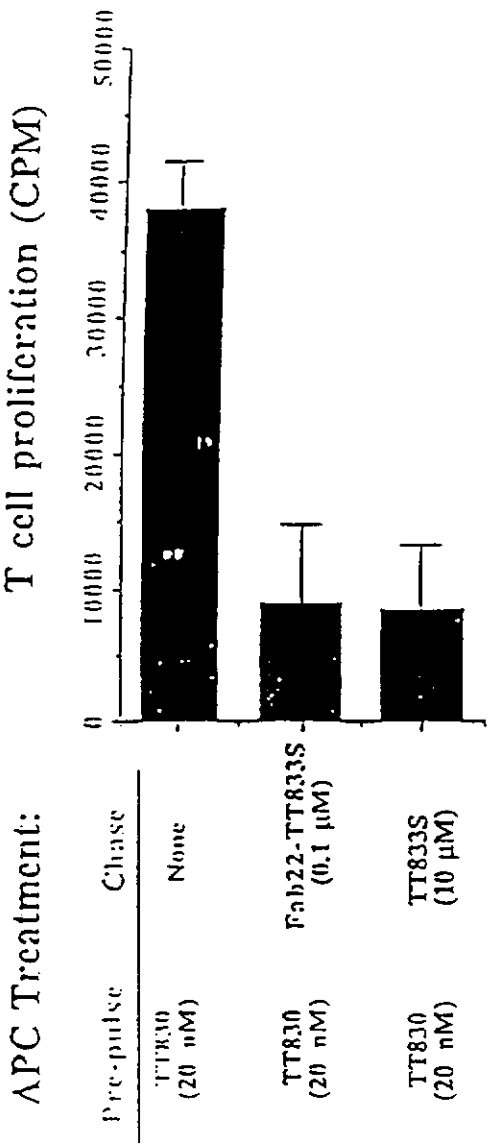

T cell proliferation (CPM)
0
10000
20000
30000
40000
50000
APC Treatment:
Pre-pulse
Chase
TT830 (20 nM)
None
TT830 (20 nM)
Fab22-TT833S (0.1 µM)
TT830 (20 nM)
TT833S (10 µM)

FIGURE 34

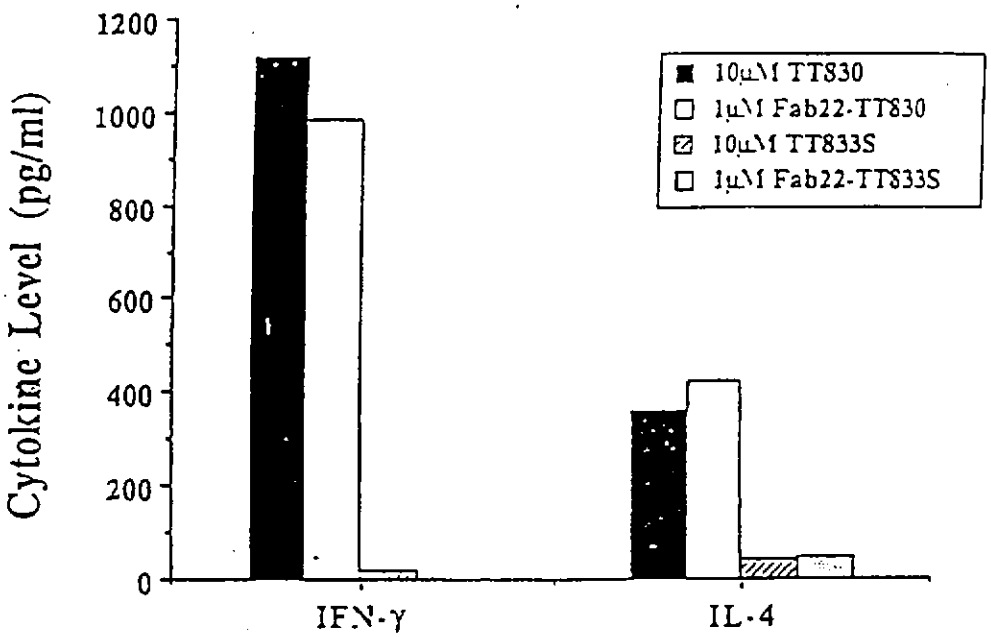

Cytokine Level (pg/ml)
1200
1000
800
600
400
200
0
10μM TT830
1μM Fab22-TT830
10μM TT833S
1μM Fab22-TT833S
IFN-γ
IL-4

FIGURE 35

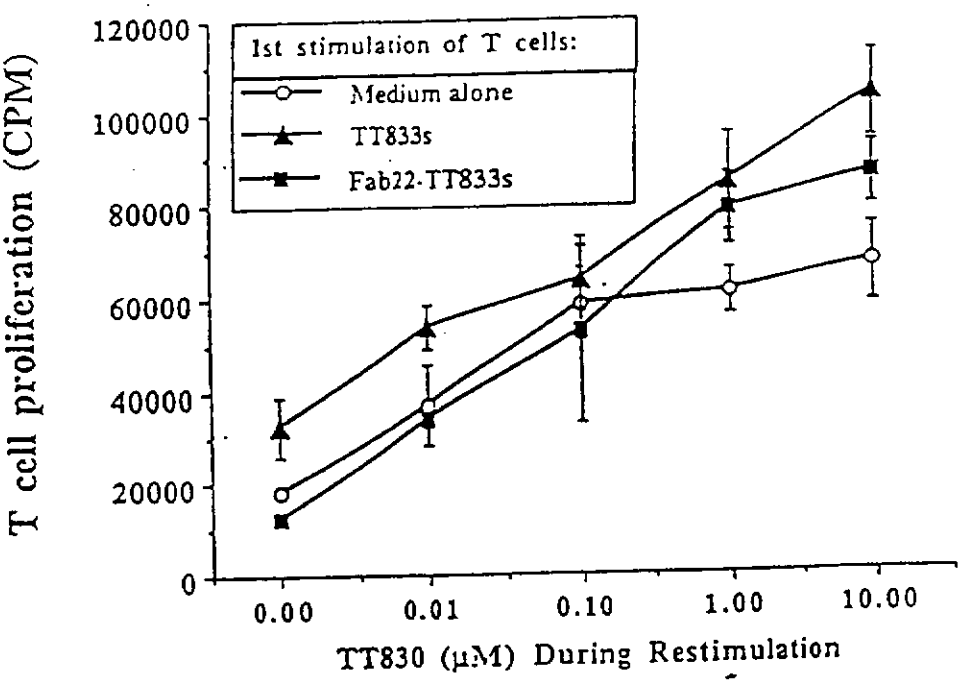

T cell proliferation (CPM)
120000
100000
80000
60000
40000
20000
0
1st stimulation of T cells:
Medium alone
TT833s
Fab22-TT833s
0.00
0.01
0.10
1.00
10.00
TT830 (μM) During Restimulation

FIGURE 36

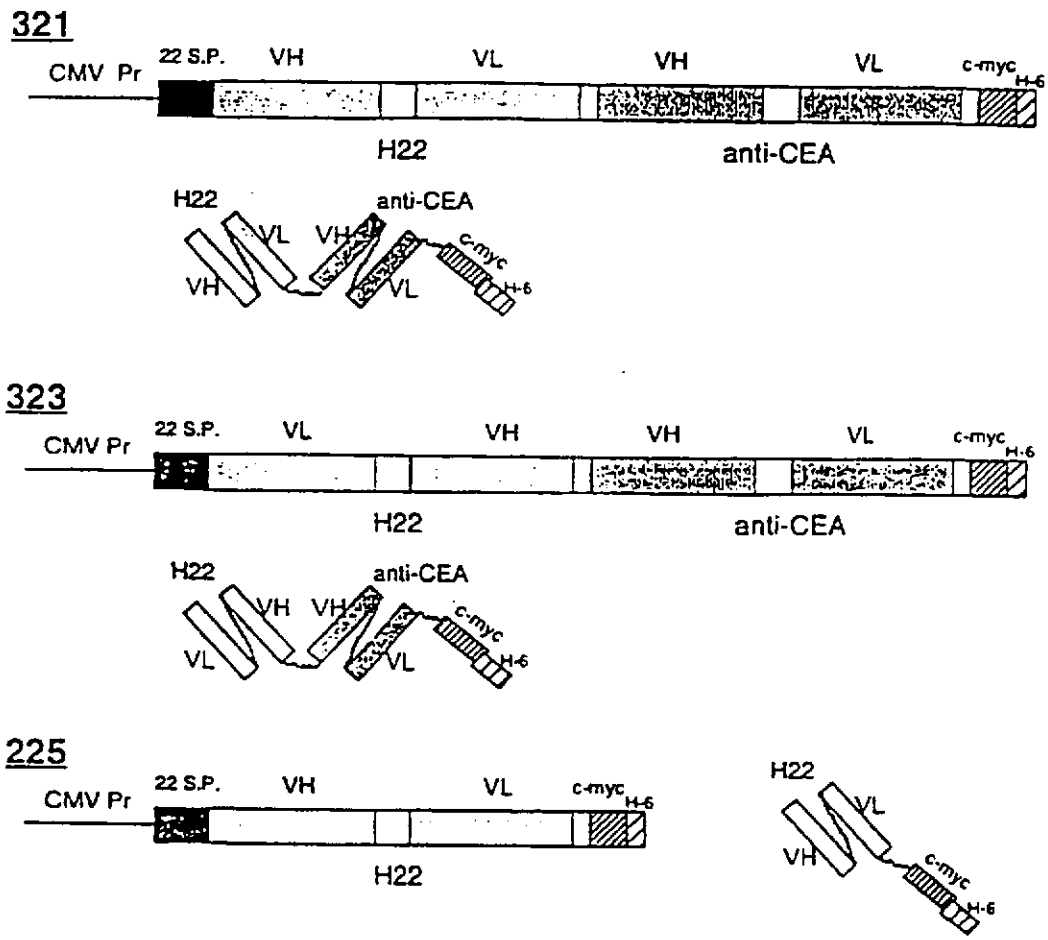

321
CMV Pr
22 S.P.
VH
VL
VH
VL
c-myc
H-6
H22
anti-CEA
H22
anti-CEA
VL
VH
VH
VL
c-myc
H-6
323
CMV Pr
22 S.P.
VL
VH
VH
VL
c-myc
H-6
H22
anti-CEA
H22
anti-CEA
VH
VH
VL
VL
c-myc
H-6
225
CMV Pr
22 S.P.
VH
VL
c-myc
H-6
H22
H22
VL
VH
c-myc
H-6
= (Gly-4, Ser)-3 linker
= (Gly-4, Ser)-1 linker

FIGURE 37

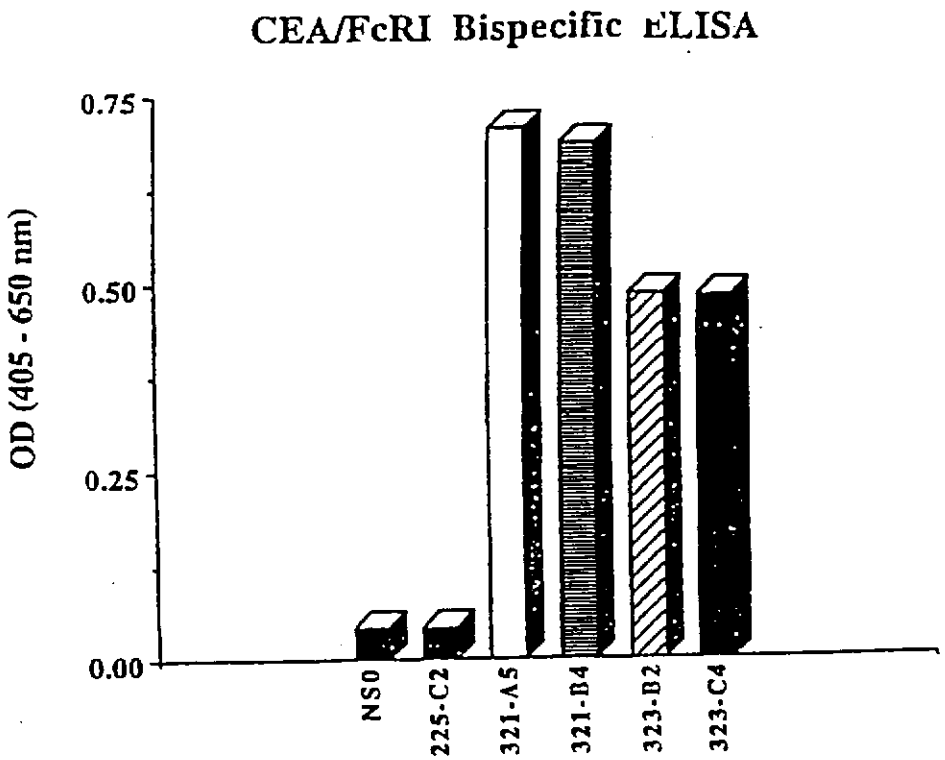

CEA/FcRI Bispecific ELISA
OD (405 - 650 nm)
0.75
0.50
0.25
0.00
NS0
225-C2
321-A5
321-B4
323-B2
323-C4

FIGURE 38

225 SEQ ID NO:13

Sequence Range: 1 to 923

```
         10          20          30          40          50
         .   .       .   .       .   .       .   .       .   .
AAGCTTCACC ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTG GCC ACA GCT
TTCGAAGTGG TAC CCT ACC TCG ACA TAG TAG GAG AAG AAC CAC CGG TGT CGA
           Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala>
           ___a___a___a_TRANSLATION OF H22 SFV (225)__a___a___a___>

        60  Signal   70          HzzVH 80          90          100
         .   .  Sequence|  .   .       .   .       .   .       .   .
ACC GGT GTC CAC TCC GAT ATC CAA CTG GTG GAG AGC GGT GGA GGT GTT
TGG CCA CAG GTG AGG CTA TAG GTT GAC CAC CTC TCG CCA CCT CCA CAA
Thr Gly Val His Ser Asp Ile Gln Leu Val Glu Ser Gly Gly Gly Val>
___a___a___a___a_TRANSLATION OF H22 SFV (225)__a___a___a___a___>
                    Glu 120  Val
           110          120         130         140
            .   .       .   .       .   .       .   .
GTG CAA CCT GGC CGG TCC CTG CGC CTG TCC TGC TCC TCG TCT GGC TTC
CAC GTT GGA CCG GCC AGG GAC GCG GAC AGG ACG AGG AGC AGA CCG AAG
Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Ser Ser Ser Gly Phe>
___a___a___a___a_TRANSLATION OF H22 SFV (225)__a___a___a___a___>

150         160         170         180         190
 .   .       .   .       .   .       .   .       .   .
ATT TTC AGT GAC AAT TAC ATG TAT TGG GTG AGA CAG GCA CCT GGA AAA
TAA AAG TCA CTG TTA ATG TAC ATA ACC CAC TCT GTC CGT GGA CCT TTT
Ile Phe Ser Asp Asn Tyr Met Tyr Trp Val Arg Gln Ala Pro Gly Lys>
___a___a___a___a_TRANSLATION OF H22 SFV (225)__a___a___a___a___>

  200         210         220         230         240
   .   .       .   .       .   .       .   .       .   .
GGT CTT GAG TGG GTT GCA ACC ATT AGT GAT GGT GGT AGT TAC ACC TAC
CCA GAA CTC ACC CAA CGT TGG TAA TCA CTA CCA CCA TCA ATG TGG ATG
Gly Leu Glu Trp Val Ala Thr Ile Ser Asp Gly Gly Ser Tyr Thr Tyr>
___a___a___a___a_TRANSLATION OF H22 SFV (225)__a___a___a___a___>

    250         260         270         280         290
     .   .       .   .       .   .       .   .       .   .
TAT CCA GAC AGT GTG AAG GGA AGA TTT ACA ATA TCG AGA GAC AAC AGC
ATA GGT CTG TCA CAC TTC CCT TCT AAA TGT TAT AGC TCT CTG TTG TCG
Tyr Pro Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser>
___a___a___a___a_TRANSLATION OF H22 SFV (225)__a___a___a___a___>

        300         310         320         330         340
         .   .       .   .       .   .       .   .       .   .
AAG AAC ACA TTG TTC CTG CAA ATG GAC AGC CTG AGA CCC GAA GAC ACC
TTC TTG TGT AAC AAG GAC GTT TAC CTG TCG GAC TCT GGG CTT CTG TGG
Lys Asn Thr Leu Phe Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr>
___a___a___a___a_TRANSLATION OF H22 SFV (225)__a___a___a___a___>

            350         360         370         380
             .   .       .   .       .   .       .   .
GGG GTC TAT TTT TGT GCA AGA GGC TAC TAT AGG TAC GAG GGG GCT ATG
CCC CAG ATA AAA ACA CGT TCT CCG ATG ATA TCC ATG CTC CCC CGA TAC
Gly Val Tyr Phe Cys Ala Arg Gly Tyr Tyr Arg Tyr Glu Gly Ala Met>
___a___a___a___a_TRANSLATION OF H22 SFV (225)__a___a___a___a___>

390         400         410         420  HzzVH  430  Linker
 .   .       .   .       .   .       .   .       .   .
GAC TAC TGG GGC CAA GGG ACC CCG GTC ACC GTG AGC TCA|GGA GGT GGC
CTG ATG ACC CCG GTT CCC TGG GGC CAG TGG CAC TCG AGT|CCT CCA CCG
```

FIGURE 39A

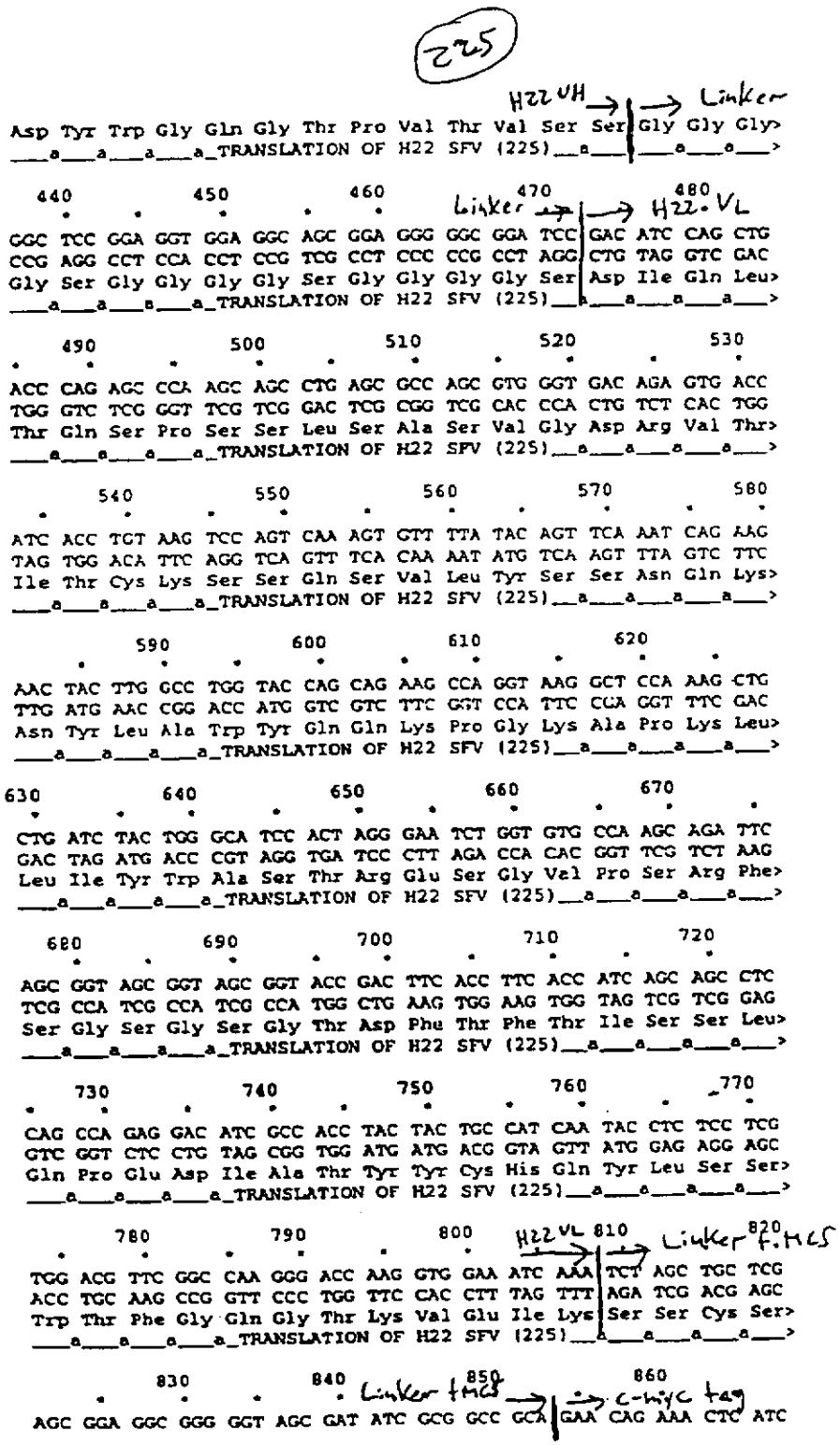

225
H22 VH
Linker
Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly Gly>
___a___a___a___a_TRANSLATION OF H22 SFV (225)__a___a___a___a___>
440 450 460 470 480
Linker
H22 VL
GGC TCC GGA GGT GGA GGC AGC GGA GGG GGC GGA TCC GAC ATC CAG CTG
CCG AGG CCT CCA CCT CCG TCG CCT CCC CCG CCT AGG CTG TAG GTC GAC
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Leu>
___a___a___a___a_TRANSLATION OF H22 SFV (225)__a___a___a___a___>
490 500 510 520 530
ACC CAG AGC CCA AGC AGC CTG AGC GCC AGC GTG GGT GAC AGA GTG ACC
TGG GTC TCG GGT TCG TCG GAC TCG CGG TCG CAC CCA CTG TCT CAC TGG
Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr>
___a___a___a___a_TRANSLATION OF H22 SFV (225)__a___a___a___a___>
540 550 560 570 580
ATC ACC TGT AAG TCC AGT CAA AGT GTT TTA TAC AGT TCA AAT CAG AAG
TAG TGG ACA TTC AGG TCA GTT TCA CAA AAT ATG TCA AGT TTA GTC TTC
Ile Thr Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser Ser Asn Gln Lys>
___a___a___a___a_TRANSLATION OF H22 SFV (225)__a___a___a___a___>
590 600 610 620
AAC TAC TTG GCC TGG TAC CAG CAG AAG CCA GGT AAG GCT CCA AAG CTG
TTG ATG AAC CGG ACC ATG GTC GTC TTC GGT CCA TTC CGA GGT TTC GAC
Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu>
___a___a___a___a_TRANSLATION OF H22 SFV (225)__a___a___a___a___>
630 640 650 660 670
CTG ATC TAC TGG GCA TCC ACT AGG GAA TCT GGT GTG CCA AGC AGA TTC
GAC TAG ATG ACC CGT AGG TGA TCC CTT AGA CCA CAC GGT TCG TCT AAG
Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val Pro Ser Arg Phe>
___a___a___a___a_TRANSLATION OF H22 SFV (225)__a___a___a___a___>
680 690 700 710 720
AGC GGT AGC GGT AGC GGT ACC GAC TTC ACC TTC ACC ATC AGC AGC CTC
TCG CCA TCG CCA TCG CCA TGG CTG AAG TGG AAG TGG TAG TCG TCG GAG
Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu>
___a___a___a___a_TRANSLATION OF H22 SFV (225)__a___a___a___a___>
730 740 750 760 770
CAG CCA GAG GAC ATC GCC ACC TAC TAC TGC CAT CAA TAC CTC TCC TCG
GTC GGT CTC CTG TAG CGG TGG ATG ATG ACG GTA GTT ATG GAG AGG AGC
Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys His Gln Tyr Leu Ser Ser>
___a___a___a___a_TRANSLATION OF H22 SFV (225)__a___a___a___a___>
780 790 800 H22 VL 810 Linker 820 f.HC5
TGG ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA TCT AGC TGC TCG
ACC TGC AAG CCG GTT CCC TGG TTC CAC CTT TAG TTT AGA TCG ACG AGC
Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Ser Ser Cys Ser>
___a___a___a___a_TRANSLATION OF H22 SFV (225)__a___a___a___a___>
830 840 Linker fHC5 850 860 c-myc tag
AGC GGA GGC GGG GGT AGC GAT ATC GCG GCC GCA GAA CAG AAA CTC ATC

FIGURE 39B

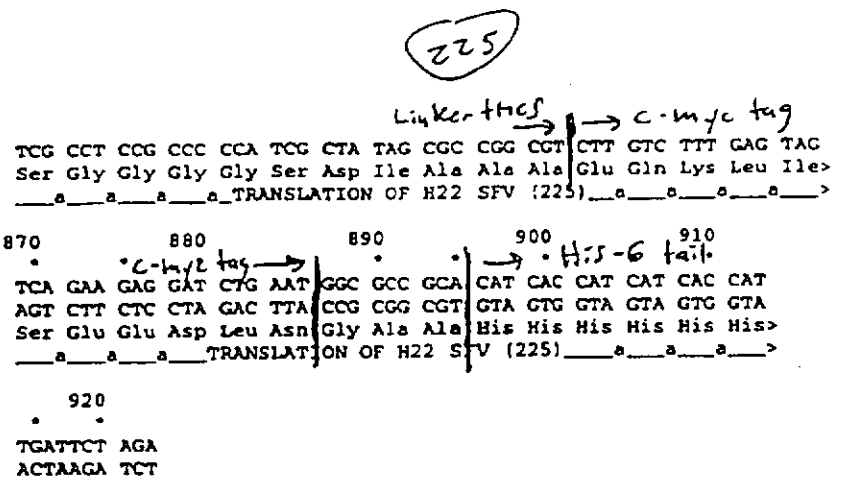

225
Linker-thcs
c-myc tag
TCG CCT CCG CCC CCA TCG CTA TAG CGC CGG CGT CTT GTC TTT GAG TAG
Ser Gly Gly Gly Gly Ser Asp Ile Ala Ala Ala Glu Gln Lys Leu Ile>
___a___a___a___a_TRANSLATION OF H22 SFV (225)__a___a___a___a___>
870
880
890
900
910
c-myc tag
His-6 tail
TCA GAA GAG GAT CTG AAT GGC GCC GCA CAT CAC CAT CAT CAC CAT
AGT CTT CTC CTA GAC TTA CCG CGG CGT GTA GTG GTA GTA GTG GTA
Ser Glu Glu Asp Leu Asn Gly Ala Ala His His His His His His>
___a___a___a___TRANSLATION OF H22 SFV (225)____a___a___a___>
920
TGATTCT AGA
ACTAAGA TCT

FIGURE 39C

321 SEQ ID NO: 15

Sequence Range: 1 to 1679

```
          10          20          30          40          50
          .   .       .   .       .   .       .   .       .   .
AAGCTTCACC ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTG GCC ACA GCT
TTCGAAGTGG TAC CCT ACC TCG ACA TAG TAG GAG AAG AAC CAC CGG TGT CGA
           Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala>
           ___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) [A]___a___>

          60 Signal      70 H22 VH  80          90          100
          .   Sequence>  .   .       .   .       .   .       .   .
ACC GGT GTC CAC TCC GAT ATC CAA CTG GTG GAG AGC GGT GGA GGT GTT
TGG CCA CAG GTG AGG CTA TAG GTT GAC CAC CTC TCG CCA CCT CCA CAA
Thr Gly Val His Ser Asp Ile Gln Leu Val Glu Ser Gly Gly Gly Val>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) [A]___a___a___>
                    Gln  Val.

          110         120         130         140
          .   .       .   .       .   .       .   .       .
GTG CAA CCT GGC CGG TCC CTG CGC CTG TCC TGC TCC TCG TCT GGC TTC
CAC GTT GGA CCG GCC AGG GAC GCG GAC AGG ACG AGG AGC AGA CCG AAG
Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Ser Ser Ser Gly Phe>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) [A]___a___a___>

150         160         170         180         190
  .   .       .   .       .   .       .   .       .   .
ATT TTC AGT GAC AAT TAC ATG TAT TGG GTG AGA CAG GCA CCT GGA AAA
TAA AAG TCA CTG TTA ATG TAC ATA ACC CAC TCT GTC CGT GGA CCT TTT
Ile Phe Ser Asp Asn Tyr Met Tyr Trp Val Arg Gln Ala Pro Gly Lys>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) [A]___a___a___>

  200         210         220         230         240
    .   .       .   .       .   .       .   .       .   .
GGT CTT GAG TGG GTT GCA ACC ATT AGT GAT GGT GGT AGT TAC ACC TAC
CCA GAA CTC ACC CAA CGT TGG TAA TCA CTA CCA CCA TCA ATG TGG ATG
Gly Leu Glu Trp Val Ala Thr Ile Ser Asp Gly Gly Ser Tyr Thr Tyr>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) [A]___a___a___>

    250         260         270         280         290
  .   .       .   .       .   .       .   .       .   .
TAT CCA GAC AGT GTG AAG GGA AGA TTT ACA ATA TCG AGA GAC AAC AGC
ATA GGT CTG TCA CAC TTC CCT TCT AAA TGT TAT AGC TCT CTG TTG TCG
Tyr Pro Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) [A]___a___a___>

      300         310         320         330         340
    .   .       .   .       .   .       .   .       .   .
AAG AAC ACA TTG TTC CTG CAA ATG GAC AGC CTG AGA CCC GAA GAC ACC
TTC TTG TGT AAC AAG GAC GTT TAC CTC TCG GAC TCT GGG CTT CTG TGG
Lys Asn Thr Leu Phe Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) [A]___a___a___>

          350         360         370         380
          .   .       .   .       .   .       .   .       .
GGG GTC TAT TTT TGT GCA AGA GGC TAC TAT AGG TAC GAG GGG GCT ATG
CCC CAG ATA AAA ACA CGT TCT CCG ATG ATA TCC ATG CTC CCC CGA TAC
Gly Val Tyr Phe Cys Ala Arg Gly Tyr Tyr Arg Tyr Glu Gly Ala Met>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) [A]___a___a___>

390         400         410         420 H22 VH  430 Linker
  .   .       .   .       .   .       .   .       .   .
GAC TAC TGG GGC CAA GGG ACC CCG GTC ACC GTG AGC TCA GGA GGT GGC
CTG ATG ACC CCG GTT CCC TGG GGC CAG TGG CAC TCG AGT CCT CCA CCG
```

FIGURE 40A

H22 VH → | → Linker

Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser | Gly Gly Gly>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) [A]___a___a___>

440 450 460 Linker 470 480 H22 VL

GGC TCC GGA GGT GGA GGC AGC GGA GGG GGC GGA TCC | GAC ATC CAG CTG
CCG AGG CCT CCA CCT CCG TCG CCT CCC CCG CCT AGG | CTG TAG GTC GAC
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser | Asp Ile Gln Leu>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) [A]___a___a___>

490 500 510 520 530

ACC CAG AGC CCA AGC AGC CTG AGC GCC AGC GTG GGT GAC AGA GTG ACC
TGG GTC TCG GGT TCG TCG GAC TCG CGG TCG CAC CCA CTG TCT CAC TGG
Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) [A]___a___a___>

540 550 560 570 580

ATC ACC TGT AAG TCC AGT CAA AGT GTT TTA TAC AGT TCA AAT CAG AAG
TAG TGG ACA TTC AGG TCA GTT TCA CAA AAT ATG TCA AGT TTA GTC TTC
Ile Thr Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser Ser Asn Gln Lys>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) [A]___a___a___>

590 600 610 620

AAC TAC TTG GCC TGG TAC CAG CAG AAG CCA GGT AAG GCT CCA AAG CTG
TTG ATG AAC CGG ACC ATG GTC GTC TTC GGT CCA TTC CGA GGT TTC GAC
Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) [A]___a___a___>

630 640 650 660 670

CTG ATC TAC TGG GCA TCC ACT AGG GAA TCT GGT GTG CCA AGC AGA TTC
GAC TAG ATG ACC CGT AGG TGA TCC CTT AGA CCA CAC GGT TCG TCT AAG
Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val Pro Ser Arg Phe>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) [A]___a___a___>

680 690 700 710 720

AGC GGT AGC GGT AGC GGT ACC GAC TTC ACC TTC ACC ATC AGC AGC CTC
TCG CCA TCG CCA TCG CCA TGG CTG AAG TGG AAG TGG TAG TCG TCG GAG
Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) [A]___a___a___>

730 740 750 760 770

CAG CCA GAG GAC ATC GCC ACC TAC TAC TGC CAT CAA TAC CTC TCC TCG
GTC GGT CTC CTG TAG CGG TGG ATG ATG ACG GTA GTT ATG GAG AGG AGC
Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys His Gln Tyr Leu Ser Ser>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) [A]___a___a___>

780 790 800 H22 VL → 810 → Linker → 820

TGG ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA | TCT AGC TGC TCG
ACC TGC AAG CCG GTT CCC TGG TTC CAC CTT TAG TTT | AGA TCG ACG AGC
Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys | Ser Ser Cys Ser>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) [A]___a___a___>

830 Linker → 840 → MFE-23 850 VH 860

AGC GGA GGC GGG GGT AGC | GAT ATC AAA CTG CAG CAG TCT GGG GCA GAA

FIGURE 40B

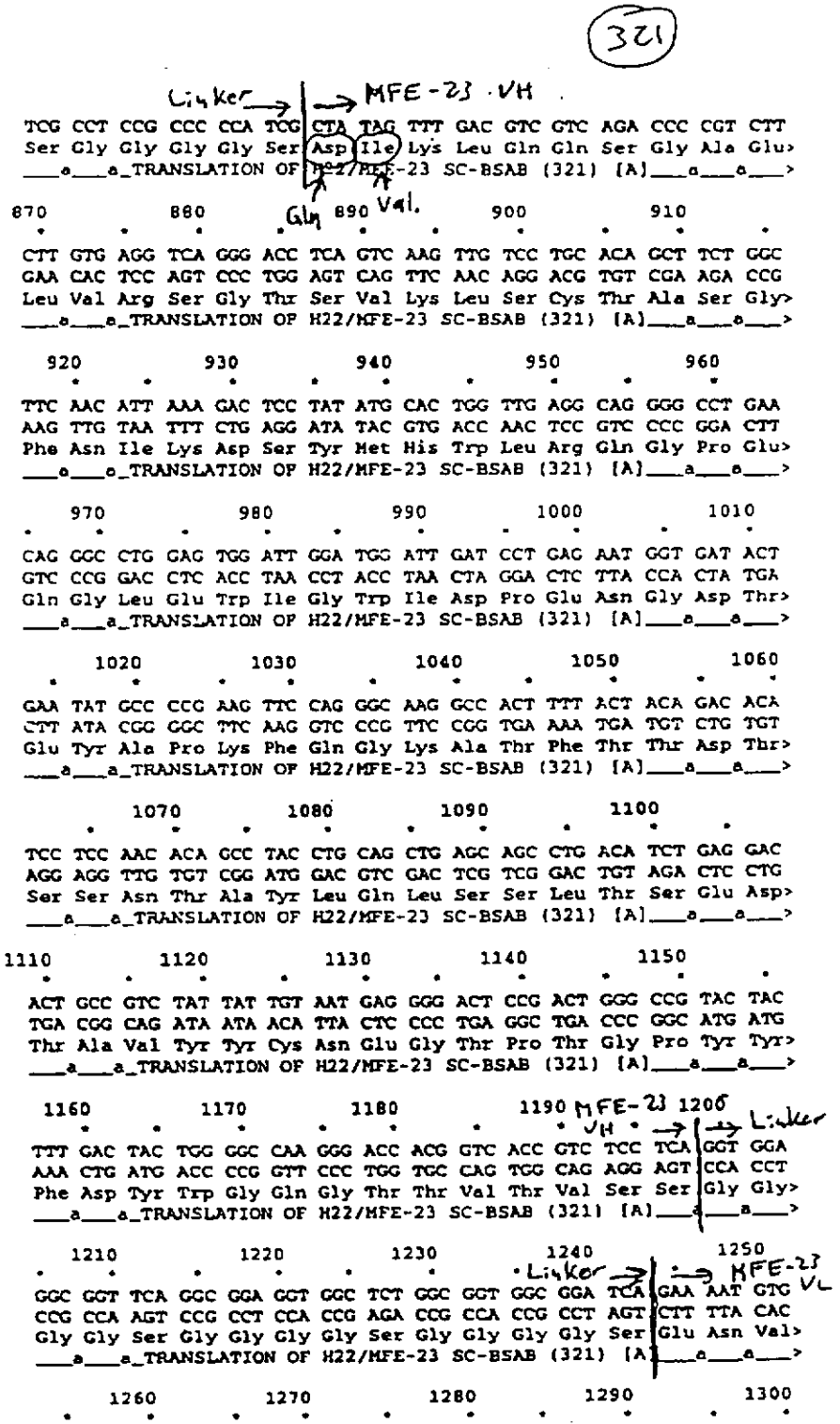

321
Linker
MFE-23 VH
TCG CCT CCG CCC CCA TCG CTA TAG TTT GAC GTC GTC AGA CCC CGT CTT
Ser Gly Gly Gly Gly Ser Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) [A]___a___a___>
Gln
Val.
870 880 890 900 910
CTT GTG AGG TCA GGG ACC TCA GTC AAG TTG TCC TGC ACA GCT TCT GGC
GAA CAC TCC AGT CCC TGG AGT CAG TTC AAC AGG ACG TGT CGA AGA CCG
Leu Val Arg Ser Gly Thr Ser Val Lys Leu Ser Cys Thr Ala Ser Gly>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) [A]___a___a___>
920 930 940 950 960
TTC AAC ATT AAA GAC TCC TAT ATG CAC TGG TTG AGG CAG GGG CCT GAA
AAG TTG TAA TTT CTG AGG ATA TAC GTG ACC AAC TCC GTC CCC GGA CTT
Phe Asn Ile Lys Asp Ser Tyr Met His Trp Leu Arg Gln Gly Pro Glu>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) [A]___a___a___>
970 980 990 1000 1010
CAG GGC CTG GAG TGG ATT GGA TGG ATT GAT CCT GAG AAT GGT GAT ACT
GTC CCG GAC CTC ACC TAA CCT ACC TAA CTA GGA CTC TTA CCA CTA TGA
Gln Gly Leu Glu Trp Ile Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) [A]___a___a___>
1020 1030 1040 1050 1060
GAA TAT GCC CCG AAG TTC CAG GGC AAG GCC ACT TTT ACT ACA GAC ACA
CTT ATA CGG GGC TTC AAG GTC CCG TTC CGG TGA AAA TGA TGT CTG TGT
Glu Tyr Ala Pro Lys Phe Gln Gly Lys Ala Thr Phe Thr Thr Asp Thr>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) [A]___a___a___>
1070 1080 1090 1100
TCC TCC AAC ACA GCC TAC CTG CAG CTG AGC AGC CTG ACA TCT GAG GAC
AGG AGG TTG TGT CGG ATG GAC GTC GAC TCG TCG GAC TGT AGA CTC CTG
Ser Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) [A]___a___a___>
1110 1120 1130 1140 1150
ACT GCC GTC TAT TAT TGT AAT GAG GGG ACT CCG ACT GGG CCG TAC TAC
TGA CGG CAG ATA ATA ACA TTA CTC CCC TGA GGC TGA CCC GGC ATG ATG
Thr Ala Val Tyr Tyr Cys Asn Glu Gly Thr Pro Thr Gly Pro Tyr Tyr>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) [A]___a___a___>
1160 1170 1180 1190 MFE-23 1200
VH
Linker
TTT GAC TAC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GGA
AAA CTG ATG ACC CCG GTT CCC TGG TGC CAG TGG CAG AGG AGT CCA CCT
Phe Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) [A]___a___a___>
1210 1220 1230 1240 1250
Linker
MFE-23
VL
GGC GGT TCA GGC GGA GGT GGC TCT GGC GGT GGC GGA TCA GAA AAT GTG
CCG CCA AGT CCG CCT CCA CCG AGA CCG CCA CCG CCT AGT CTT TTA CAC
Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Asn Val>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) [A]___a___a___>
1260 1270 1280 1290 1300

FIGURE 40C

(321)

```
CTC ACC CAG TCT CCA GCA ATC ATG TCT GCA TCT CCA GGG GAG AAG GTC
GAG TGG GTC AGA GGT CGT TAG TAC AGA CGT AGA GGT CCC CTC TTC CAG
Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) (A)___a___a___>

        1310        1320        1330        1340
         .           .           .           .           .
ACC ATA ACC TGC AGT GCC AGC TCA AGT GTA AGT TAC ATG CAC TGG TTC
TGG TAT TGG ACG TCA CGG TCG AGT TCA CAT TCA ATG TAC GTG ACC AAG
Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met His Trp Phe>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) (A)___a___a___>

1350        1360        1370        1380        1390
  .           .           .           .           .           .
CAG CAG AAG CCA GGC ACT TCT CCC AAA CTC TGG ATT TAT AGC ACA TCC
GTC GTC TTC GGT CCG TGA AGA GGG TTT GAG ACC TAA ATA TCG TGT AGG
Gln Gln Lys Pro Gly Thr Ser Pro Lys Leu Trp Ile Tyr Ser Thr Ser>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) (A)___a___a___>

 1400        1410        1420        1430        1440
   .           .           .           .           .           .
AAC CTG GCT TCT GGA GTC CCT GCT CGC TTC AGT GGC AGT GGA TCT GGG
TTG GAC CGA AGA CCT CAG GGA CGA GCG AAG TCA CCG TCA CCT AGA CCC
Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) (A)___a___a___>

   1450        1460        1470        1480        1490
    .           .           .           .           .           .
ACC TCT TAC TCT CTC ACA ATC AGC CGA ATG GAG GCT GAA GAT GCT GCC
TGG AGA ATG AGA GAG TGT TAG TCG GCT TAC CTC CGA CTT CTA CGA CGG
Thr Ser Tyr Ser Leu Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) (A)___a___a___>

      1500        1510        1520        1530        1540
       .           .           .           .           .           .
ACT TAT TAC TGC CAG CAA AGG AGT AGT TAC CCA CTC ACG TTC GGT GCT
TGA ATA ATG ACG GTC GTT TCC TCA TCA ATG GGT GAG TGC AAG CCA CGA
Thr Tyr Tyr Cys Gln Gln Arg Ser Ser Tyr Pro Leu Thr Phe Gly Ala>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) (A)___a___a___>

        1550        1560   MFE-23 1570     Linker 1580
         .           .           .           .  VL  .  →        →  .           .           .
GGC ACC AAG CTG GAG CTG AAA CGG GCG GCA|GGC TCG AGC GGA GGC GGG
CCG TGG TTC GAC CTC GAC TTT GCC CGC CGT|CCG AGC TCG CCT CCG CCC
Gly Thr Lys Leu Glu Leu Lys Arg Ala Ala|Gly Ser Ser Gly Gly Gly>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) (A)___a___a___>

1590        1600   Linker 1610   c-myc 1620        1630
  .           .           .           .  →        →  .  tag  .           .           .
GGT AGC GAT ATC GCG GCC GCA|GAA CAG AAA CTC ATC TCA GAA GAG GAT
CCA TCG CTA TAG CGC CGG CGT|CTT GTC TTT GAG TAG AGT CTT CTC CTA
Gly Ser Asp Ile Ala Ala Ala|Glu Gln Lys Leu Ile Ser Glu Glu Asp>
___a___a_TRANSLATION OF H22/MFE-23 SC-BSAB (321) (A)___a___a___>

 1640        1650        1660        1670
c-myc →  .           .  |→ -His-6-tag|  .           .           .
CTG AAT|GGC GCC GCA|CAT CAC CAT CAT CAC CAT T GATTCTAGA
GAC TTA|CCG CGG CGT|GTA GTG GTA GTA GTG GTA A CTAAGATCT
Leu Asn|Gly Ala Ala|His His His His His His>
___TRANSLATION OF H22/MFE-23 SC-BSAB (3____>
```

FIGURE 40D

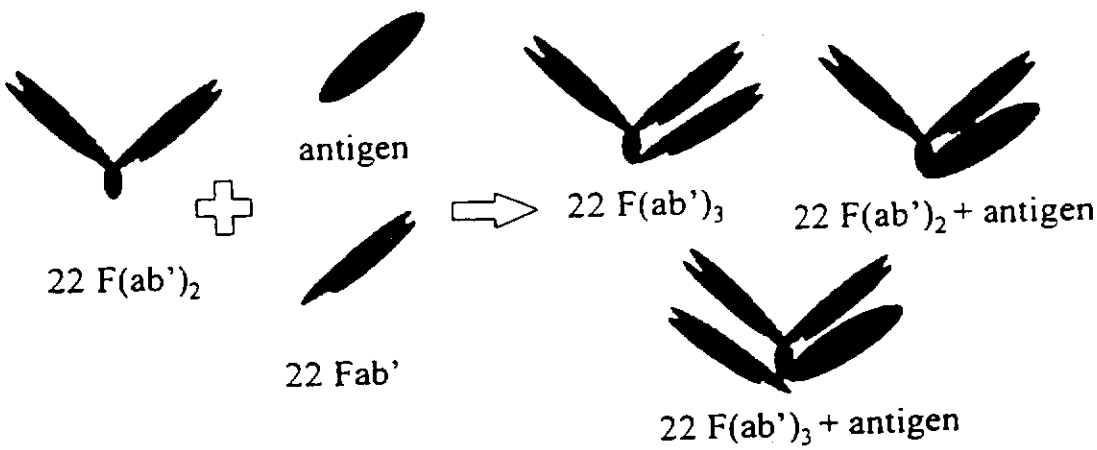

antigen
22 F(ab')2
22 Fab'
22 F(ab')3
22 F(ab')2 + antigen
22 F(ab')3 + antigen

FIGURE 41

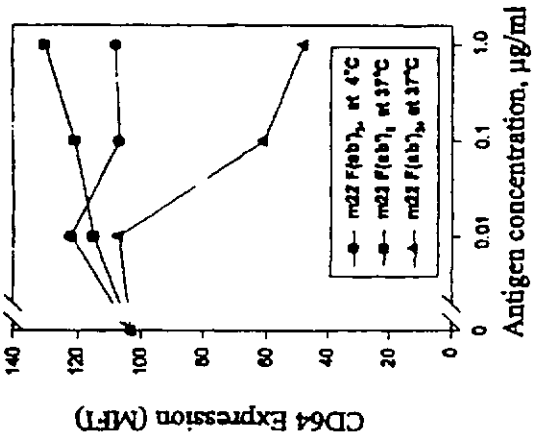


FIGURE 42B

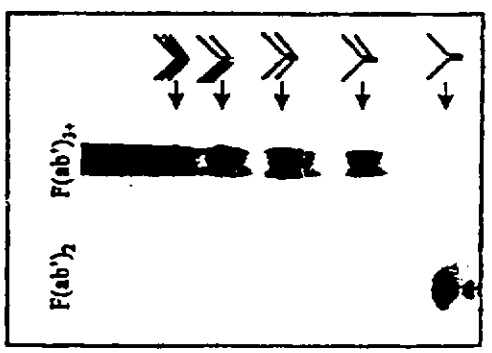


FIGURE 42A

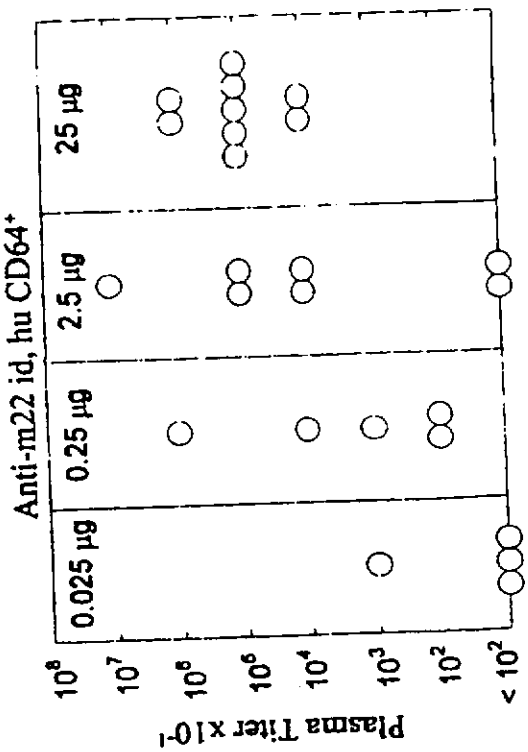


FIGURE 43B

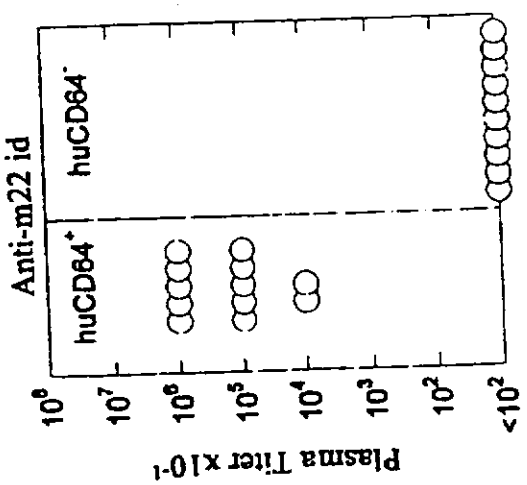


FIGURE 43A

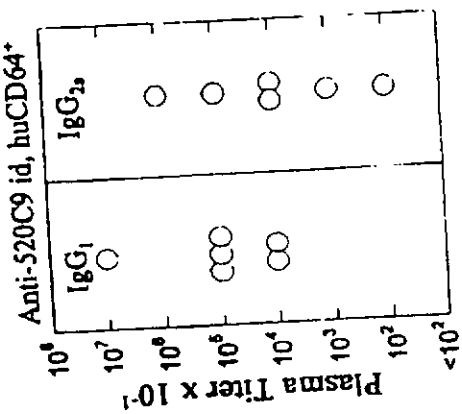


FIGURE 44B

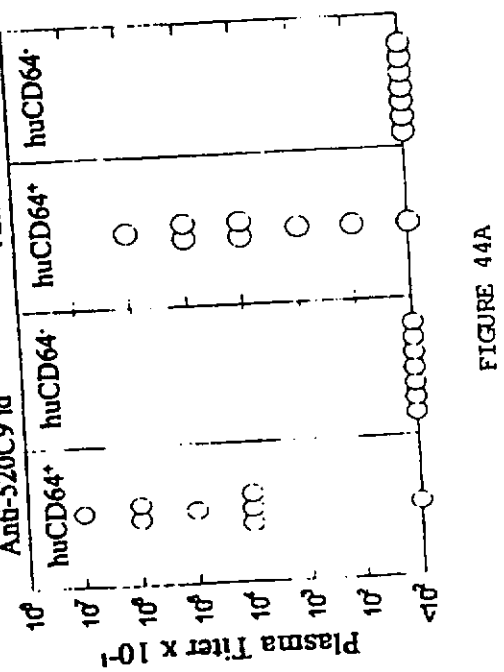


FIGURE 44A

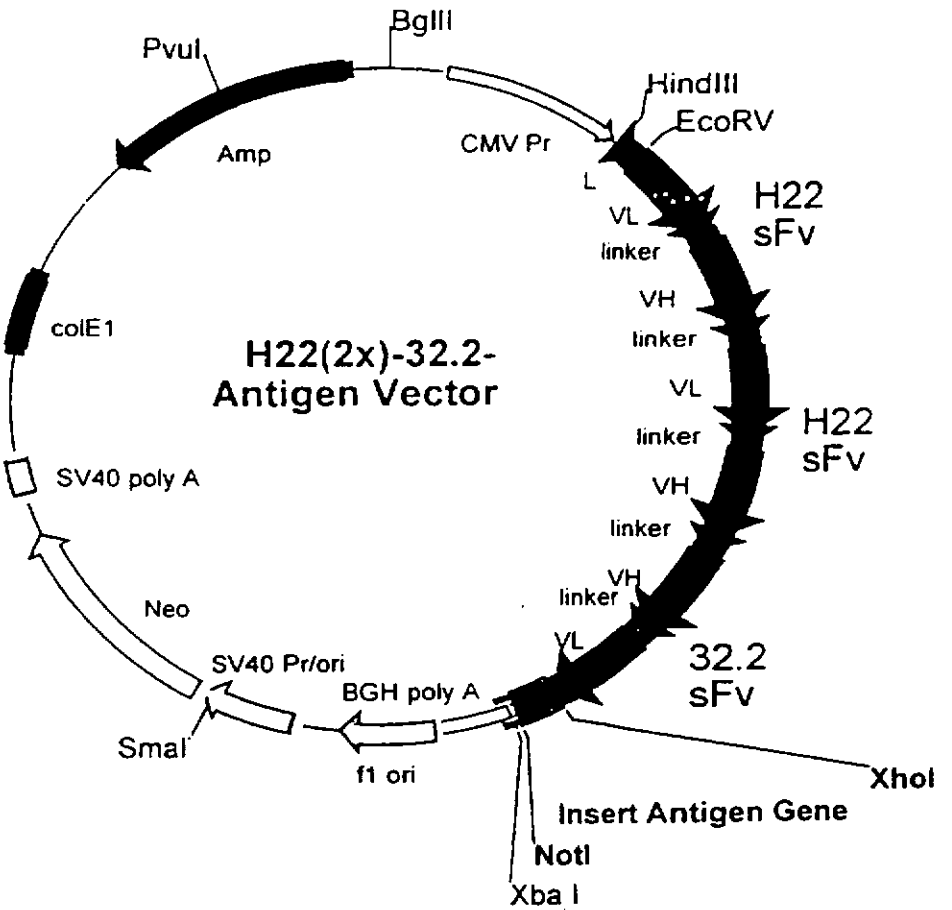


FIGURE 45A

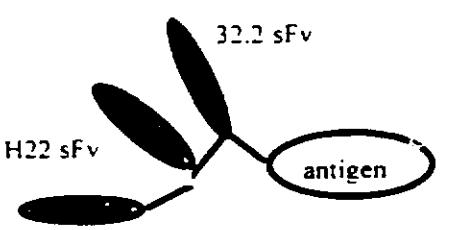


FIGURE 45B

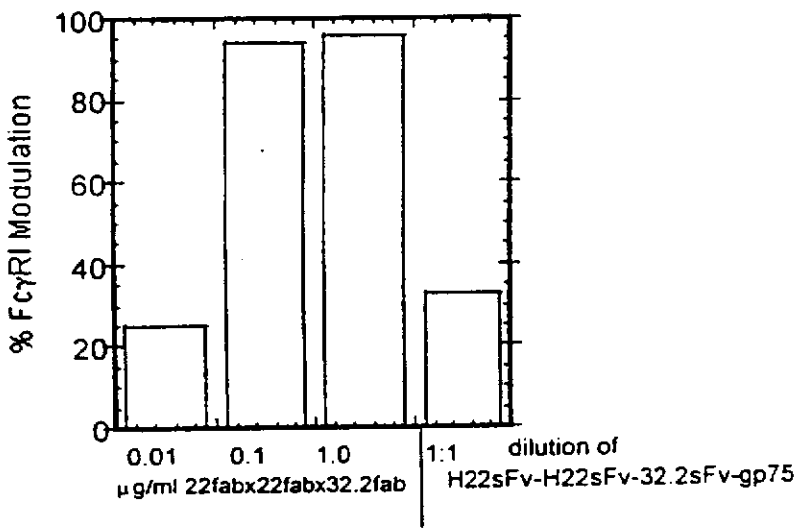

100
80
60
40
20
0
% FcγRI Modulation
0.01
0.1
1.0
1:1
dilution of
µg/ml 22fabx22fabx32.2fab
H22sFv-H22sFv-32.2sFv-gp75

FIGURE 46

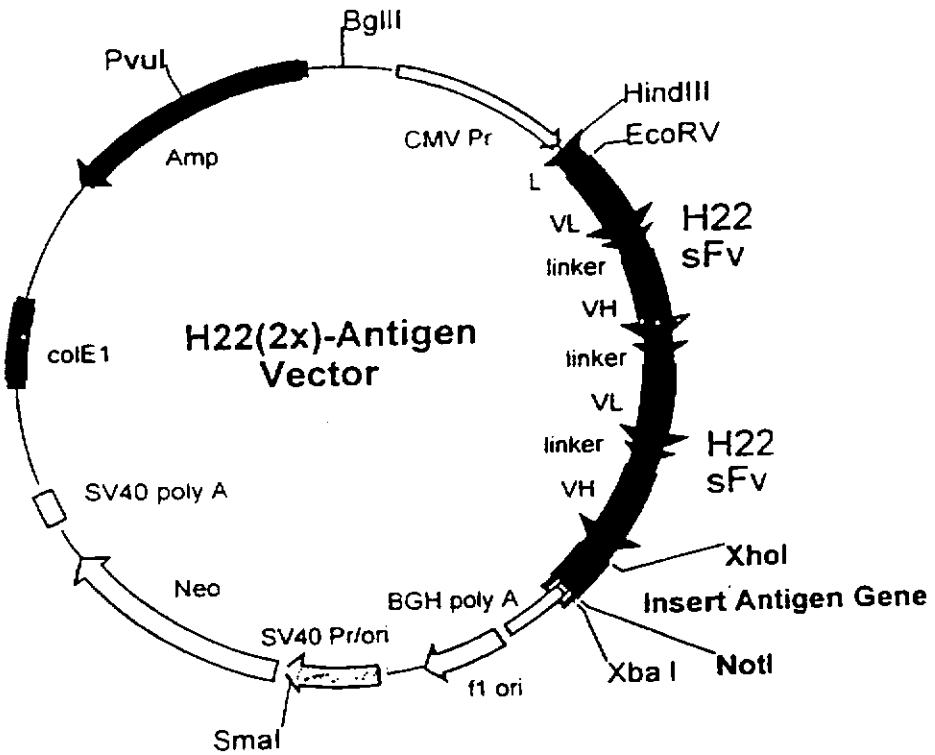


FIGURE 47A

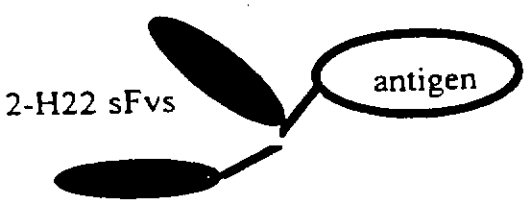


FIGURE 47B

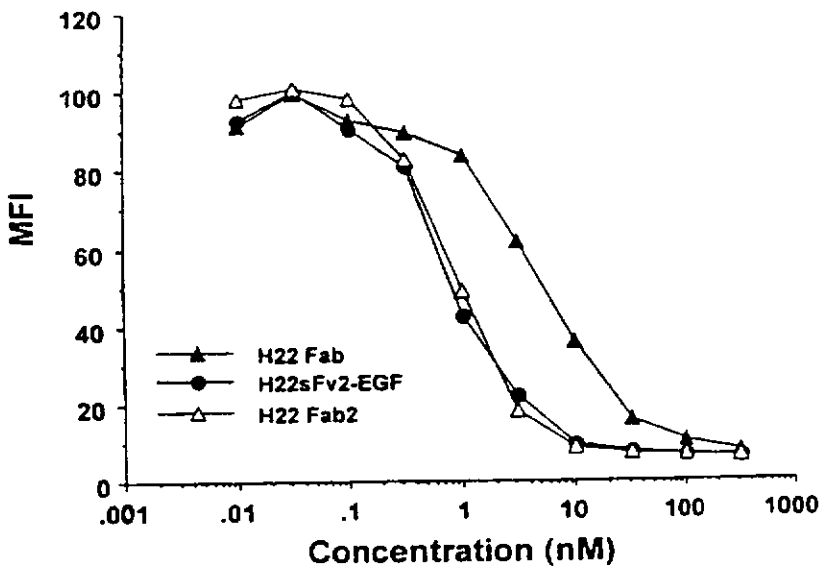

120
100
80
60
40
20
0
MFI
H22 Fab
H22sFv2-EGF
H22 Fab2
.001
.01
.1
1
10
100
1000
Concentration (nM)

FIGURE 48

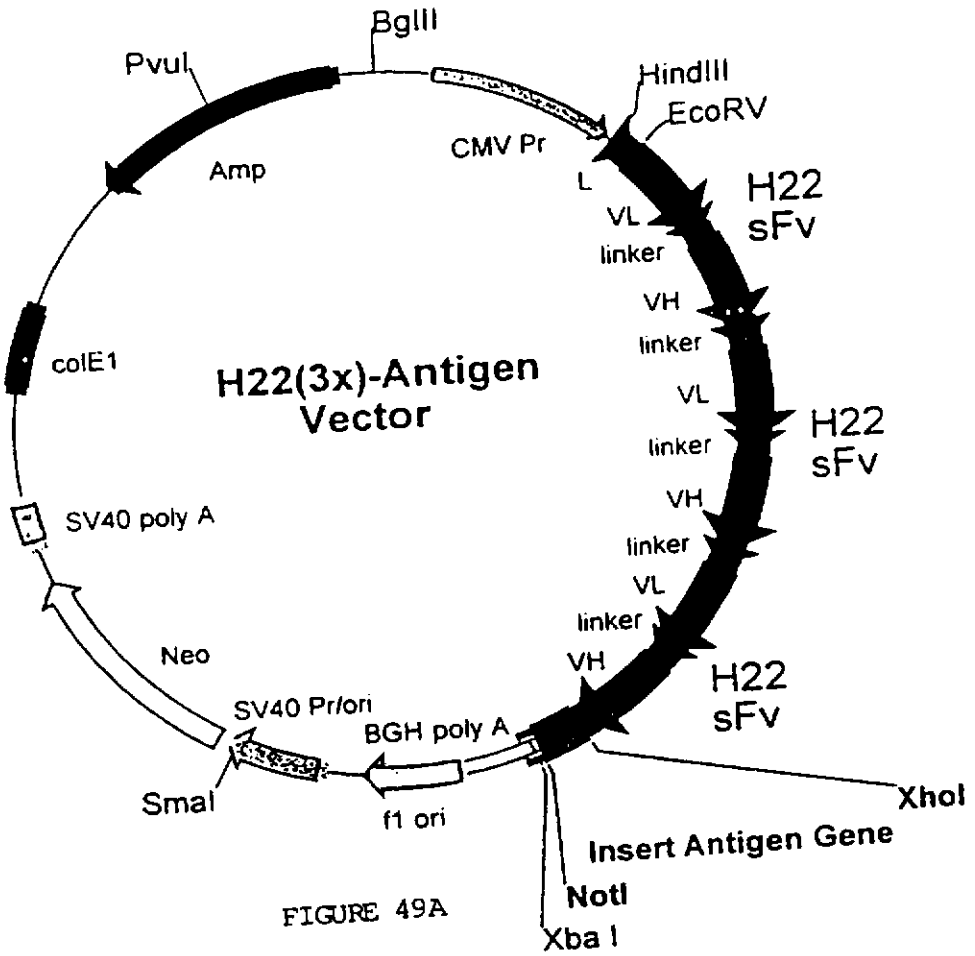


FIGURE 49A

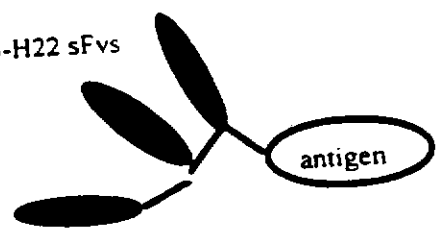


FIGURE 49B

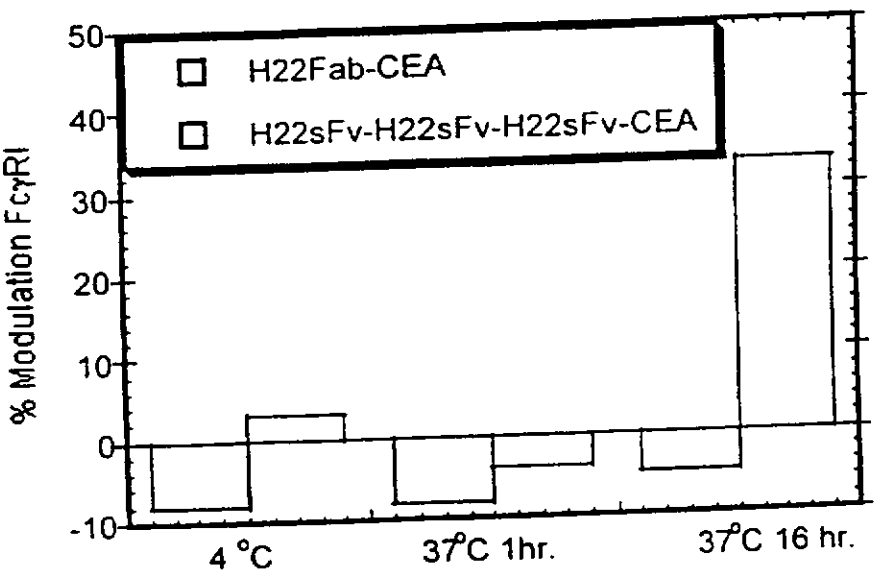
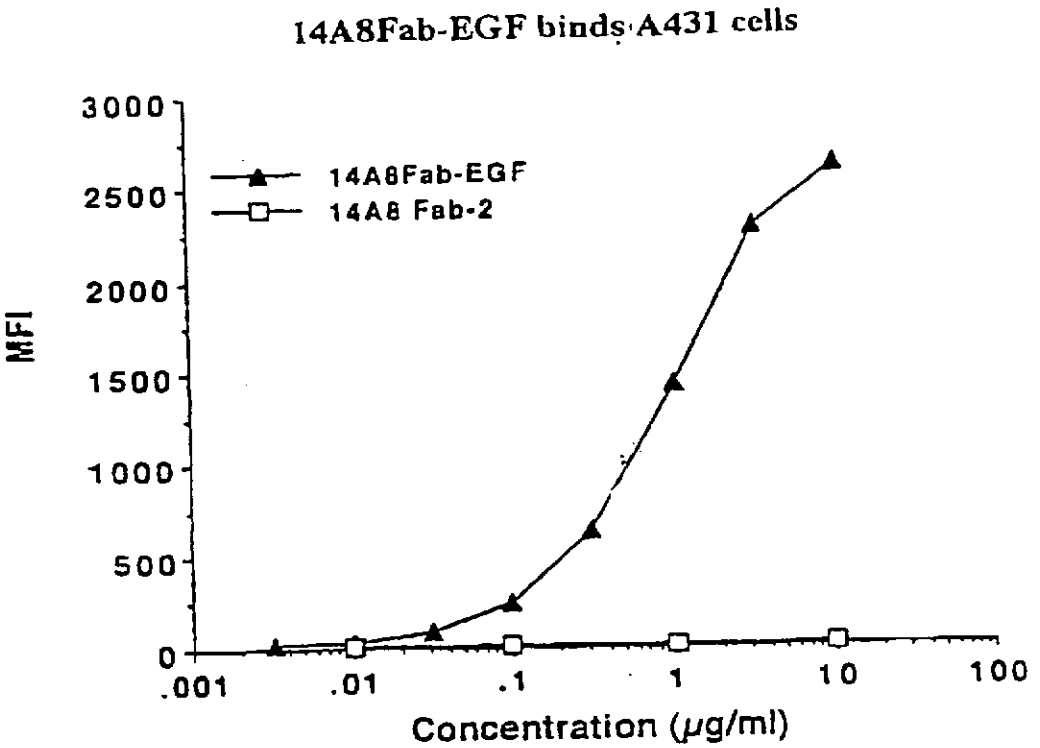

50
40
30
20
10
0
-10
% Modulation FcγRI
H22Fab-CEA
H22sFv-H22sFv-H22sFv-CEA
4 °C
37°C 1hr.
37°C 16 hr.

FIGURE 50

CMV Pr
L
VL
linker
VH
EGF
pJZ909
BGH poly A
Neomycin
anti-CD89 sFv - EGF Vector
Transfection
NS0 Myeloma Line
Selection
anti-CD89 sFv
EGF
anti-CD89 sFv - EGF Fusion Protein
CMV Pr
L
VL
CL
pJZ907
BGH poly A
hygromycin
anti-CD89 Light Chain Vector
CMV Pr
L
VH
CH1
H
EGF
pJZ906
BGH poly A
Neo
anti-CD89 Fd - EGF Vector
Transfection
NS0 Myeloma Line
Selection
anti-CD89 Fab'
SH
EGF
anti-CD89 Fab' - EGF Fusion Protein

FIGURE 51

A



B

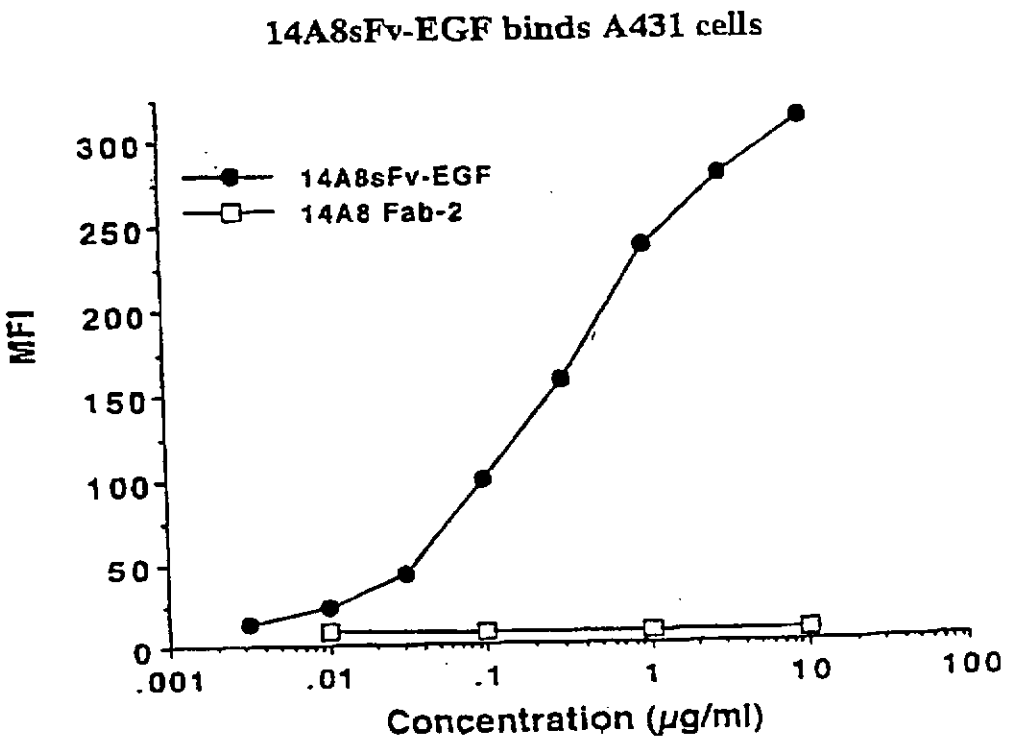


FIGURE 52A and FIGURE 52B

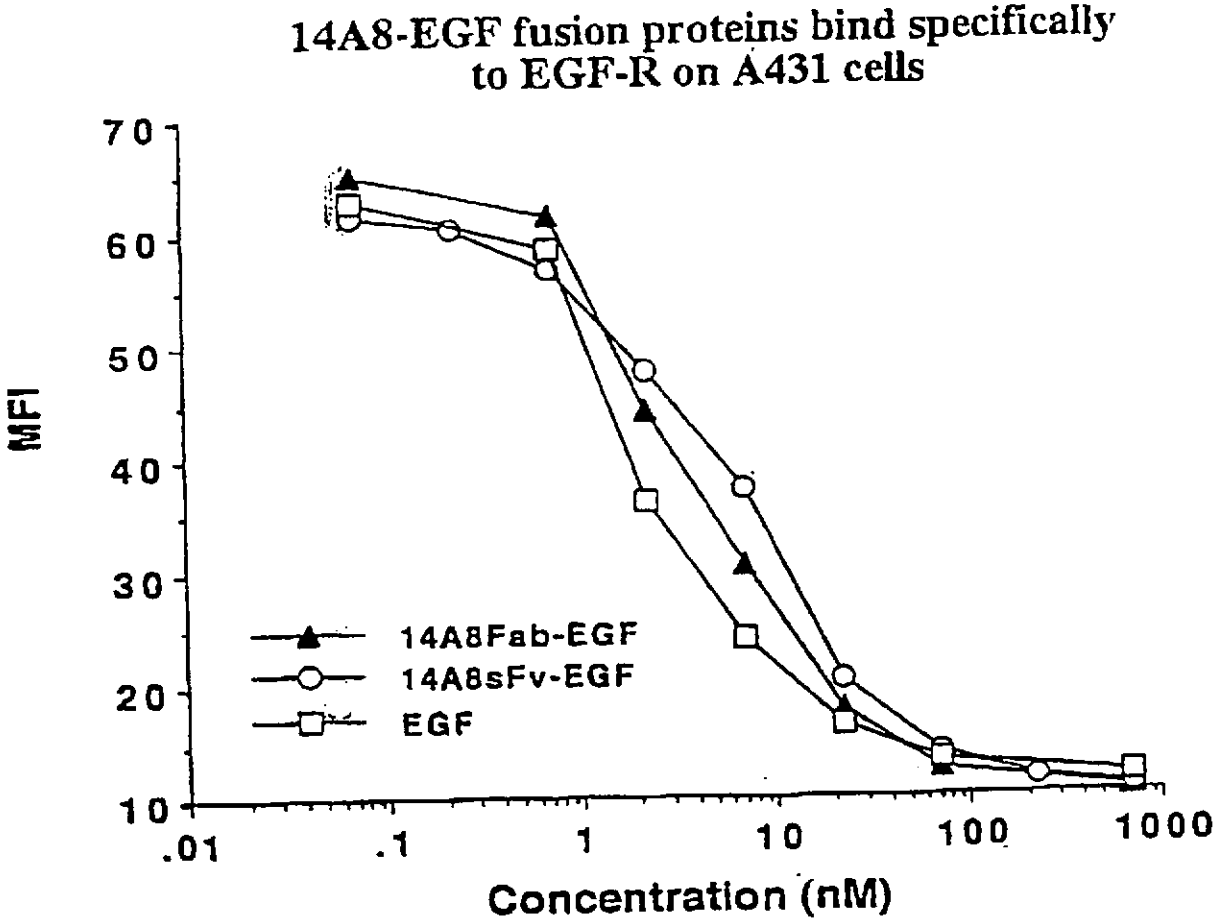
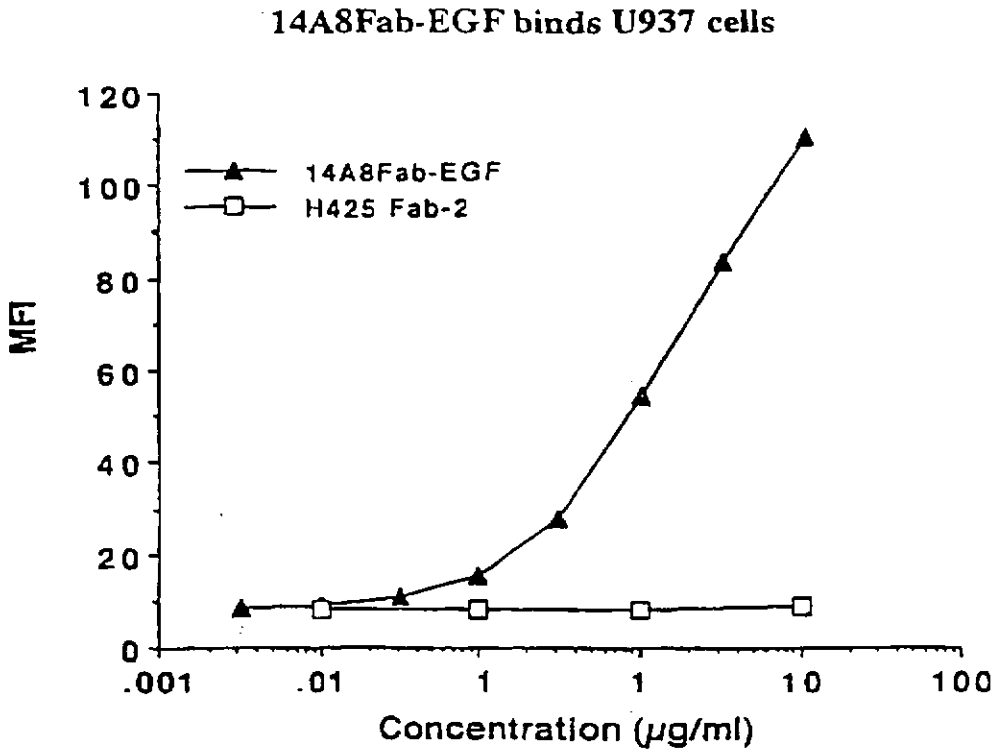

14A8-EGF fusion proteins bind specifically to EGF-R on A431 cells
MFI
70
60
50
40
30
20
10
.01
.1
1
10
100
1000
Concentration (nM)
14A8Fab-EGF
14A8sFv-EGF
EGF

FIGURE 52C

A


14A8Fab-EGF binds U937 cells
120
100
80
60
40
20
0
MFI
14A8Fab-EGF
H425 Fab-2
.001
.01
1
1
10
100
Concentration (μg/ml)

B

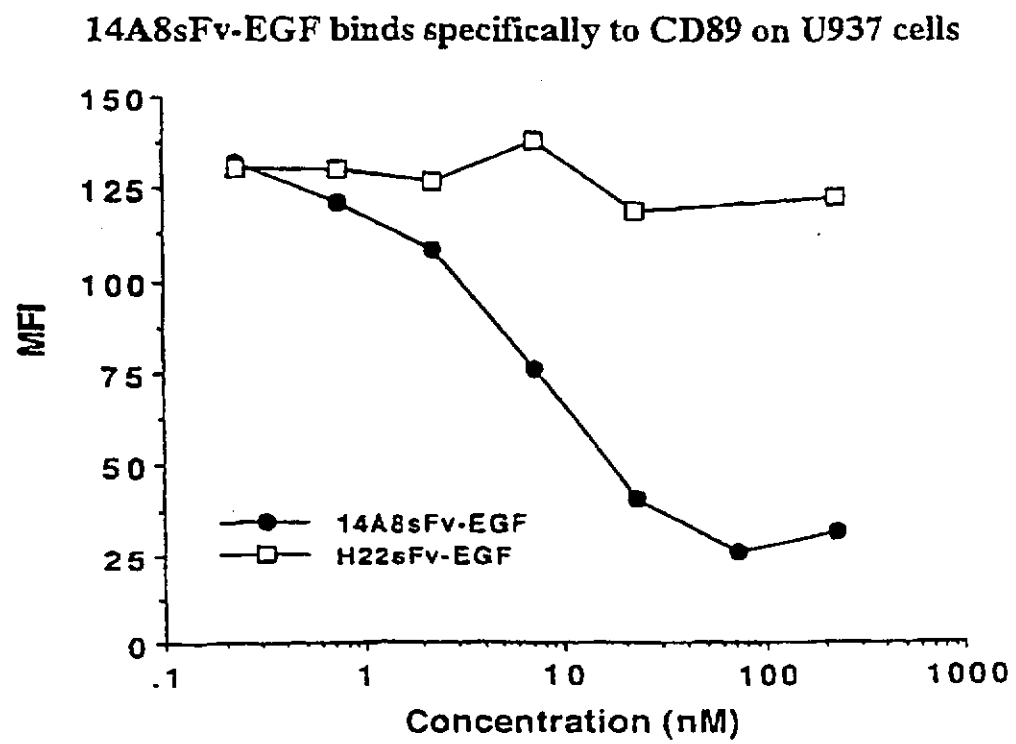

14A8sFv-EGF binds specifically to CD89 on U937 cells
150
125
100
75
50
25
0
MFI
14A8sFv-EGF
H22sFv-EGF
.1
1
10
100
1000
Concentration (nM)

FIGURE 53

A

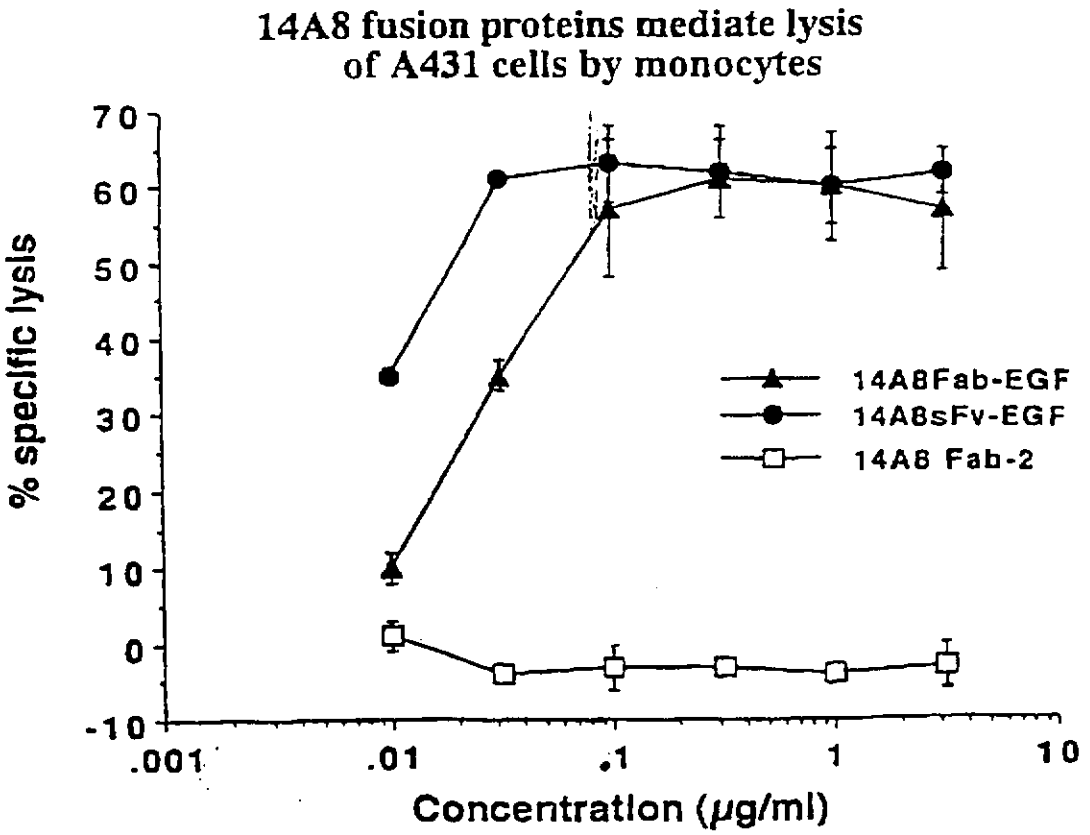

14A8 fusion proteins mediate lysis of A431 cells by monocytes
% specific lysis
70
60
50
40
30
20
10
0
-10
.001
.01
.1
1
10
Concentration (μg/ml)
14A8Fab-EGF
14A8sFv-EGF
14A8 Fab-2

FIGURE 54A

B

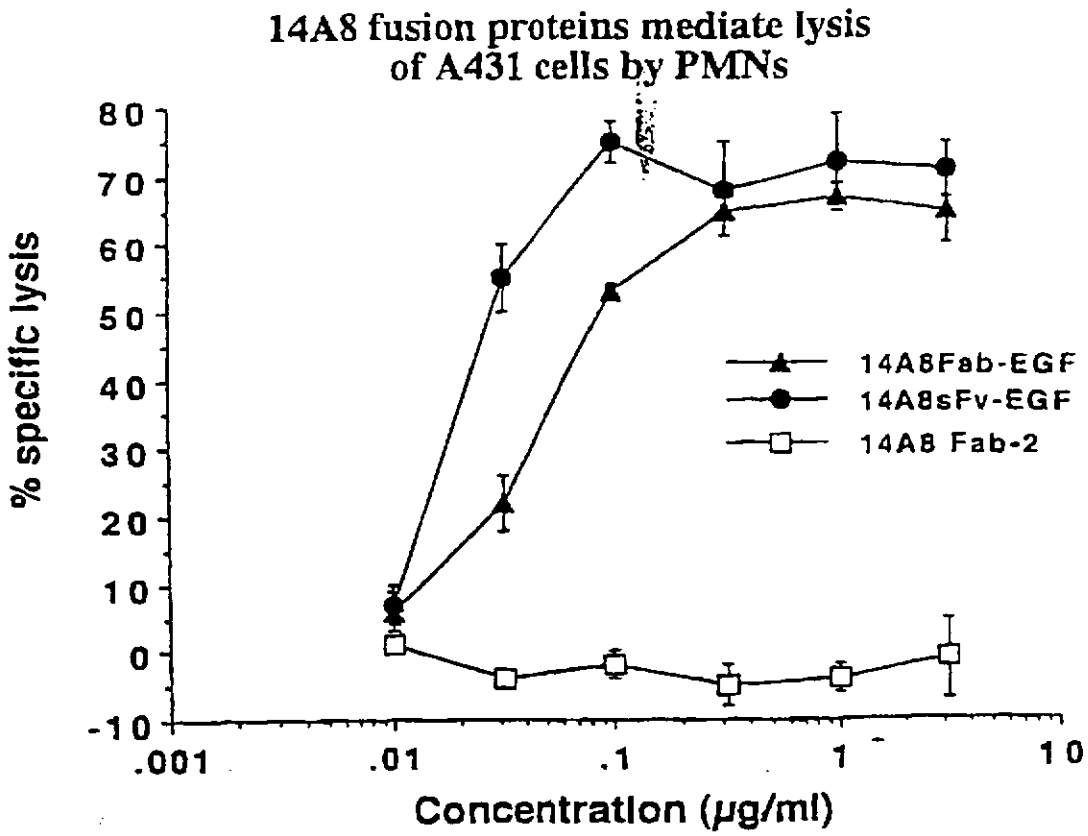

14A8 fusion proteins mediate lysis
of A431 cells by PMNs
% specific lysis
80
70
60
50
40
30
20
10
0
-10
.001
.01
.1
1
10
Concentration (µg/ml)
14A8Fab-EGF
14A8sFv-EGF
14A8 Fab-2

FIGURE 54B

C

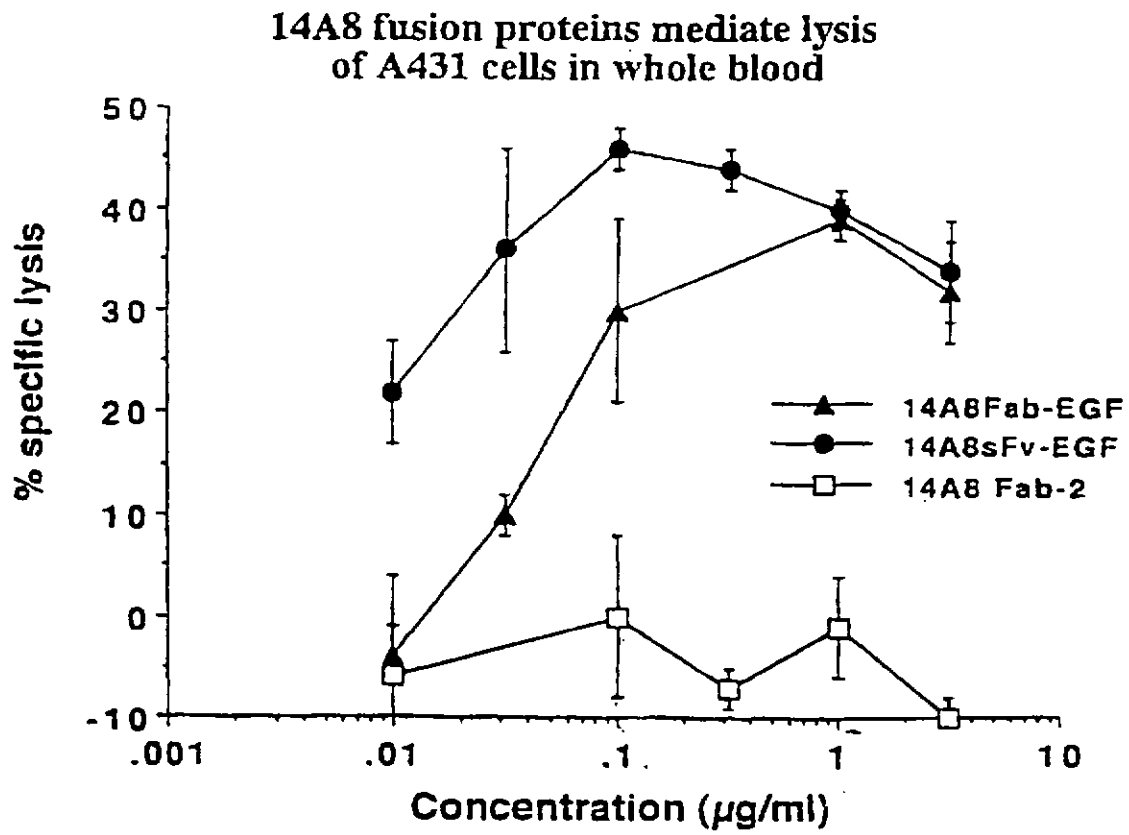

14A8 fusion proteins mediate lysis
of A431 cells in whole blood
% specific lysis
50
40
30
20
10
0
-10
.001
.01
.1
1
10
Concentration (μg/ml)
14A8Fab-EGF
14A8sFv-EGF
14A8 Fab-2

FIGURE 54C

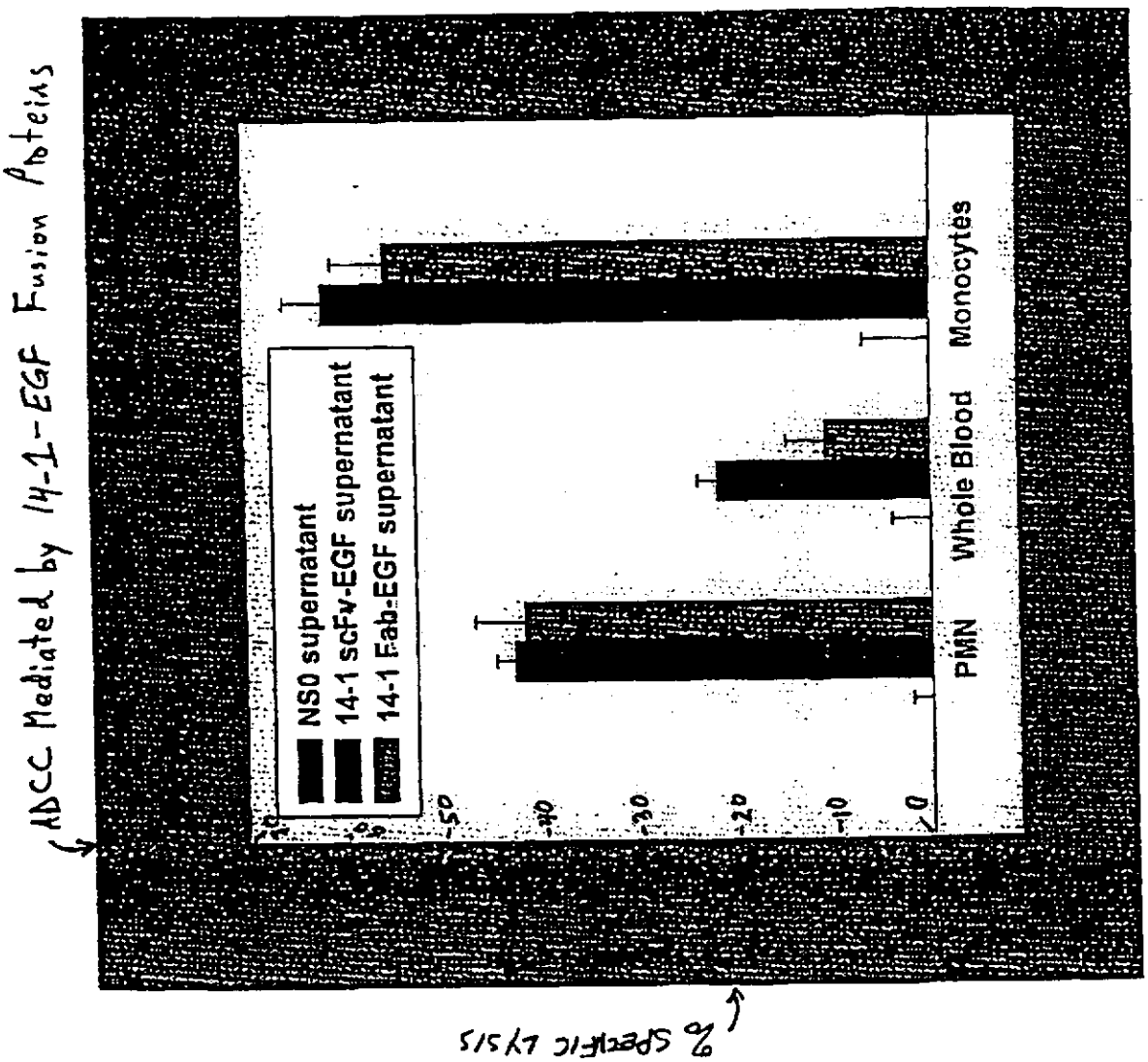
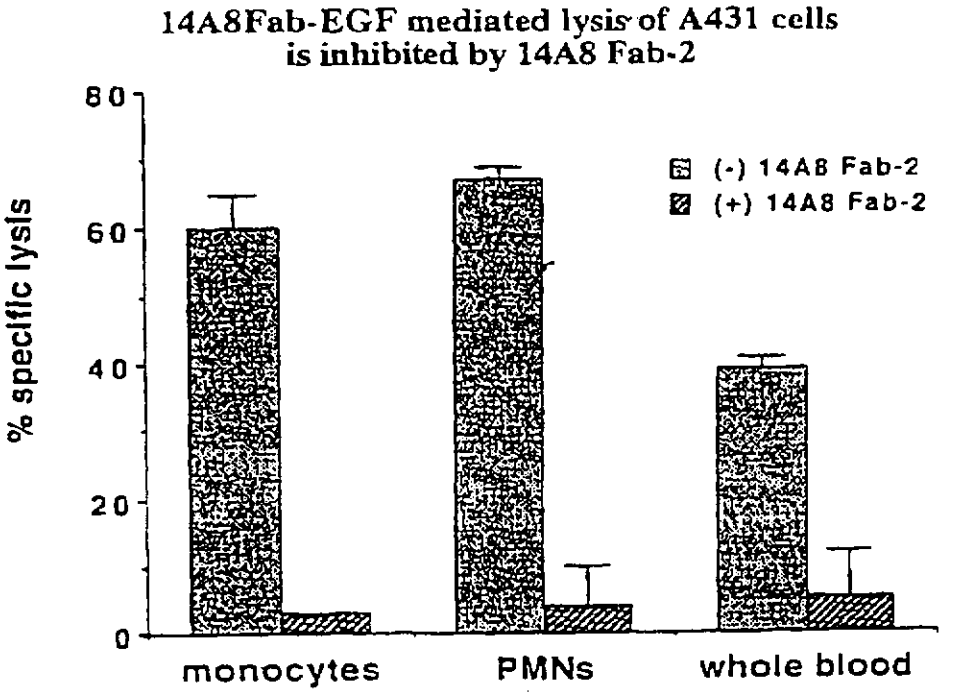

ADCC Mediated by 14-1-EGF Fusion Proteins
NS0 supernatant
14-1 scFv-EGF supernatant
14-1 Fab-EGF supernatant
PMN
Whole Blood
Monocytes
0
-10
-20
-30
-40
-50
% Specific Lysis

FIGURE 55

A


14A8Fab-EGF mediated lysis of A431 cells
is inhibited by 14A8 Fab-2
% specific lysis
80
60
40
20
0
(-) 14A8 Fab-2
(+) 14A8 Fab-2
monocytes
PMNs
whole blood

B

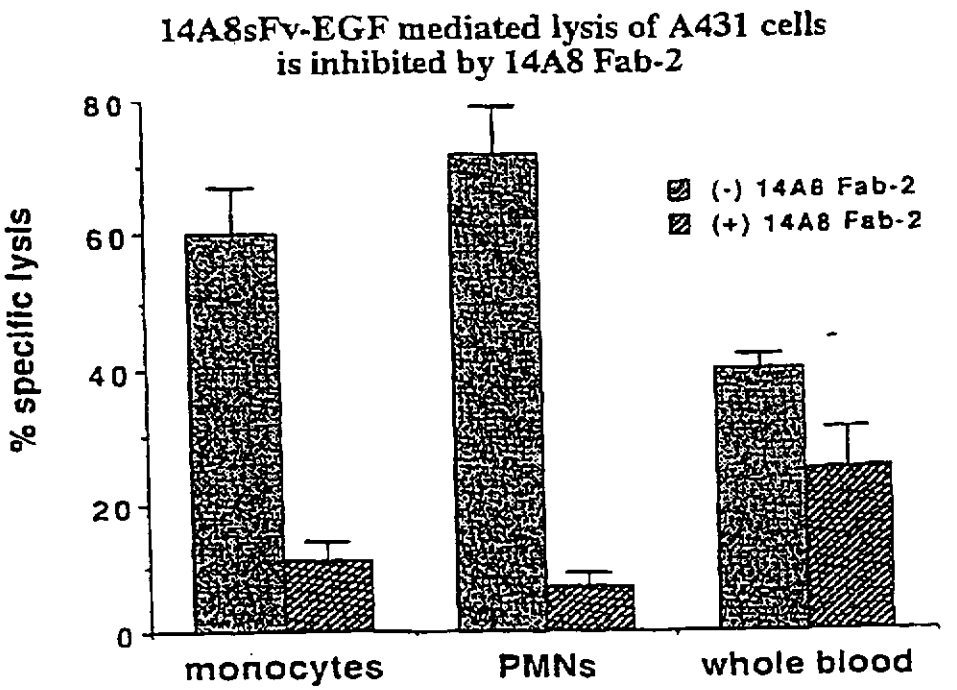

14A8sFv-EGF mediated lysis of A431 cells
is inhibited by 14A8 Fab-2
% specific lysis
80
60
40
20
0
(-) 14A8 Fab-2
(+) 14A8 Fab-2
monocytes
PMNs
whole blood

FIGURE 56

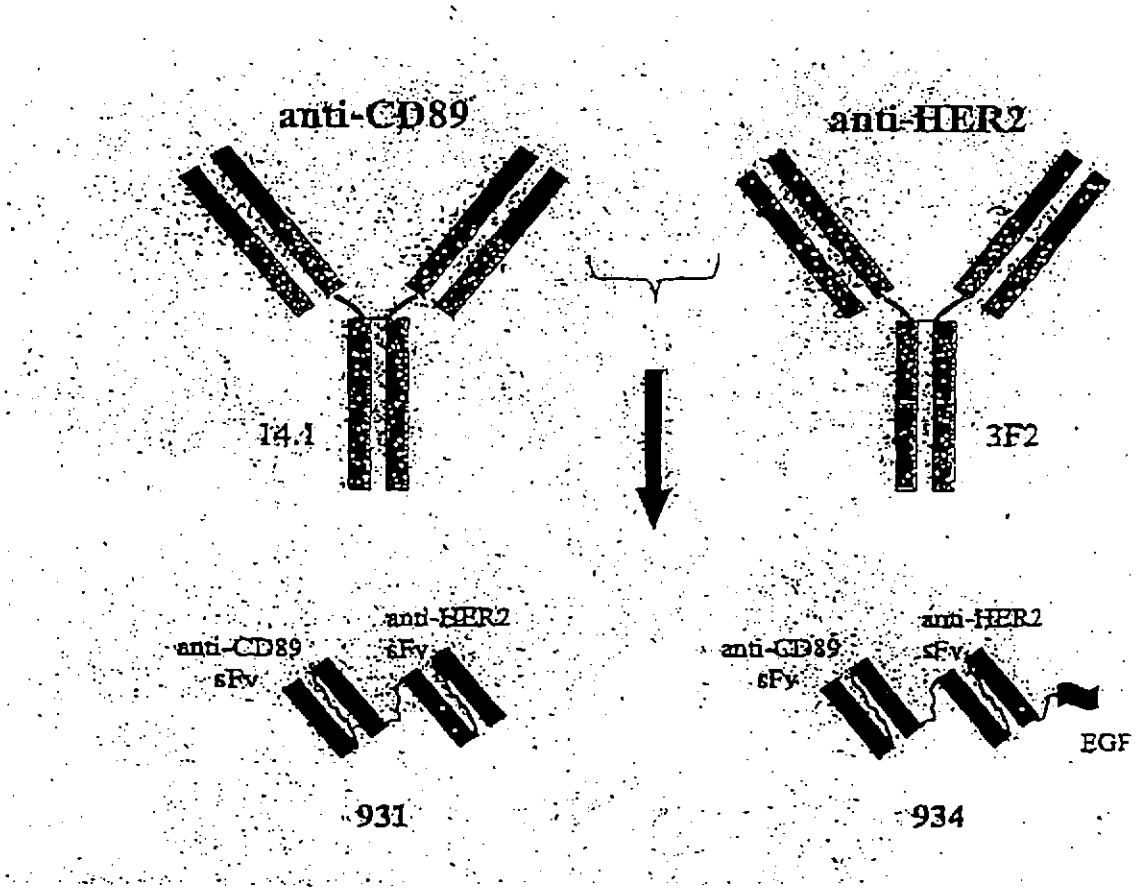

anti-CD89
anti-HER2
14.1
3F2
anti-CD89 sFv
anti-HER2 sFv
931
anti-CD89 sFv
anti-HER2 sFv
EGF
934

FIGURE 57

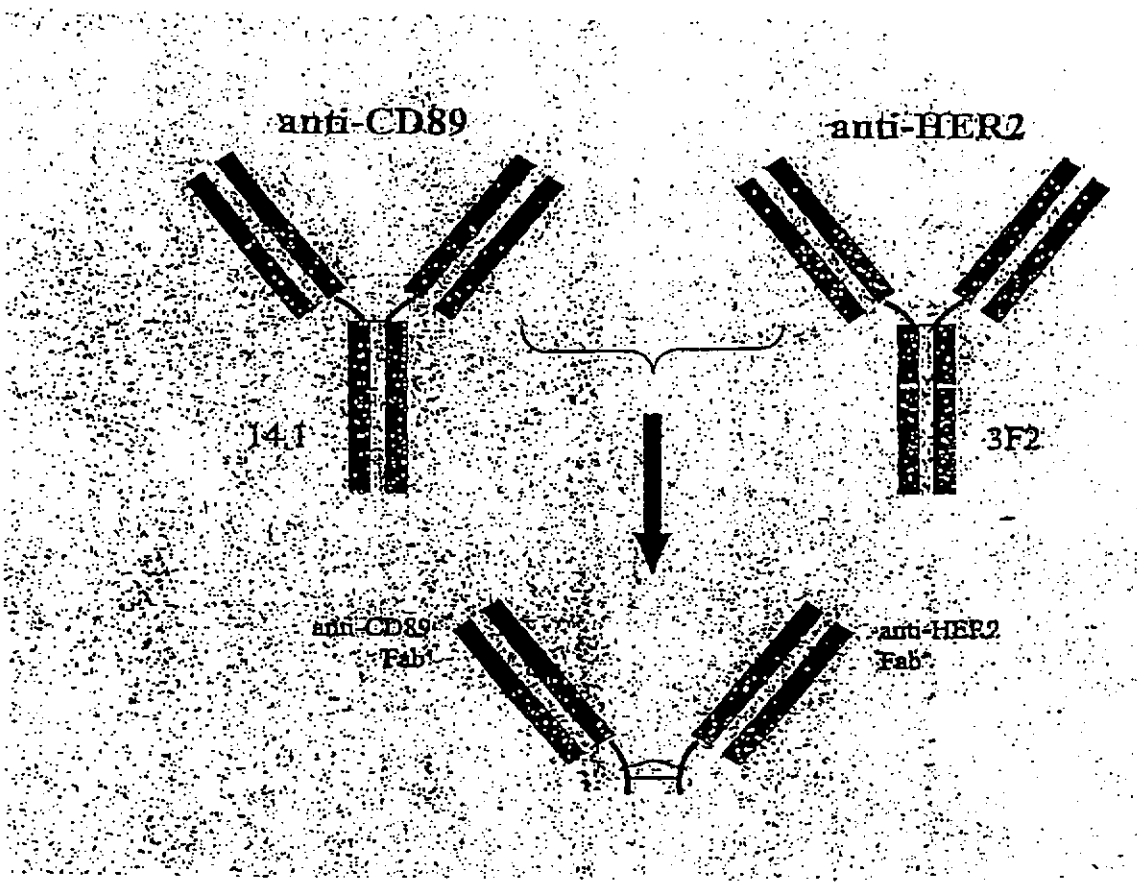
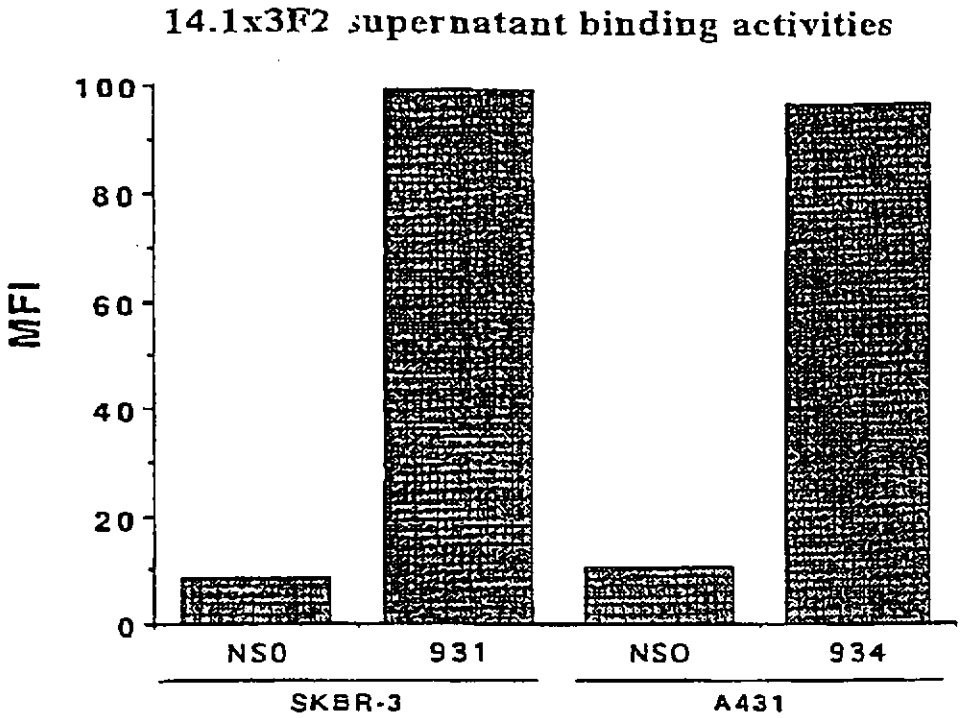


FIGURE 58


14.1x3F2 supernatant binding activities
(A)
MFI
100
80
60
40
20
0
NSO
931
NSO
934
SKBR-3
A431

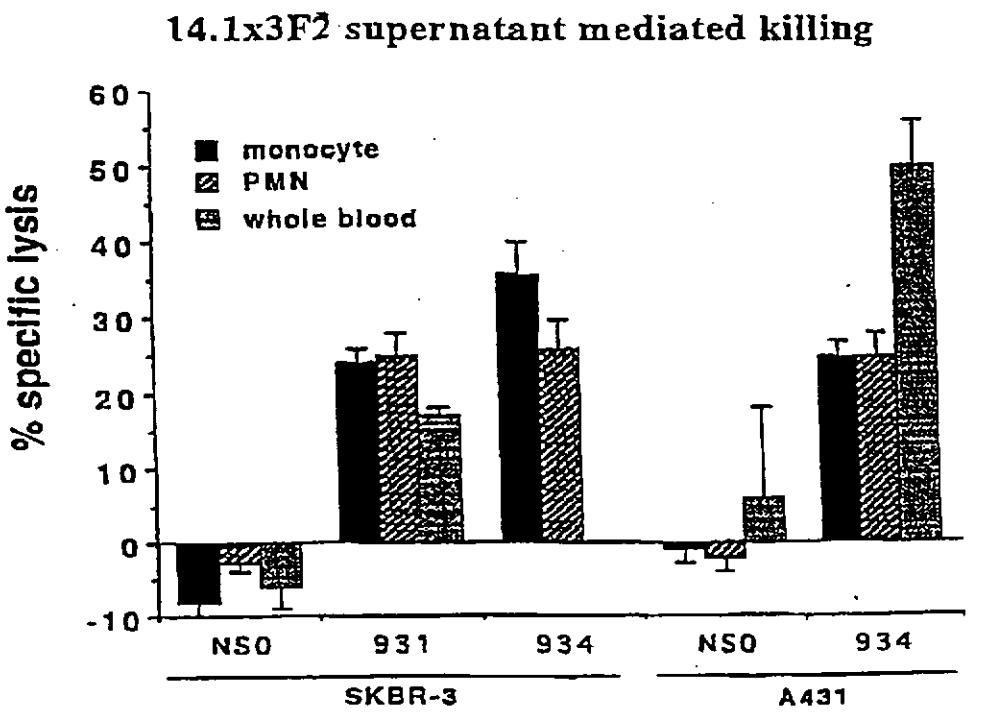
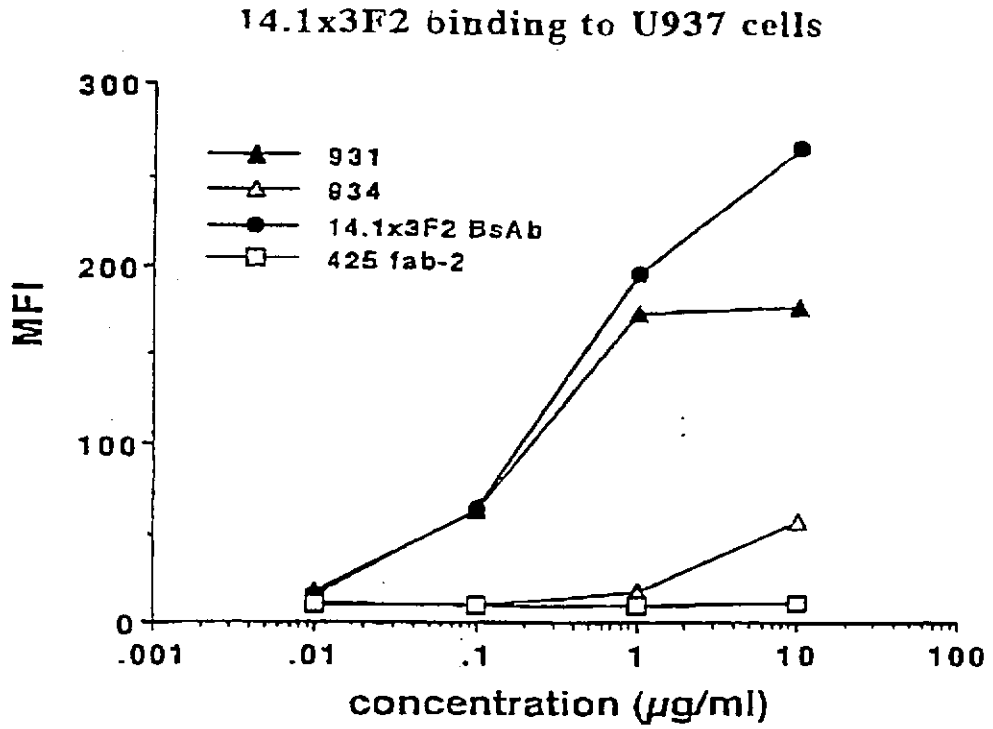

14.1x3F2 supernatant mediated killing
(B)
% specific lysis
60
50
40
30
20
10
0
-10
monocyte
PMN
whole blood
NSO
931
934
NSO
934
SKBR-3
A431

FIGURE 59

A)


14.1x3F2 binding to U937 cells
MFI
300
200
100
0
931
934
14.1x3F2 BsAb
425 fab-2
.001
.01
.1
1
10
100
concentration (μg/ml)

B)

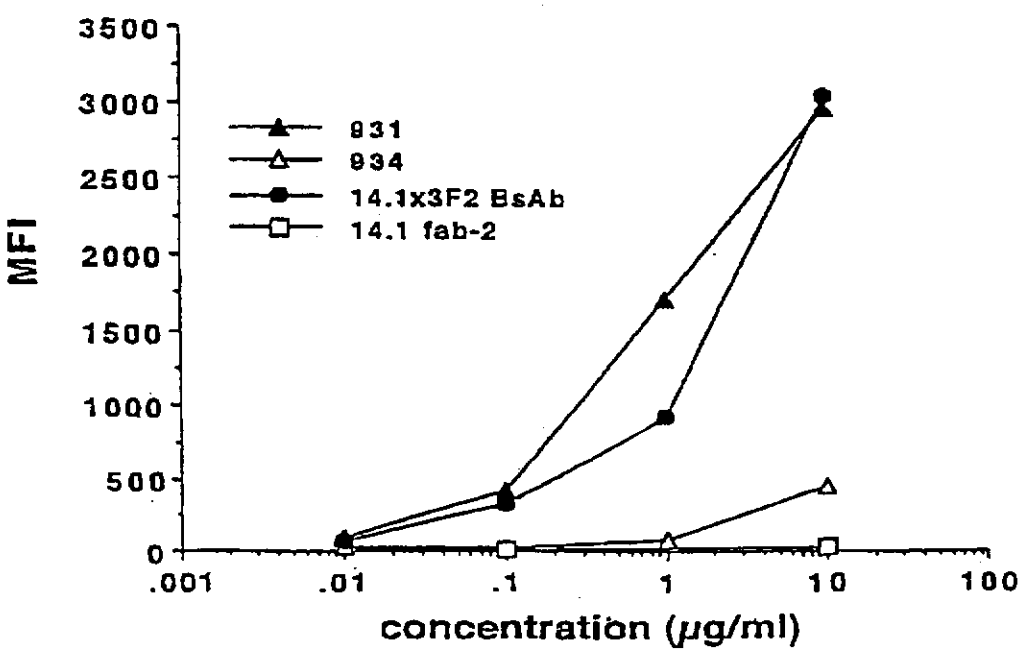

14.1x3F2 binding to SKBR-3 cells
MFI
3500
3000
2500
2000
1500
1000
500
0
931
934
14.1x3F2 BsAb
14.1 fab-2
.001
.01
.1
1
10
100
concentration (μg/ml)

FIGURE 60

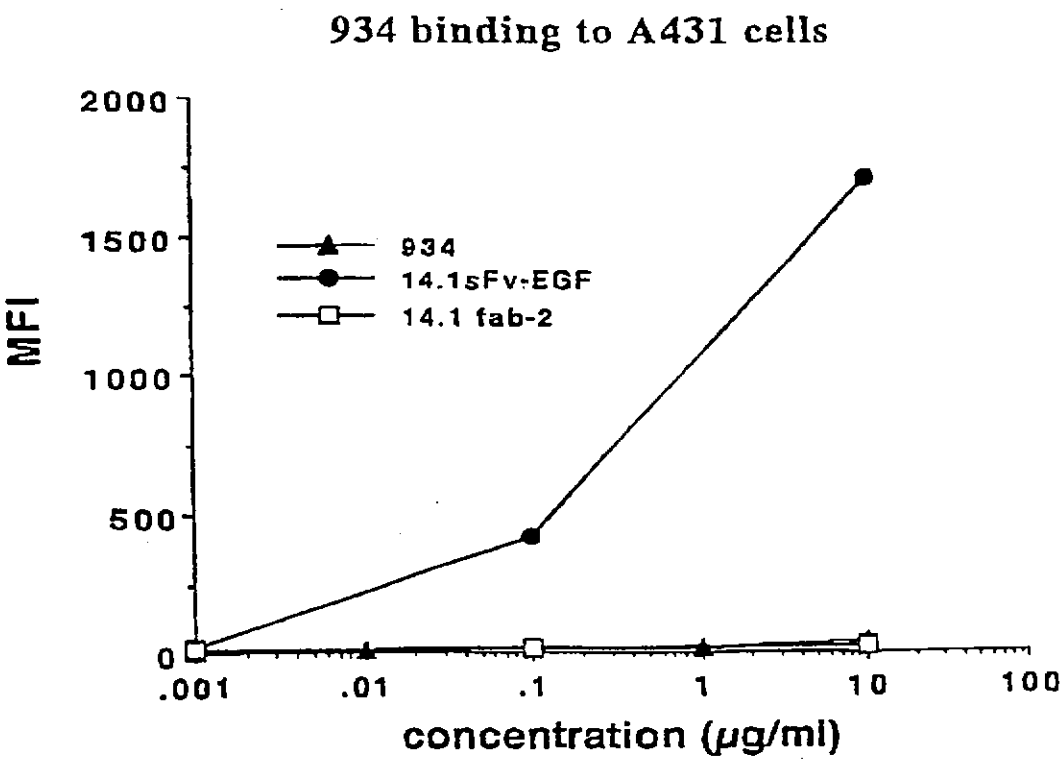
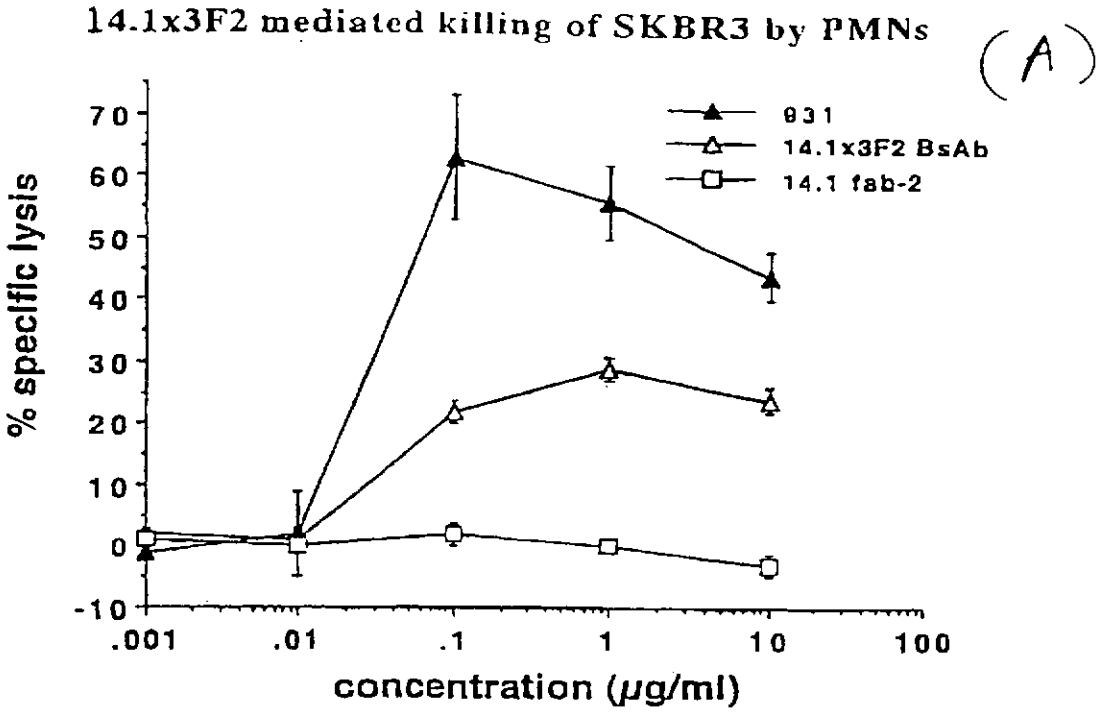

934 binding to A431 cells
2000
1500
1000
500
0
MFI
934
14.1sFv-EGF
14.1 fab-2
.001
.01
.1
1
10
100
concentration (μg/ml)

FIGURE 61


14.1x3F2 mediated killing of SKBR3 by PMNs
(A)
% specific lysis
70
60
50
40
30
20
10
0
-10
.001
.01
.1
1
10
100
concentration (μg/ml)
931
14.1x3F2 BsAb
14.1 fab-2

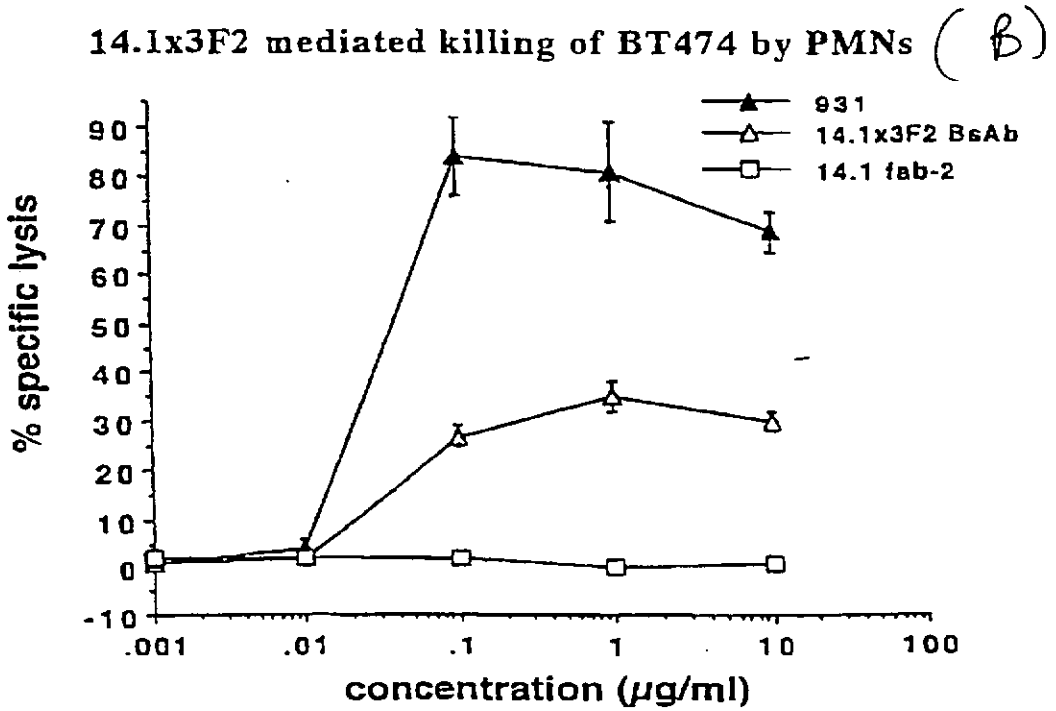
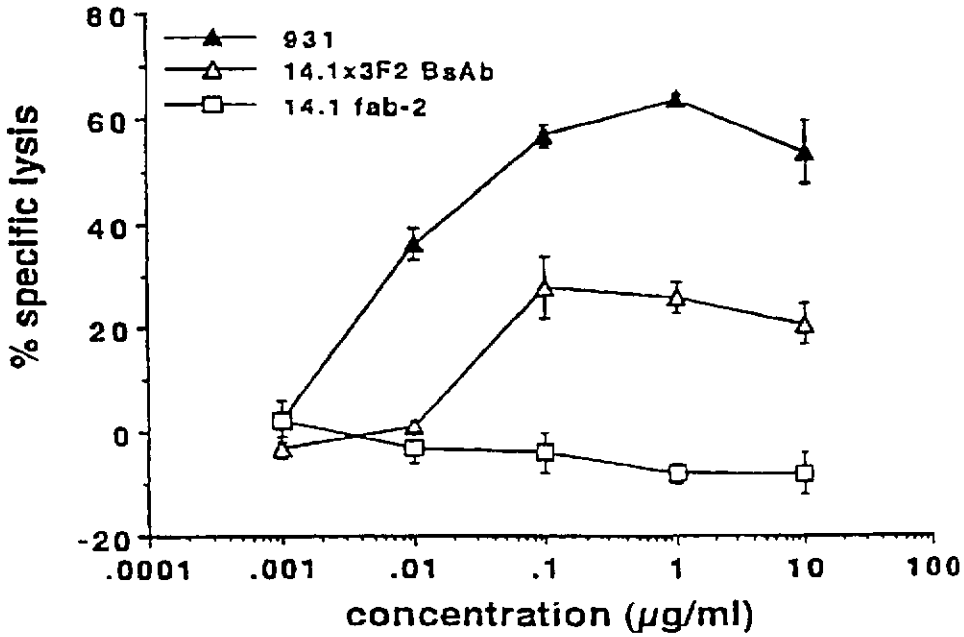

14.1x3F2 mediated killing of BT474 by PMNs
(B)
% specific lysis
90
80
70
60
50
40
30
20
10
0
-10
.001
.01
.1
1
10
100
concentration (μg/ml)
931
14.1x3F2 BsAb
14.1 fab-2

FIGURE 62

14.1x3F2 mediated killing of SKBR3 by monocytes

(A)



14.1x3F2 mediated killing of BT474 by monocytes

(B)

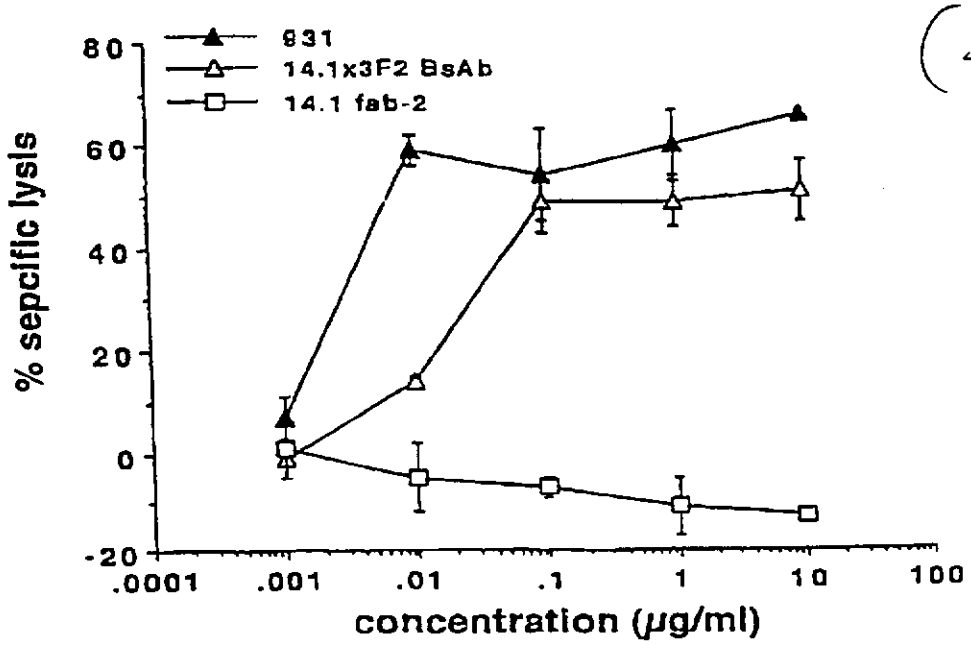


FIGURE 63

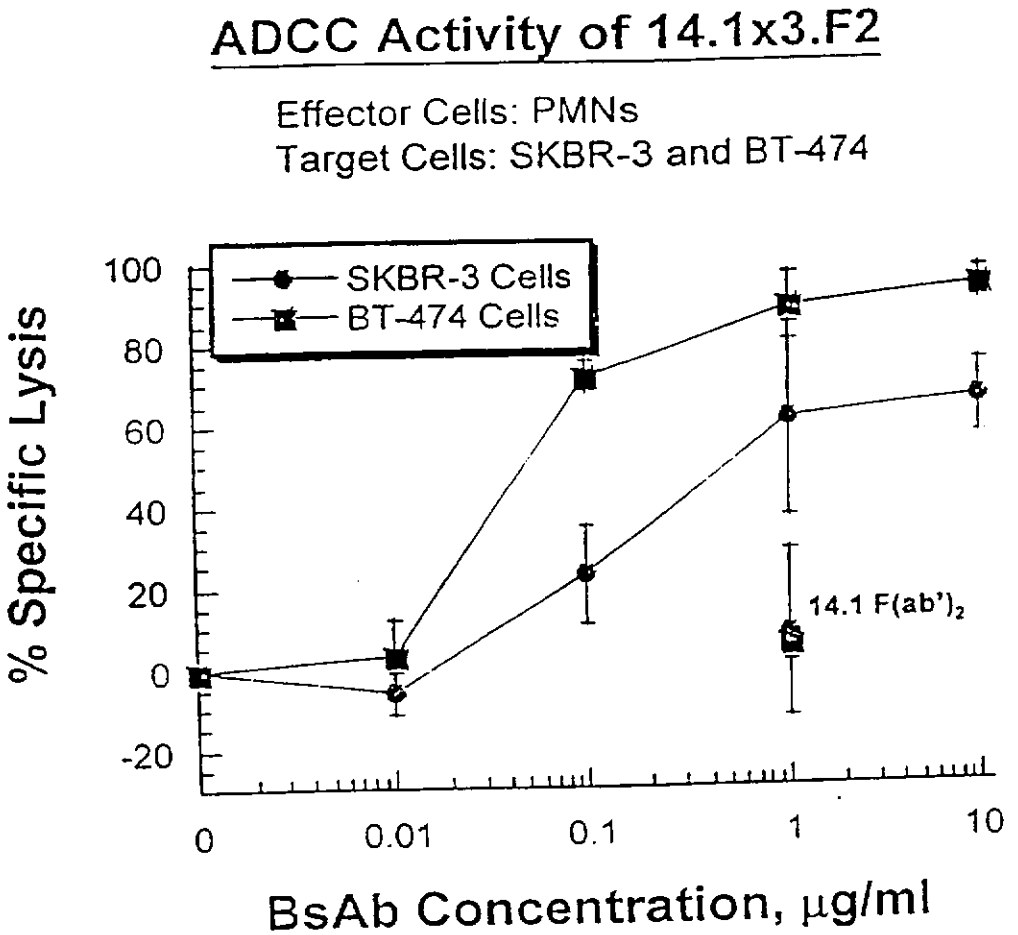

ADCC Activity of 14.1x3.F2
Effector Cells: PMNs
Target Cells: SKBR-3 and BT-474
% Specific Lysis
100
80
60
40
20
0
-20
SKBR-3 Cells
BT-474 Cells
14.1 F(ab')2
0
0.01
0.1
1
10
BsAb Concentration, µg/ml

FIGURE 64

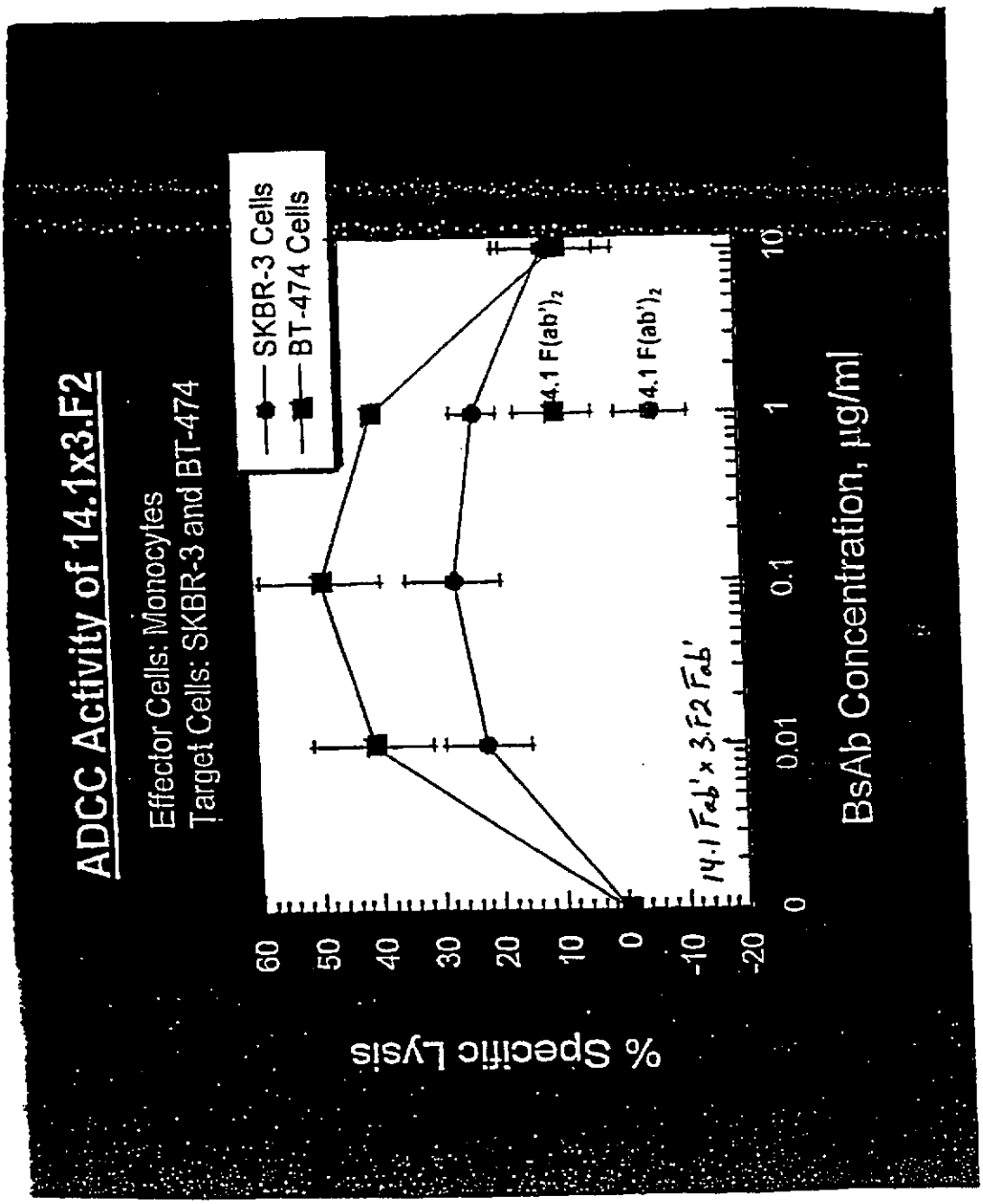

ADCC Activity of 14.1x3.F2
Effector Cells: Monocytes
Target Cells: SKBR-3 and BT-474
SKBR-3 Cells
BT-474 Cells
% Specific Lysis
60
50
40
30
20
10
0
-10
-20
0
0.01
0.1
1
10
BsAb Concentration, µg/ml
14.1 Fab' x 3.F2 Fab'
4.1 F(ab')2
4.1 F(ab')2

FIGURE 65

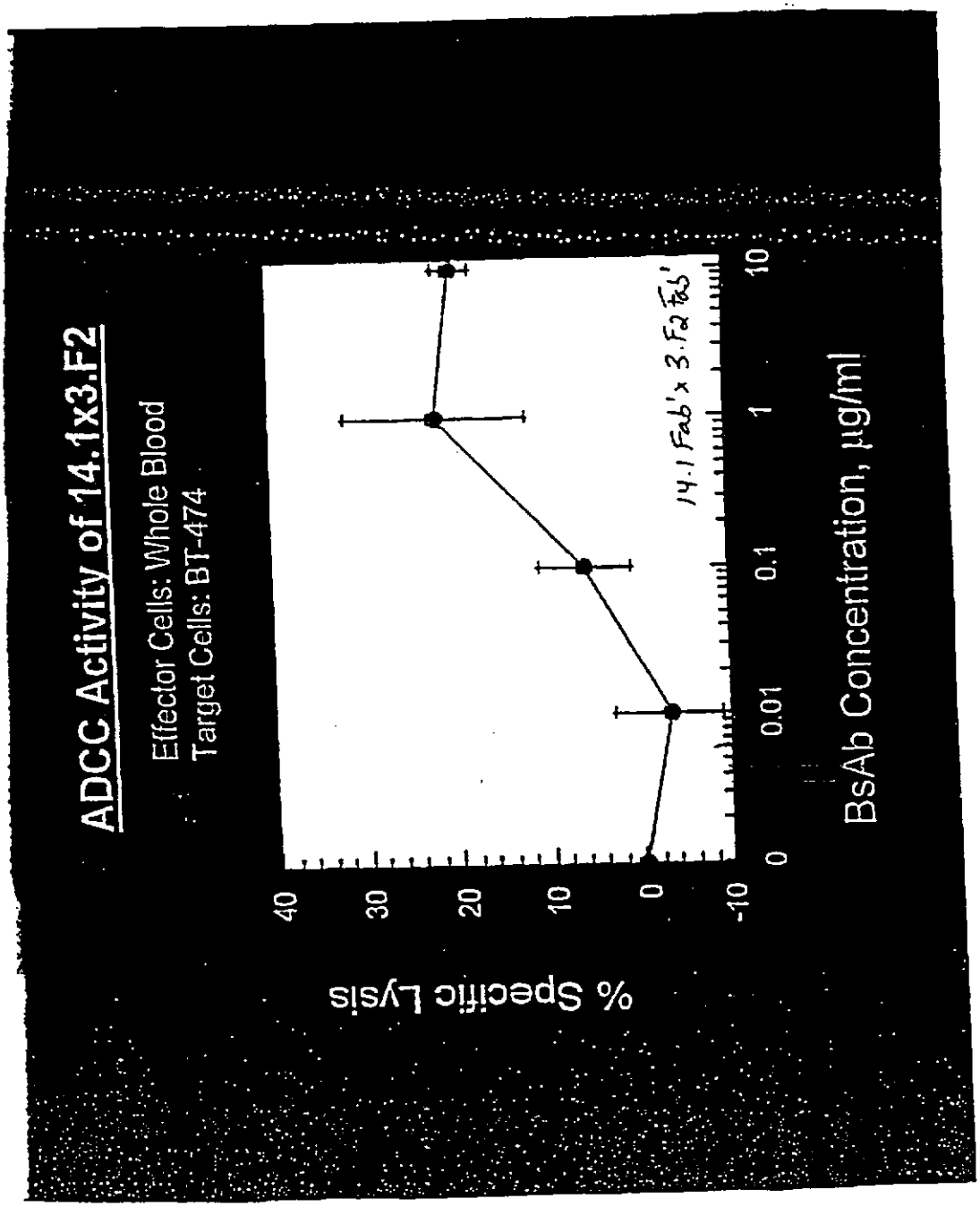

ADCC Activity of 14.1x3.F2
Effector Cells: Whole Blood
Target Cells: BT-474
% Specific Lysis
40
30
20
10
0
-10
0
0.01
0.1
1
10
BsAb Concentration, µg/ml
14.1 Fab' x 3.F2 Fab'

FIGURE 66