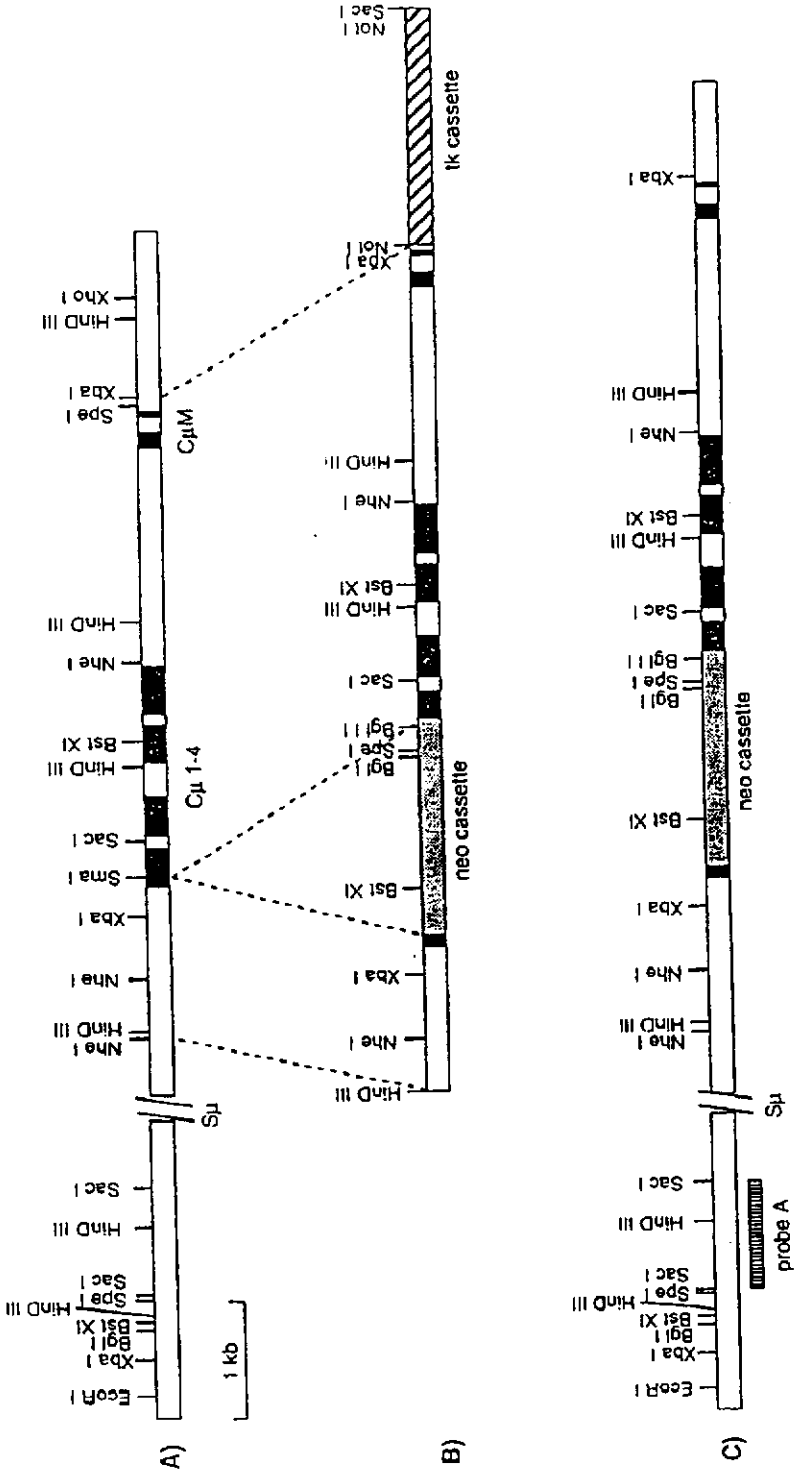


Expected Fragment Sizes (kb) using Probe A		
Restriction Digest	Fragment Length wild type	mutant
Bgl I	15.7	7.7
Bst XI	7.3	6.6
Spe I	9.9	7.6
Eco RI	12.5	14.3

FIGURE 1

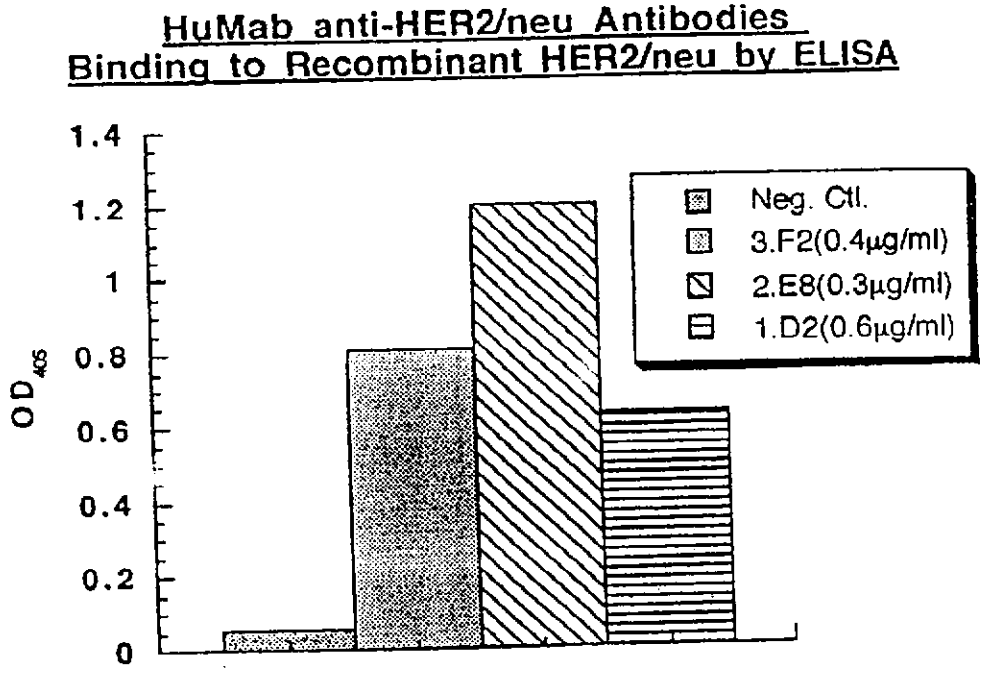

HuMab anti-HER2/neu Antibodies
Binding to Recombinant HER2/neu by ELISA
1.4
1.2
1
0.8
0.6
0.4
0.2
0
OD 405
Neg. Ctl.
3.F2(0.4µg/ml)
2.E8(0.3µg/ml)
1.D2(0.6µg/ml)

FIGURE 2 A

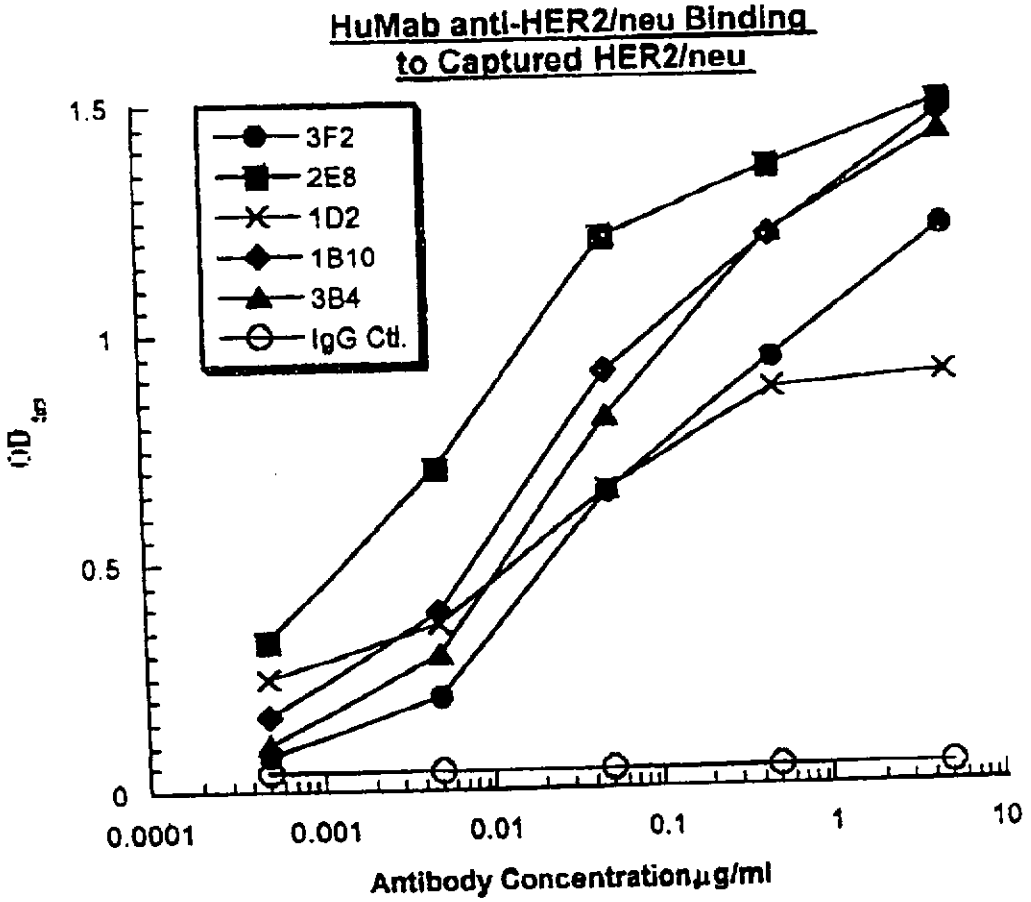

HuMab anti-HER2/neu Binding
to Captured HER2/neu
3F2
2E8
1D2
1B10
3B4
IgG Ctl.
1.5
1
0.5
0
OD 450
0.0001
0.001
0.01
0.1
1
10
Antibody Concentration µg/ml

FIGURE 2B

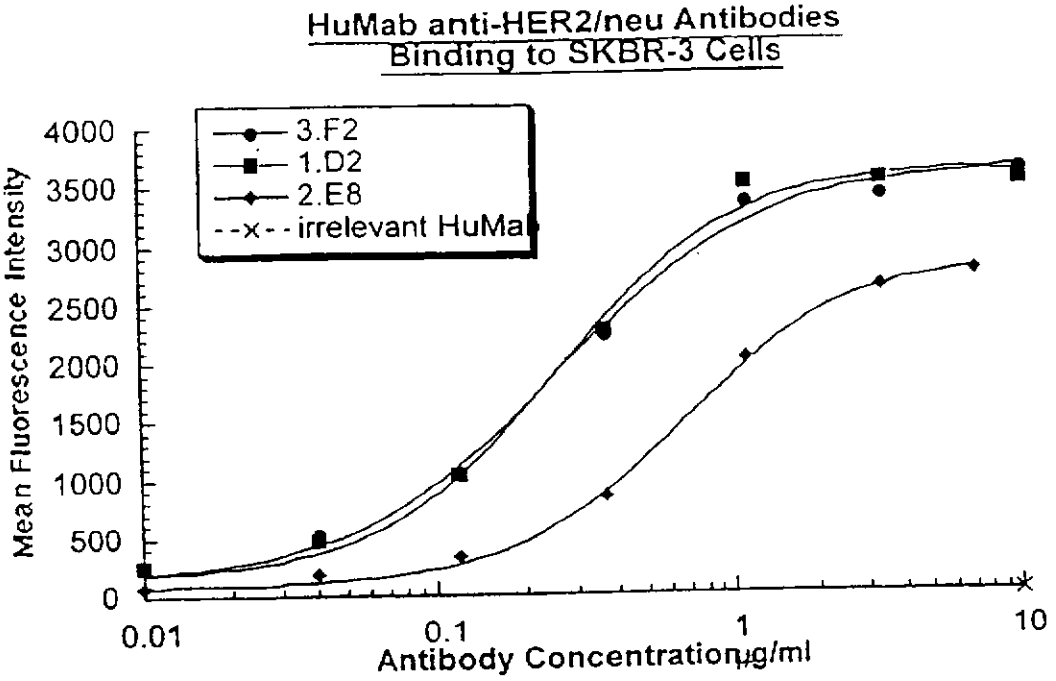

HuMab anti-HER2/neu Antibodies
Binding to SKBR-3 Cells
3.F2
1.D2
2.E8
--x-- irrelevant HuMab
Mean Fluorescence Intensity
4000
3500
3000
2500
2000
1500
1000
500
0
0.01
0.1
1
10
Antibody Concentration µg/ml

FIGURE 3 A

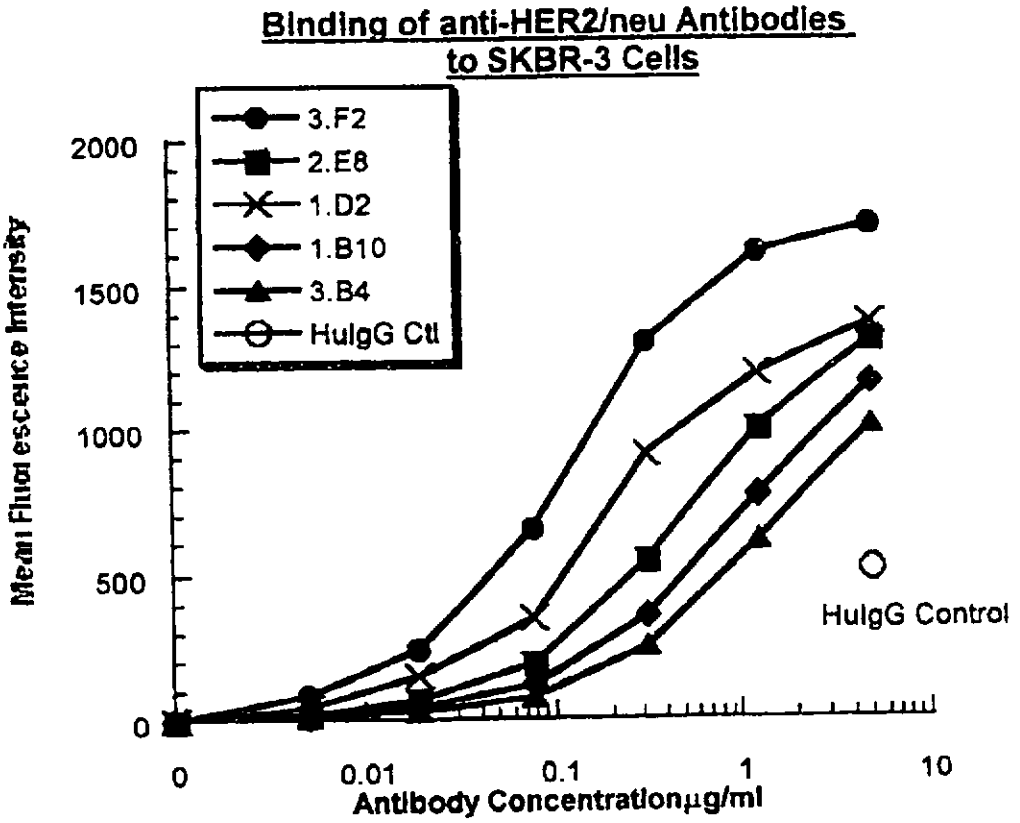

Binding of anti-HER2/neu Antibodies to SKBR-3 Cells
3.F2
2.E8
1.D2
1.B10
3.B4
HulgG Ctl
Mean Fluorescence Intensity
2000
1500
1000
500
0
0
0.01
0.1
1
10
Antibody Concentrationμg/ml
HulgG Control

FIGURE 3B

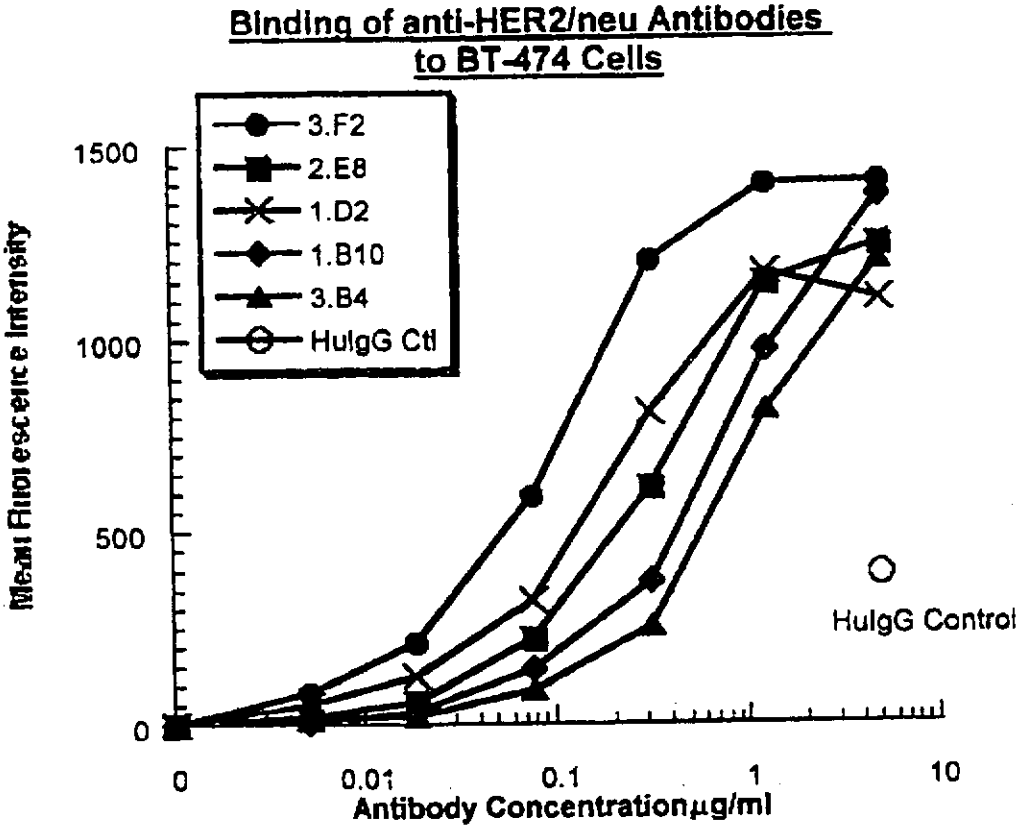

Binding of anti-HER2/neu Antibodies to BT-474 Cells
3.F2
2.E8
1.D2
1.B10
3.B4
HuIgG Ctl
Mean Fluorescence Intensity
1500
1000
500
0
0
0.01
0.1
1
10
Antibody Concentrationμg/ml
HuIgG Control

FIGURE 3C

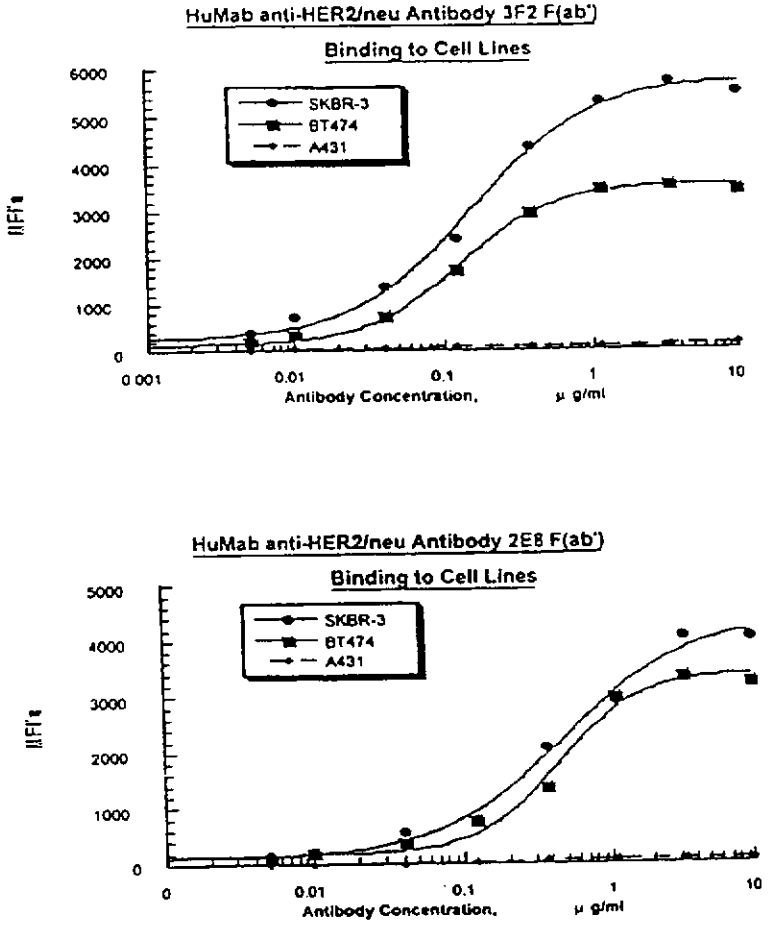

HuMab anti-HER2/neu Antibody 3F2 F(ab')
Binding to Cell Lines
SKBR-3
BT474
A431
MFI
6000
5000
4000
3000
2000
1000
0
0.001
0.01
0.1
1
10
Antibody Concentration, μ g/ml
HuMab anti-HER2/neu Antibody 2E8 F(ab')
Binding to Cell Lines
SKBR-3
BT474
A431
MFI
5000
4000
3000
2000
1000
0
0
0.01
0.1
1
10
Antibody Concentration, μ g/ml

FIGURE 4

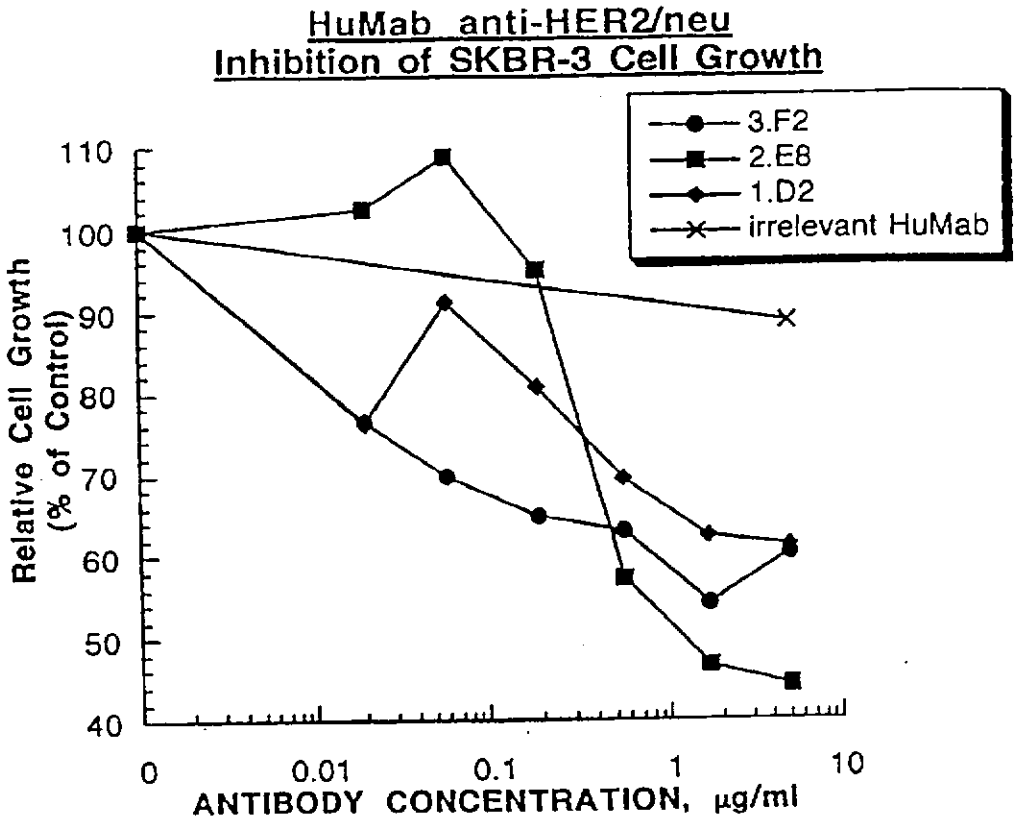

HuMab anti-HER2/neu
Inhibition of SKBR-3 Cell Growth
3.F2
2.E8
1.D2
irrelevant HuMab
Relative Cell Growth (% of Control)
110
100
90
80
70
60
50
40
0
0.01
0.1
1
10
ANTIBODY CONCENTRATION, µg/ml

FIGURE 5 A

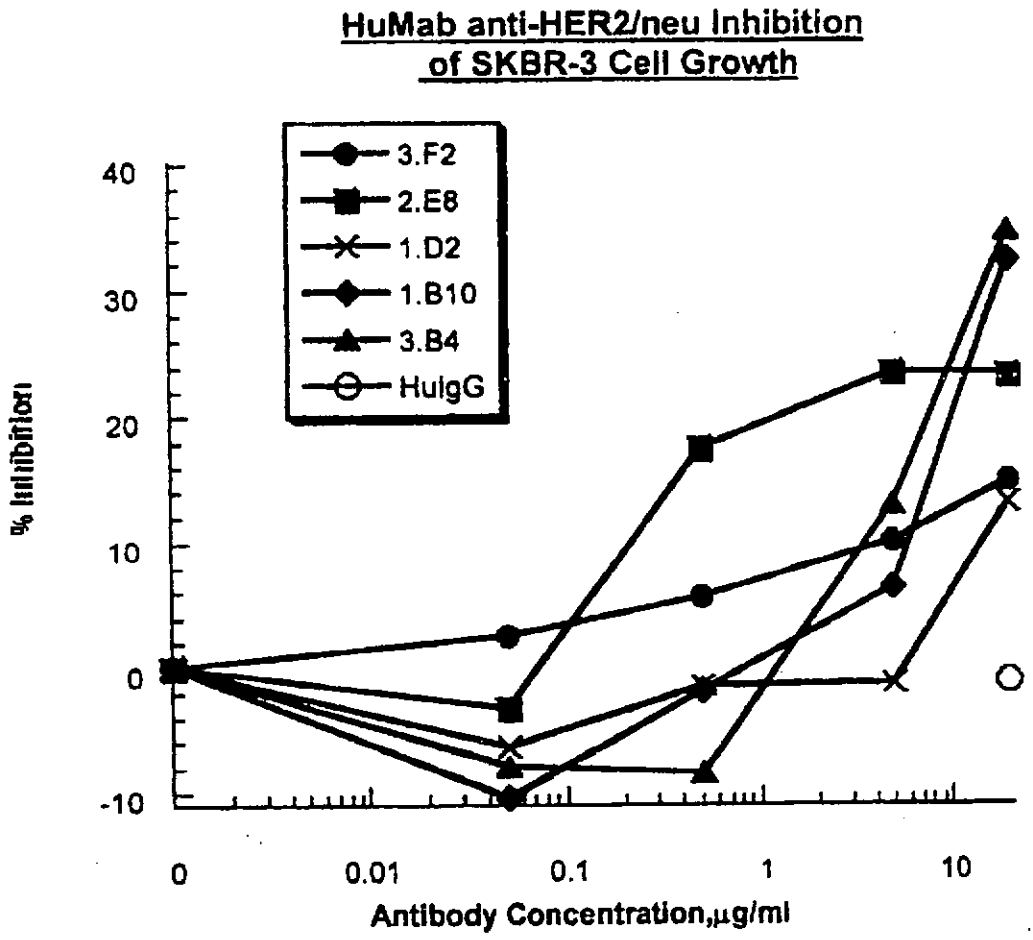

HuMab anti-HER2/neu Inhibition
of SKBR-3 Cell Growth
3.F2
2.E8
1.D2
1.B10
3.B4
HuIgG
% Inhibition
40
30
20
10
0
-10
0
0.01
0.1
1
10
Antibody Concentration,µg/ml

FIGURE 5B

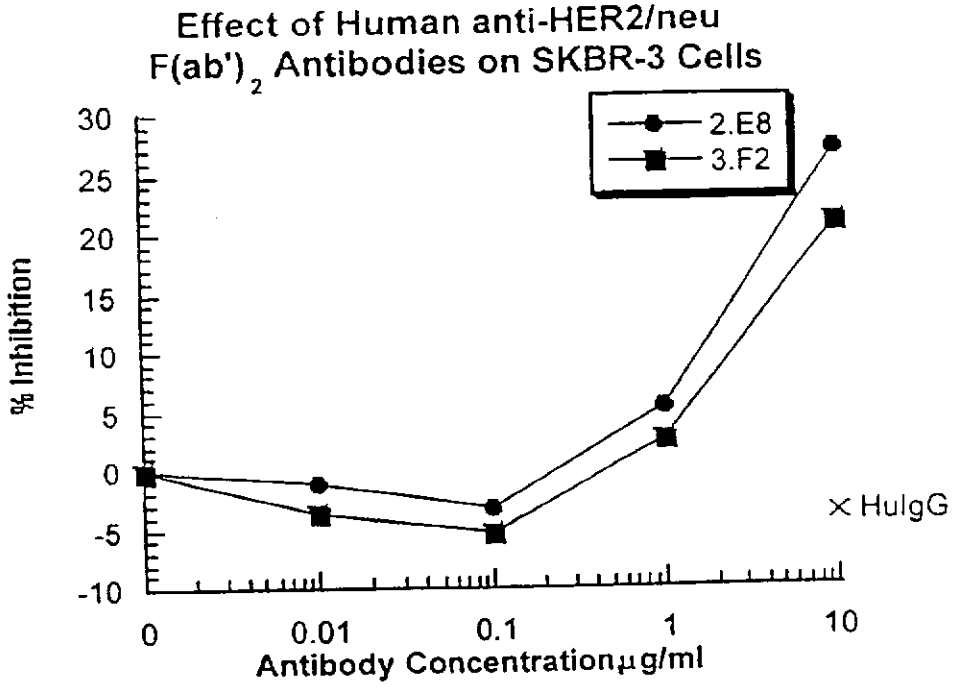

Effect of Human anti-HER2/neu
F(ab')2 Antibodies on SKBR-3 Cells
2.E8
3.F2
% Inhibition
30
25
20
15
10
5
0
-5
-10
0
0.01
0.1
1
10
Antibody Concentrationμg/ml
× HulgG

FIGURE 6

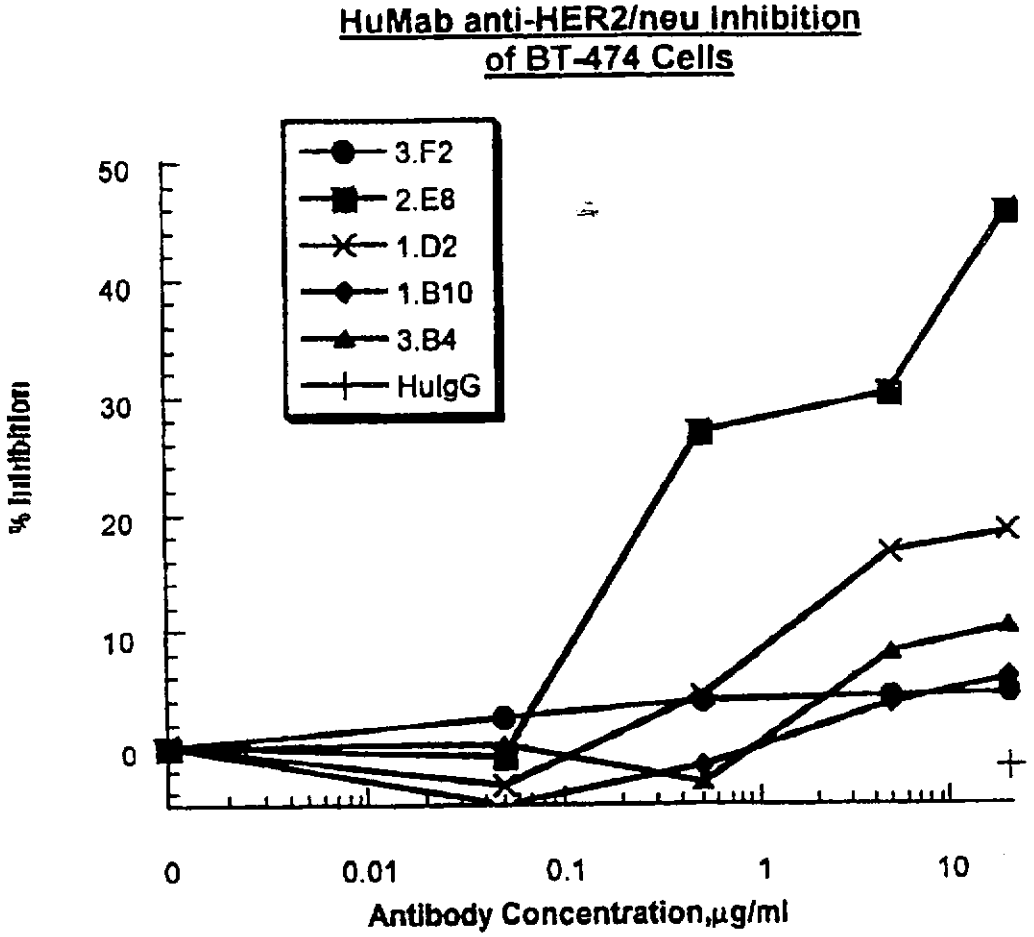

HuMab anti-HER2/neu Inhibition of BT-474 Cells
3.F2
2.E8
1.D2
1.B10
3.B4
HuIgG
% Inhibition
50
40
30
20
10
0
0
0.01
0.1
1
10
Antibody Concentration,µg/ml

FIGURE 7A

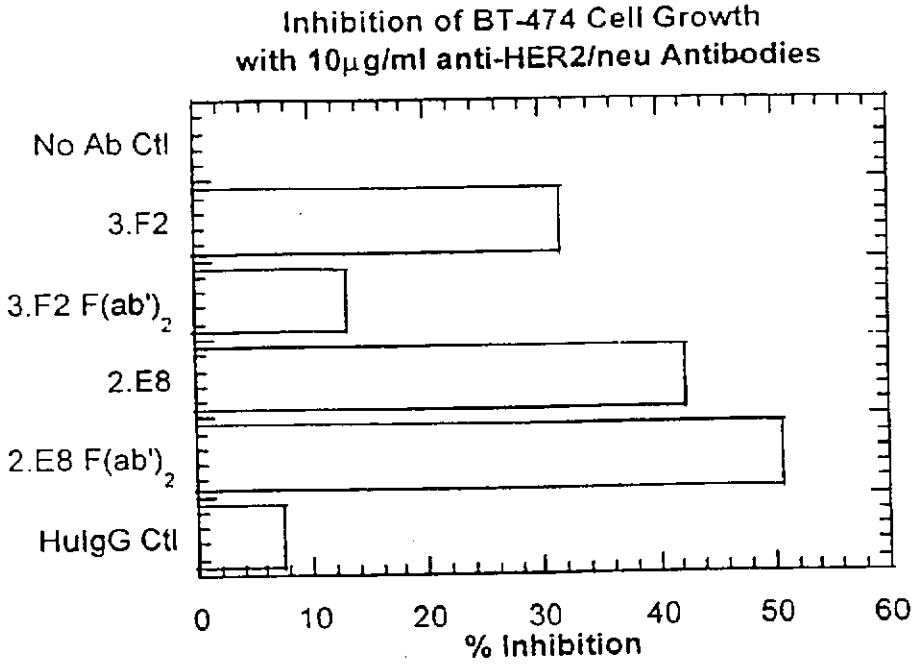

Inhibition of BT-474 Cell Growth
with 10μg/ml anti-HER2/neu Antibodies
No Ab Ctl
3.F2
3.F2 F(ab')2
2.E8
2.E8 F(ab')2
HuIgG Ctl
0
10
20
30
40
50
60
% Inhibition

FIGURE 7 B

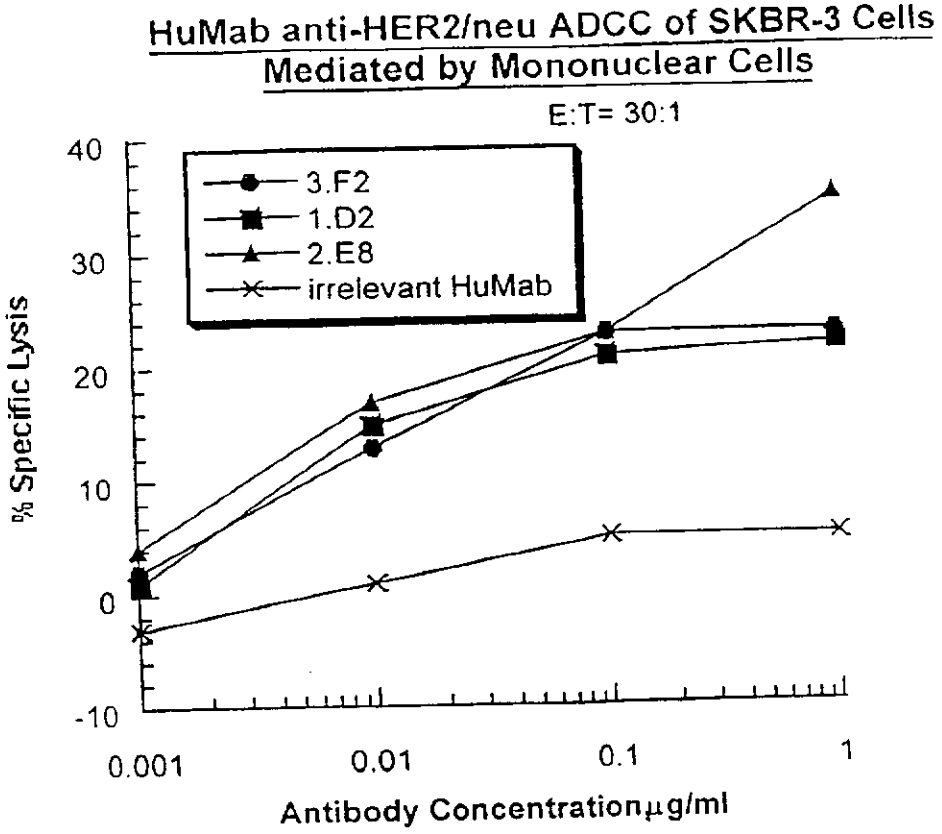

HuMab anti-HER2/neu ADCC of SKBR-3 Cells
Mediated by Mononuclear Cells
E:T= 30:1
3.F2
1.D2
2.E8
irrelevant HuMab
% Specific Lysis
40
30
20
10
0
-10
0.001
0.01
0.1
1
Antibody Concentrationμg/ml

FIGURE 8 A

ADCC of SKBR-3 Cells by γ-IFN Induced Macrophages Mediated by HuMab anti-HER2/neu Antibodies

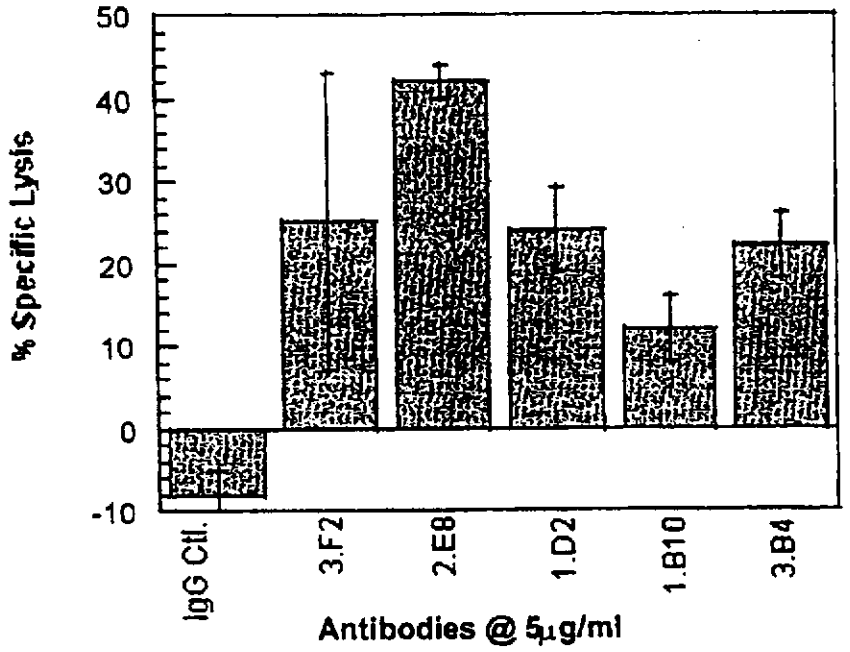


FIGURE 8B

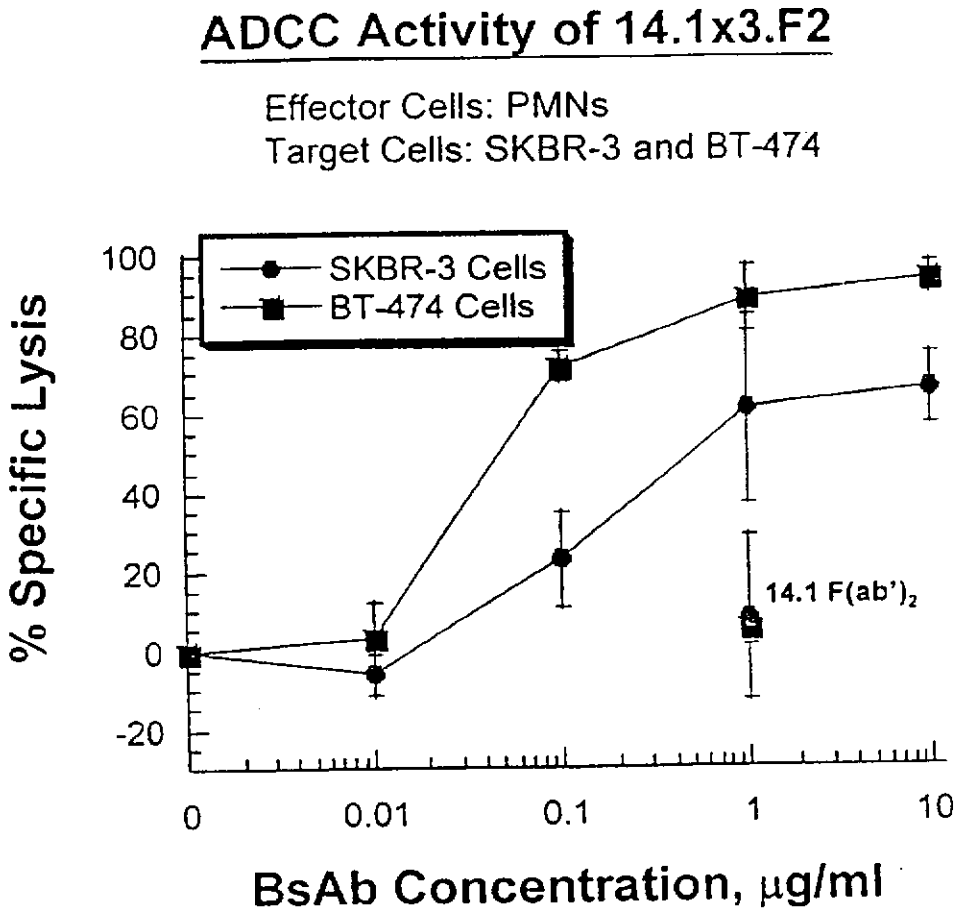

ADCC Activity of 14.1x3.F2
Effector Cells: PMNs
Target Cells: SKBR-3 and BT-474
SKBR-3 Cells
BT-474 Cells
14.1 F(ab')2
% Specific Lysis
100
80
60
40
20
0
-20
0
0.01
0.1
1
10
BsAb Concentration, µg/ml

FIGURE 9

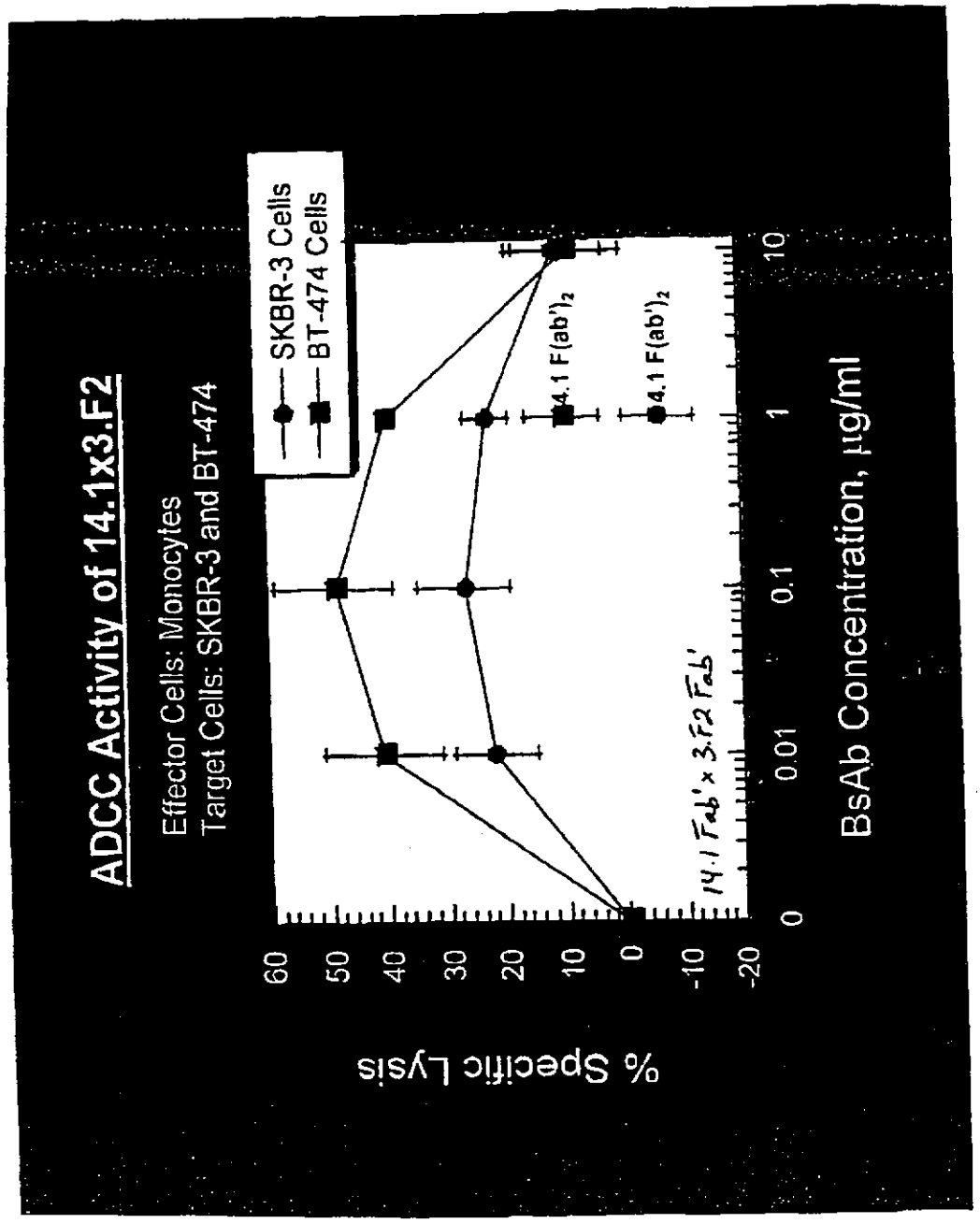

ADCC Activity of 14.1x3.F2
Effector Cells: Monocytes
Target Cells: SKBR-3 and BT-474
SKBR-3 Cells
BT-474 Cells
4.1 F(ab')2
4.1 F(ab')2
14.1 Fab' x 3.F2 Fab'
% Specific Lysis
60
50
40
30
20
10
0
-10
-20
0
0.01
0.1
1
10
BsAb Concentration, µg/ml

FIGURE 10

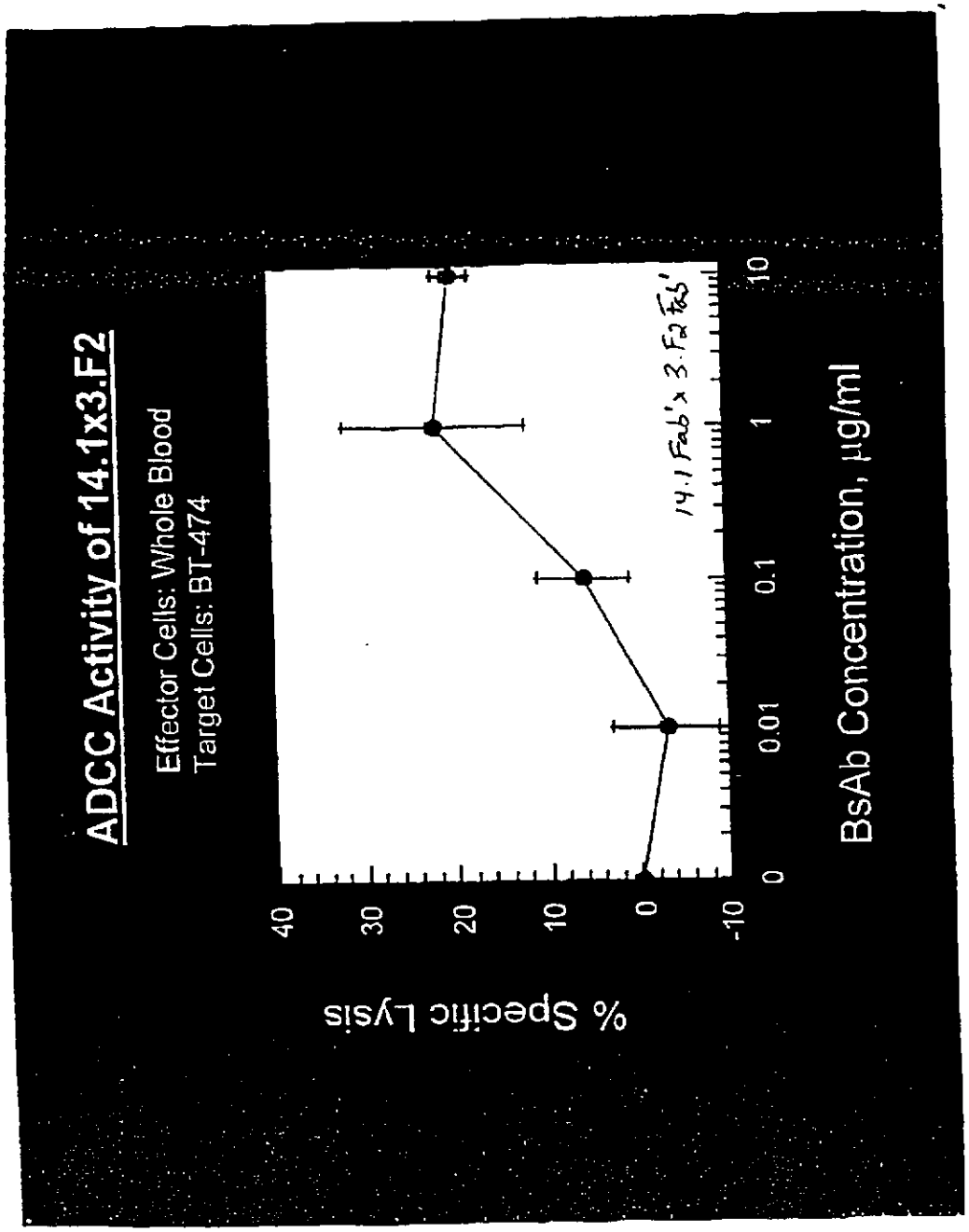

ADCC Activity of 14.1x3.F2
Effector Cells: Whole Blood
Target Cells: BT-474
% Specific Lysis
40
30
20
10
0
-10
0
0.01
0.1
1
10
BsAb Concentration, µg/ml
14.1 Fab' x 3.F2 Fab'

FIGURE 11

FIGURE 12

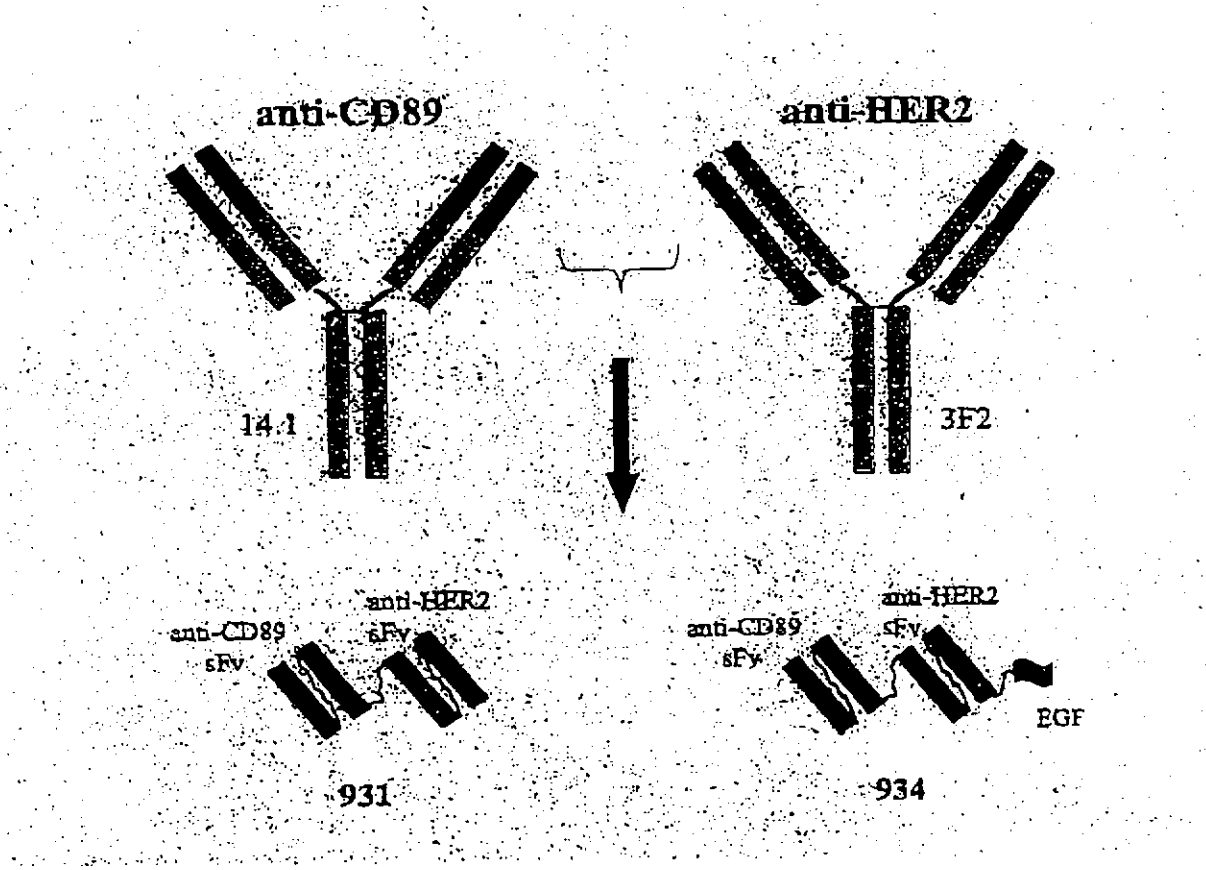

anti-CD89
anti-HER2
14.1
3F2
anti-CD89 sFv
anti-HER2 sFv
931
anti-CD89 sFv
anti-HER2 sFv
EGF
934

FIGURE 13

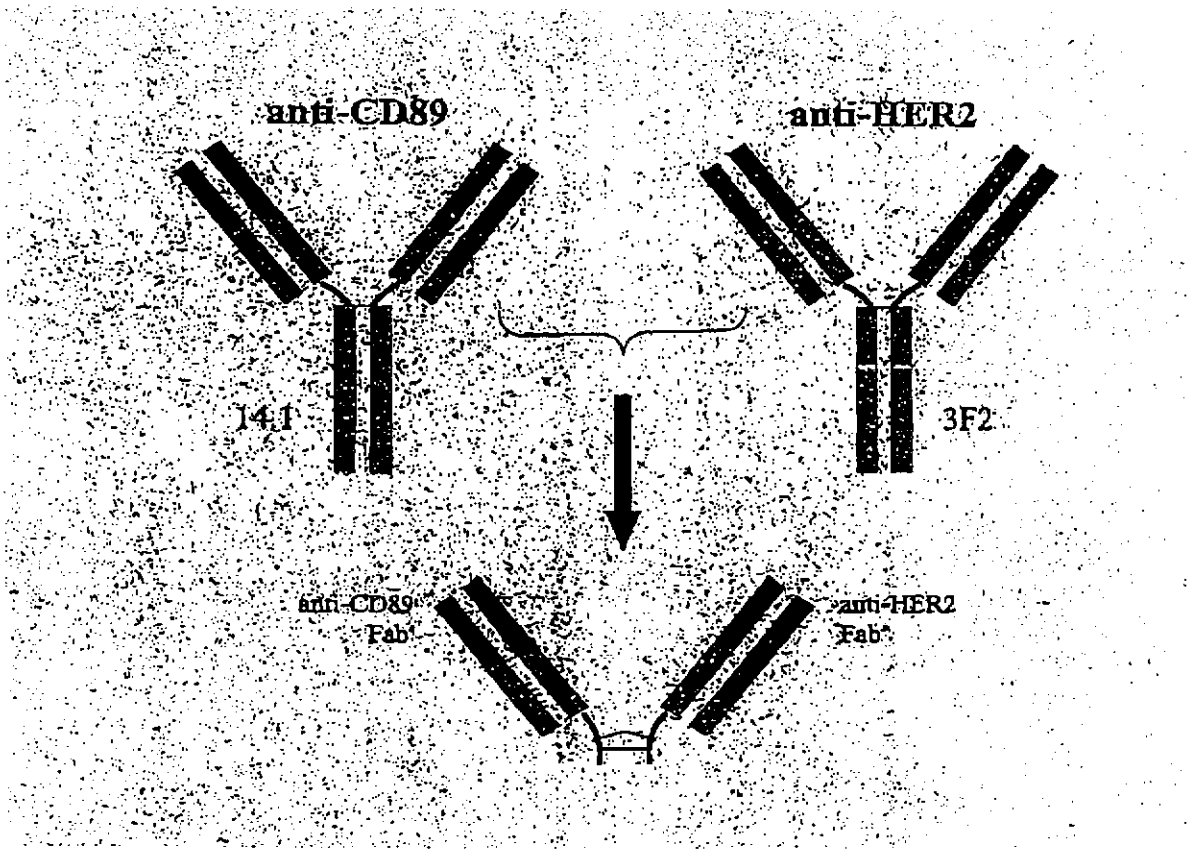
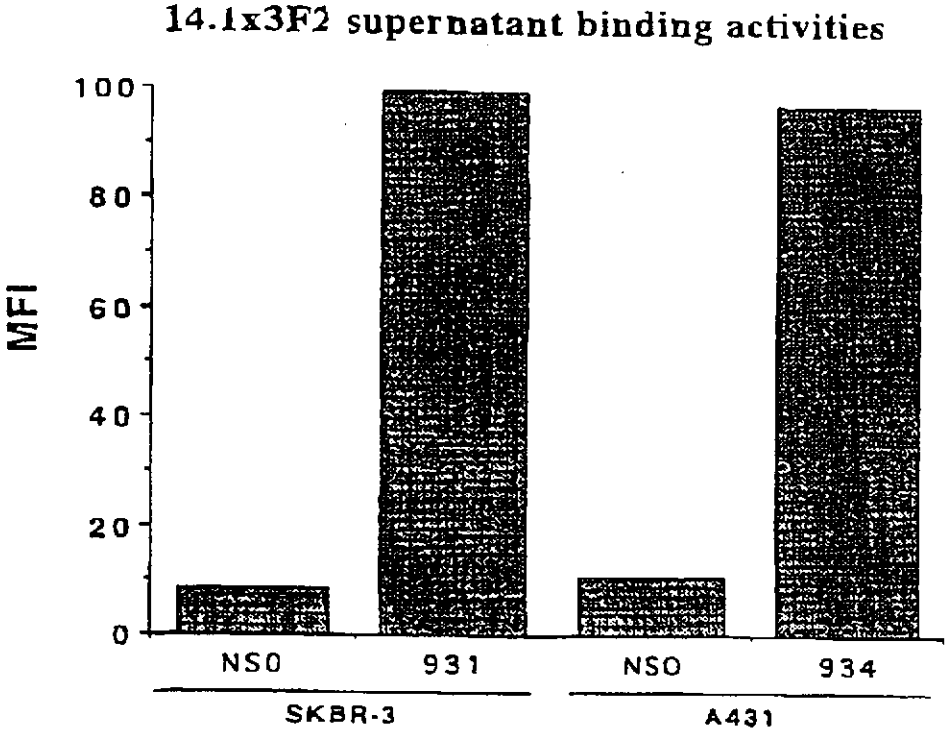


FIGURE 14


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

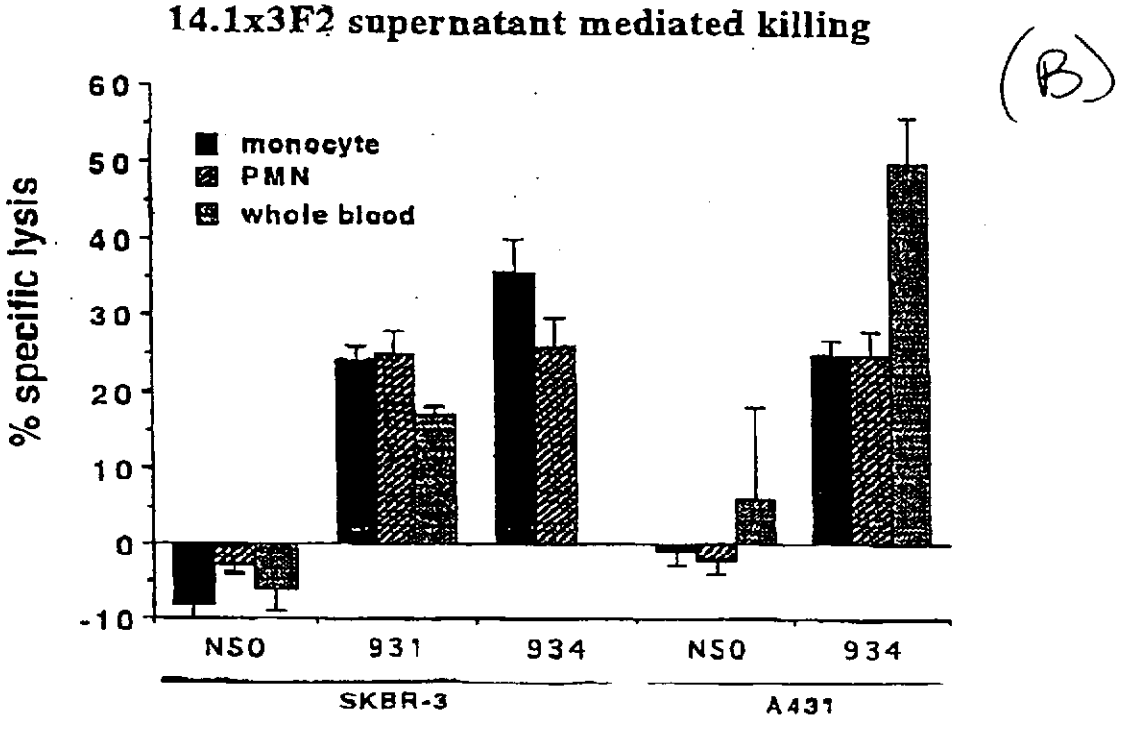
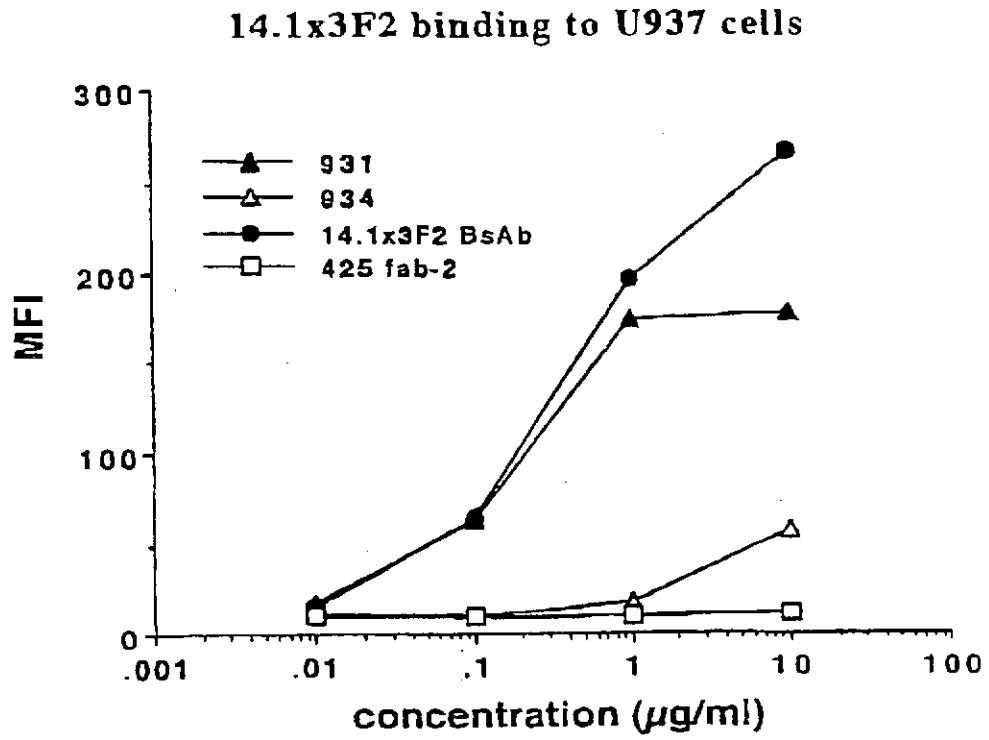

14.1x3F2 supernatant mediated killing
(B)
% specific lysis
60
50
40
30
20
10
0
-10
monocyte
PMN
whole blood
NSO
931
934
NSO
934
SKBR-3
A431

FIGURE 15

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)

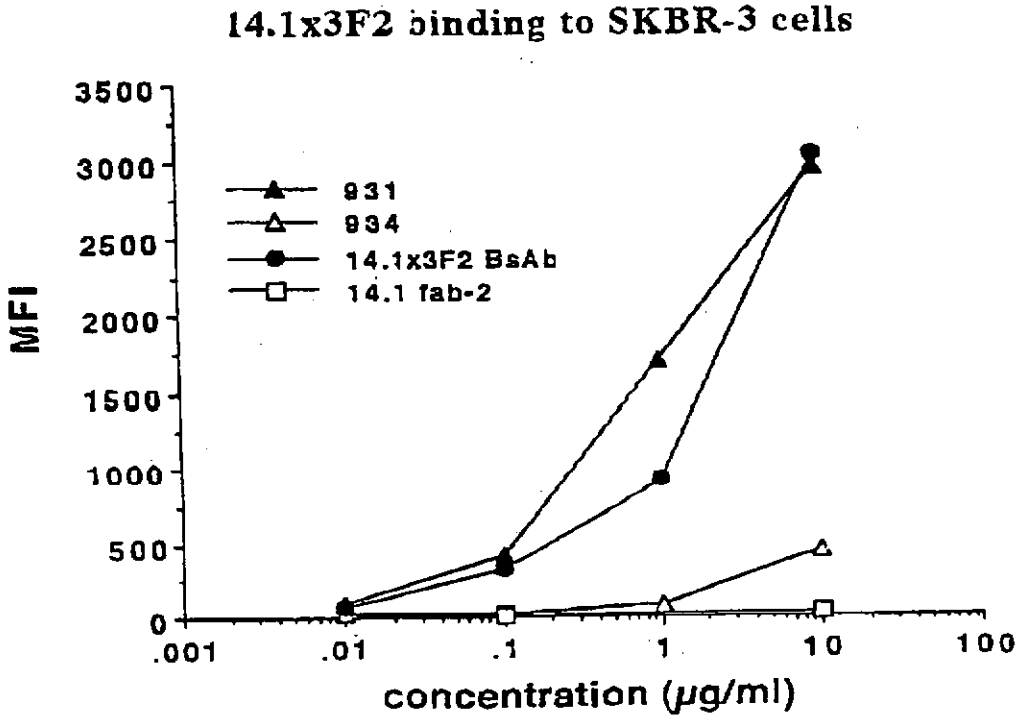

14.1x3F2 binding to SKBR-3 cells
MFI
3500
3000
2500
2000
1500
1000
500
0
931
934
14.1x3F2 BsAb
14.1 fab-2
.001
.01
.1
1
10
100
concentration (μg/ml)

FIGURE 16

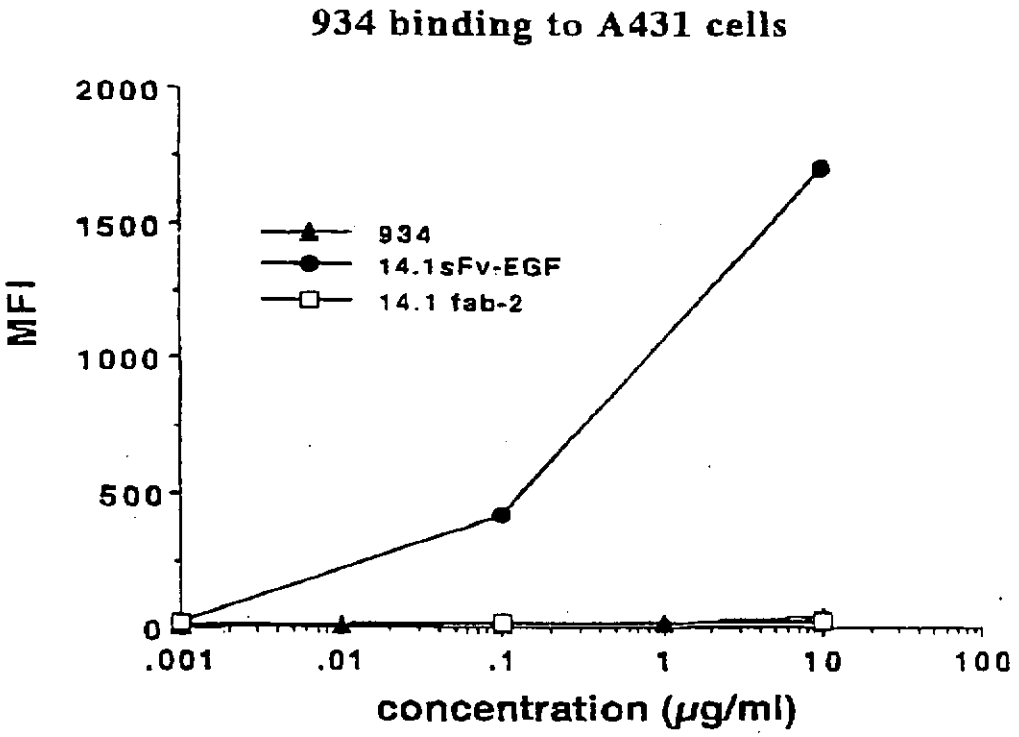
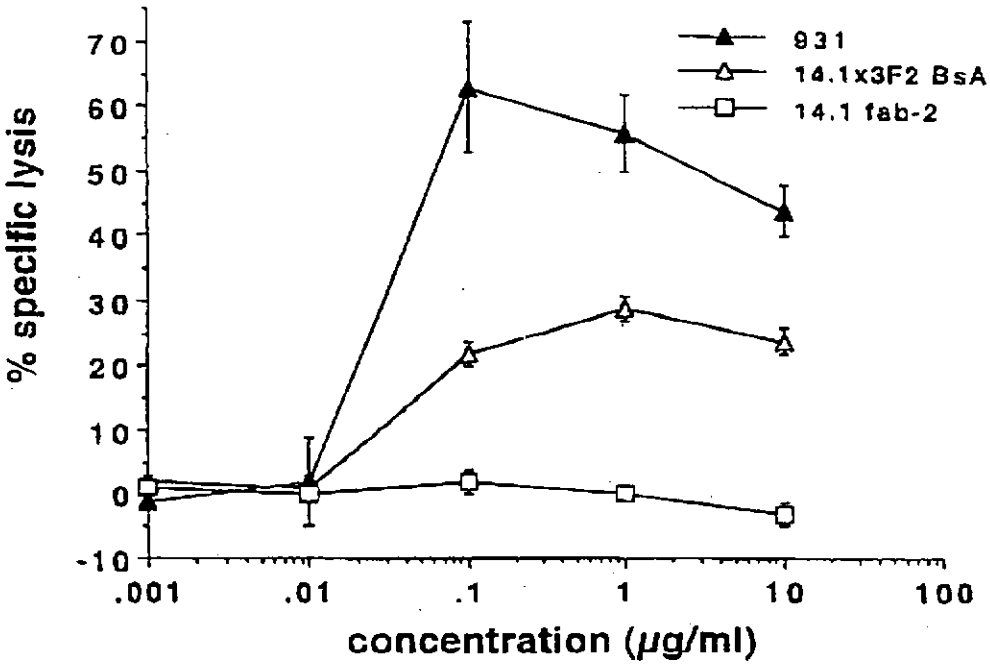

934 binding to A431 cells
2000
1500
1000
500
0
MFI
934
14.1sFv-EGF
14.1 fab-2
.001
.01
.1
1
10
100
concentration (μg/ml)

FIGURE 17

14.1x3F2 mediated killing of SKBR3 by PMNs (A)



14.1x3F2 mediated killing of BT474 by PMNs (B)

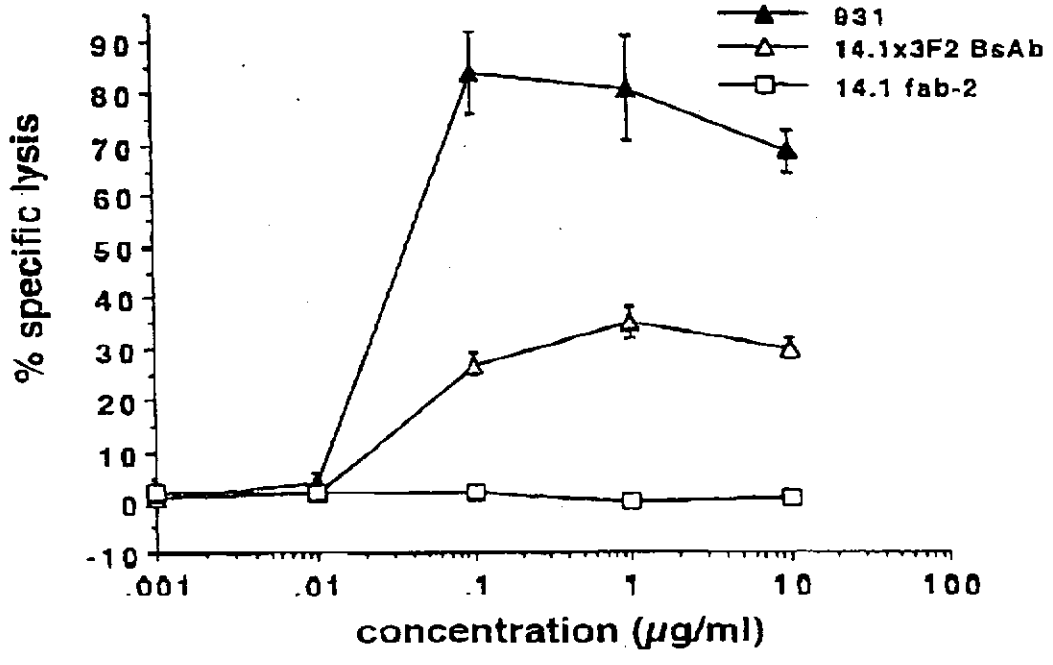
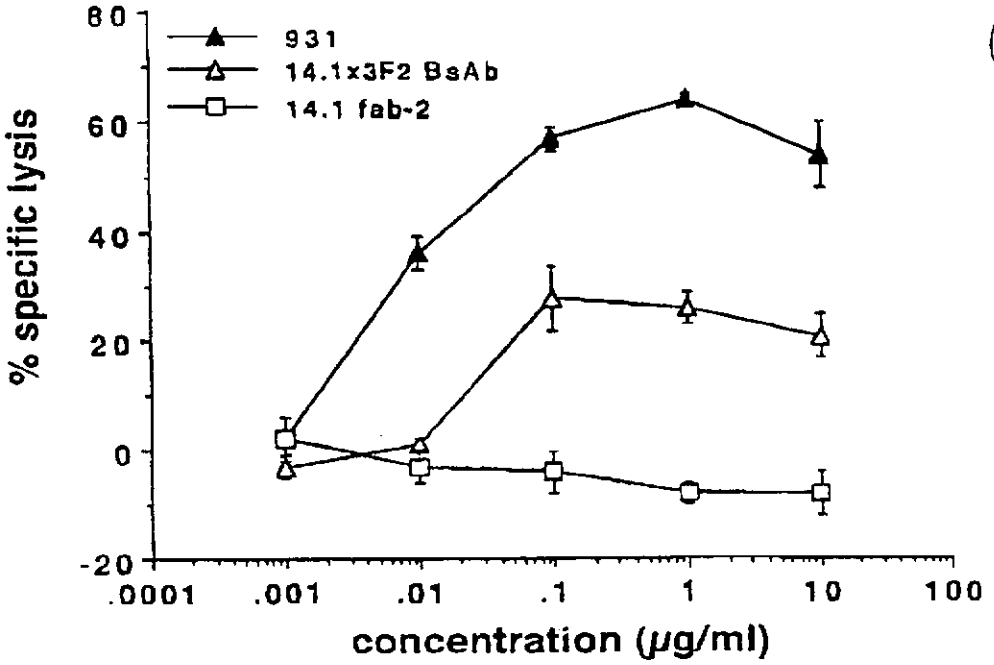


FIGURE 18

14.1x3F2 mediated killing of SKBR3 by monocytes

(A)



14.1x3F2 mediated killing of BT474 by monocytes

(B)

