Map of myc-murine APRIL construct pCCM213.10 for expression in Pichia pastoris

```
CCAAACG ATG AGA TTT CCT TCA ATT TTT ACT GCA GTT TTA TTC GCA GCA TCC TCC GCA TTA GCT GCT CCA GTC AAC ACT ACA
GGTTTGC TAC TCT AAA GGA AGT TAA AAA TGA CGT CAA AAT AAG CGT CGT AGG AGG CGT AAT CGA CGA GGT CAG TTG TGA TGT
      M   R   F   P   S   I   F   T   A   V   L   F   A   A   S   S   A   L   A   A   P   V   N   T   T

ACA GAA GAT GAA ACG GCA CAA ATT CCG GCT GAA GCT GTC ATC GGT TAC TCA GAT TTA GAA GGG GAT TTC GAT GTT GCT GTT
TGT CTT CTA CTT TGC CGT GTT TAA GGC CGA CTT CGA CAG TAG CCA ATG AGT CTA AAT CTT CCC CTA AAG CTA CAA CGA CAA
  T   E   D   E   T   A   Q   I   P   A   E   A   V   I   G   Y   S   D   L   E   G   D   F   D   V   A   V

TTG CCA TTT TCC AAC AGC ACA AAT AAC GGG TTA TTG TTT ATA AAT ACT ACT ATT GCC AGC ATT GCT GCT AAA GAA GAA GGG
AAC GGT AAA AGG TTG TCG TGT TTA TTG CCC AAT AAC AAA TAT TTA TGA TGA TAA CGG TCG TAA CGA CGA TTT CTT CTT CCC
  L   P   F   S   N   S   T   N   N   G   L   L   F   I   N   T   T   I   A   S   I   A   A   K   E   E   G

GTA TCT CTC GAG AAA AGA GAA CAA AAA CTC ATT TCT GAG GAA GAT CTG AAT AAA GAG CTC CAC TCA GTC CTG CAT CTT GTT
CAT AGA GAG CTC TTT TCT CTT GTT TTT GAG TAA AGA CTC CTT CTa GAC TTA TTT CTC GAG GTG AGT CAG GAC GTA GAA CAA
                    myc-tag ____ ____ ____
```

Map Of Flag-Human APRIL Construct PS429 For Expression In Mammalian Cells

```
TTAATCAAAAC ATG GCT ATC ATC TAC CTC ATC CTC CTG TTC ACC GCT GTG CGG GGC GAT TAC AAA GAC GAT GAC GAT
AATTAGTTTTG TAC CGA TAG TAG ATG GAG TAG GAG GAC AAG TGG CGA CAC GCC CCG CTA ATG TTT CTG CTA CTG CTA

                                                                                  FLAG-tag
        >>> M   A   I   I   Y   L   I   L   L   F   T   A   V   R   G   D   Y   K   D   D   D   D

AAA GGA CCC GGA CAG GTG CAG CTG CAG AAA CAG AAG AAG CAG CAC TCT GTC CTG CAC CTG GTT CCC ATT AAC GCC
TTT CCT GGG CCT GTC CAC GTC GAC GTC TTT GTC TTC TTC GTC GTG AGA CAG GAC GTG GAC CAA GGG TAA TTG CGG

                                    >>> human APRIL
 K   G   P   G   Q   V   Q   L   Q   K   Q   K   K   Q   H   S   V   L   H   L   V   P   I   N   A

ACC TCC AAG GAT GAC TCC GAT GTG ACA GAG GTG ATG TGG CAA CCA GCT CTT AGG CGT GGG AGA GGC CTA CAG GCC
TGG AGG TTC CTA CTG AGG CTA CAC TGT CTC CAC TAC ACC GTT GGT CGA GAA TCC GCA CCC TCT CCG GAT GTC CGG
 T   S   K   D   D   S   D   V   T   E   V   M   W   Q   P   A   L   R   R   G   R   G   L   Q   A

CAA GGA TAT GGT GTC CGA ATC CAG GAT GCT GGA GTT TAT CTG CTG TAT AGC CAG GTC CTG TTT CAA GAC GTG ACT
GTT CCT ATA CCA CAG GCT TAG GTC CTA CGA CCT CAA ATA GAC GAC ATA TCG GTC CAG GAC AAA GTT CTG CAC TGA
 Q   G   Y   G   V   R   I   Q   D   A   G   V   Y   L   L   Y   S   Q   V   L   F   Q   D   V   T

TTC ACC ATG GGT CAG GTG GTG TCT CGA GAA GGC CAA GGA AGG CAG GAG ACT CTA TTC CGA TGT ATA AGA AGT ATG
AAG TGG TAC CCA GTC CAC CAC AGA GCT CTT CCG GTT CCT TCC GTC CTC TGA GAT AAG GCT ACA TAT TCT TCA TAC
 F   T   M   G   Q   V   V   S   R   E   G   Q   G   R   Q   E   T   L   F   R   C   I   R   S   M

CCC TCC CAC CCG GAC CGG GCC TAC AAC AGC TGC TAT AGC GCA GGT GTC TTC CAT TTA CAC CAA GGG GAT ATT CTG
GGG AGG GTG GGC CTG GCC CGG ATG TTG TCG ACG ATA TCG CGT CCA CAG AAG GTA AAT GTG GTT CCC CTA TAA GAC
 P   S   H   P   D   R   A   Y   N   S   C   Y   S   A   G   V   F   H   L   H   Q   G   D   I   L

AGT GTC ATA ATT CCC CGG GCA AGG GCG AAA CTT AAC CTC TCT CCA CAT GGA ACC TTC CTG GGG TTT GTG AAA CTG
TCA CAG TAT TAA GGG GCC CGT TCC CGC TTT GAA TTG GAG AGA GGT GTA CCT TGG AAG GAC CCC AAA CAC TTT GAC
 S   V   I   I   P   R   A   R   A   K   L   N   L   S   P   H   G   T   F   L   G   F   V   K   L

TGA TCTAGAGGGCCC
ACT AGATCTCCCGGG
```

FIG. 2

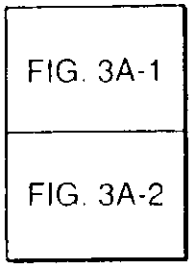

FIG. 3A

Nucleotide sequence (sequence ID #7) and
amino acid sequence (sequence id #8) of full length human BCMA.

sequence ID#7

```
   1 atgttgcagatggctgggcagtgctcccaaaatgaatattttgacagtttgttgcatgcttgcataccttgt
     tacaacgtctaccgacccgtcacgagggttttacttataaaactgtcaaacaacgtacgaacgtatggaaca
```

sequence ID#8

```
   1> M  L  Q  M  A  G  Q  C  S  Q  N  E  Y  F  D  S  L  L  H  A  C  I  P  C

  73 caacttcgatgttcttctaatactcctcctctaacatgtcagcgttattgtaatgcaagtgtgaccaattca
     gttgaagctacaagaagattatgaggaggagattgtacagtcgcaataacattacgttcacactggttaagt

  25> Q  L  R  C  S  S  N  T  P  P  L  T  C  Q  R  Y  C  N  A  S  V  T  N  S
```

FIG. 3A-1

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```
145  gtgaaaggaacgaatgcgattctctggacctgtttgggactgagcttaataatttctttggcagttttcgtg
     cactttccttgcttacgctaagagacctggacaaaccctgactcgaattattaaagaaaccgtcaaaagcac

 49>  V   K   G   T   N   A   I   L   W   T   C   L   G   L   S   L   I   I   S   L   A   V   F   V

217  ctaatgtttttgctaaggaagataagctctgaaccattaaaggacgagtttaaaaacacaggatcaggtctc
     gattacaaaaacgattccttctattcgagacttggtaatttcctgctcaaattttttgtgtcctagtccagag

 73>  L   M   F   L   L   R   K   I   S   S   E   P   L   K   D   E   F   K   N   T   G   S   G   L

289  ctgggcatggctaacattgacctggaaaagagcaggactggtgatgaaattattcttccgagaggcctcgag
     gacccgtaccgattgtaactggacctttttctcgtcctgaccactactttaataagaaggctctccggagctc

 97>  L   G   M   A   N   I   D   L   E   K   S   R   T   G   D   E   I   I   L   P   R   G   L   E

361  tacacggtggaagaatgcacctgtgaagactgcatcaagagcaaaccgaaggtcgactctgaccattgcttt
     atgtgccaccttcttacgtggacacttctgacgtagttctcgtttggcttccagctgagactggtaacgaaa

121>  Y   T   V   E   E   C   T   C   E   D   C   I   K   S   K   P   K   V   D   S   D   H   C   F

433  ccactcccagctatggaggaaggcgcaaccattcttgtcaccacgaaaacgaatgactattgcaagagcctg
     ggtgagggtcgatacctccttccgcgttggtaagaacagtggtgcttttgcttactgataacgttctcggac

145>  P   L   P   A   M   E   E   G   A   T   I   L   V   T   T   K   T   N   D   Y   C   K   S   L

505  ccagctgctttgagtgctacggagatagagaaatcaatttctgctaggtaa
     ggtcgacgaaactcacgatgcctctatctctttagttaaagacgatccatt

169>  P   A   A   L   S   A   T   E   I   E   K   S   I   S   A   R   *
```

FIG. 3A-2

FIG. 3B-1	FIG. 3B-2	FIG. 3B-3

FIG. 3B

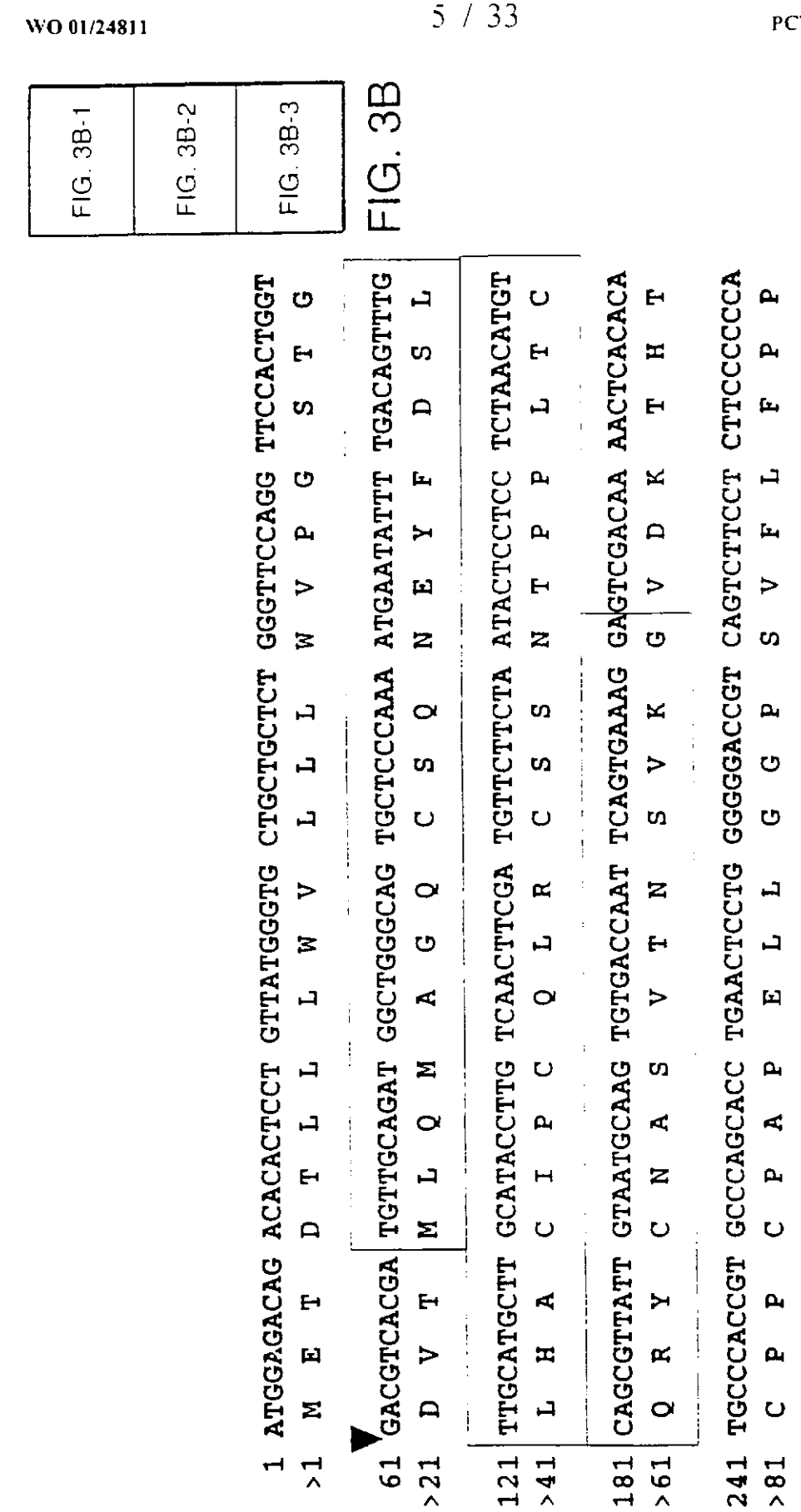

FIG. 3B-1

```
301 AAACCCAAGG ACACCCTCAT GATCTCCCGG ACCCCTGAGG TCACATGCGT GGTGGTGGAC
101   K   P   K     D   T   L   M     I   S   R     T   P   E     V   T   C   V     V   V   D

361 GTGAGCCACG AAGACCCTGA GGTCAAGTTC AACTGGTACG TGGACGGCGT GGAGGTGCAT
121   V   S   H     E   D   P   E     V   K   F     N   W   Y     V   D   G   V     E   V   H

421 AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TACAACAGCA CGTACCGTGT GGTCAGCGTC
141   N   A   K     T   K   P   R     E   E   Q     Y   N   S     T   Y   R   V     V   S   V

481 CTCACCGTCC TGCACCAGGA CTGGCTGAAT GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC
161   L   T   V     L   H   Q   D     W   L   N     G   K   E     Y   K   C   K     V   S   N

541 AAAGCCCTCC CAGCCCCCAT CGAGAAAACC ATCTCCAAAG CCAAAGGGCA GCCCCGAGAA
181   K   A   L     P   A   P   I     E   K   T     I   S   K     A   K   G   Q     P   R   E

601 CCACAGGTGT ACACCCTGCC CCCATCCCGG GATGAGCTGA CCAAGAACCA GGTCAGCCTG
201 P   Q   V     Y   T   L   P     P   S   R     D   E   L     T   K   N   Q     V   S   L

661 ACCTGCCTGG TCAAAGGCTT CTATCCCAGC GACATCGCCG TGGAGTGGGA GAGCAATGGG
221   T   C   L     V   K   G   F     Y   P   S     D   I   A     V   E   W   E     S   N   G
```

FIG. 3B-2

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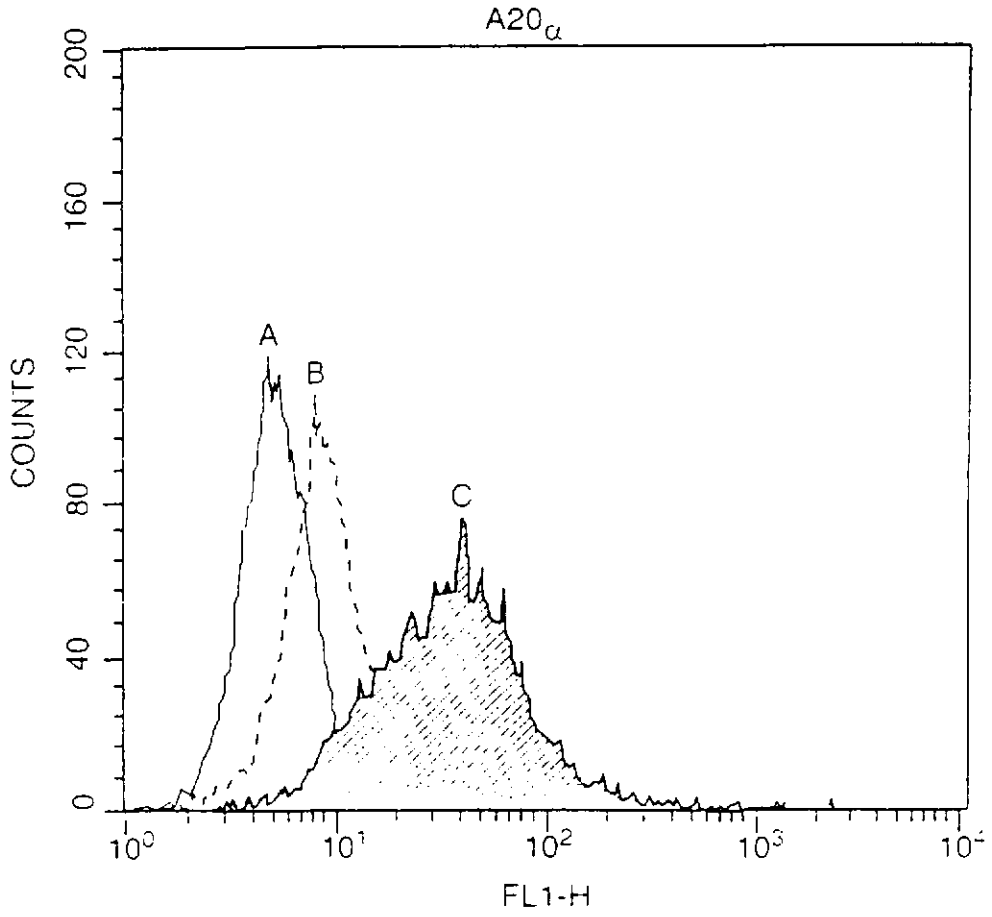
```
 721 CAGCCGGAGA ACAACTACAA GACCACGCCT CCCGTGTTGG ACTCCGACGG CTCCTTCTTC
>241  Q  P  E   N  N  Y  K   T  T  P   P  V  L   D  S  D  G   S  F  F

 781 CTCTACAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC AGGGGAACGT CTTCTCATGC
>261 L  Y  S   K  L  T  V   D  K  S   R  W  Q   Q  G  N  V   F  S  C

 841 TCCGTGATGC ATGAGGCTCT GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCC
>281  S  V  M   H  E  A  L   H  N  H   Y  T  Q   K  S  L  S   L  S  P

 901 GGGAAA
>301  G  K
```

Fig. 3B: Example of a gene encoding a huBCMA-huIgG1 fusion protein. Origin of nucleotide and amino acid sequence for hBCMA-hIgG1 fusion protein from expression vector pJST538 is as follows: signal peptide derived from a murine Ig kappa cDNA is encoded nucleotides 1-66 (amino acids 1-22); cysteine rich domain of human BCMA is boxed and encoded by nucleotides 70-222 (amino acids 24-74); hIgG1 is encoded by nucleotides 226-909 (amino acids 76-302); noncritical residues were introduced at the cloning junctions (amino acids 23 and 75). Arrowhead indicates predicted site of signal sequence cleavage.

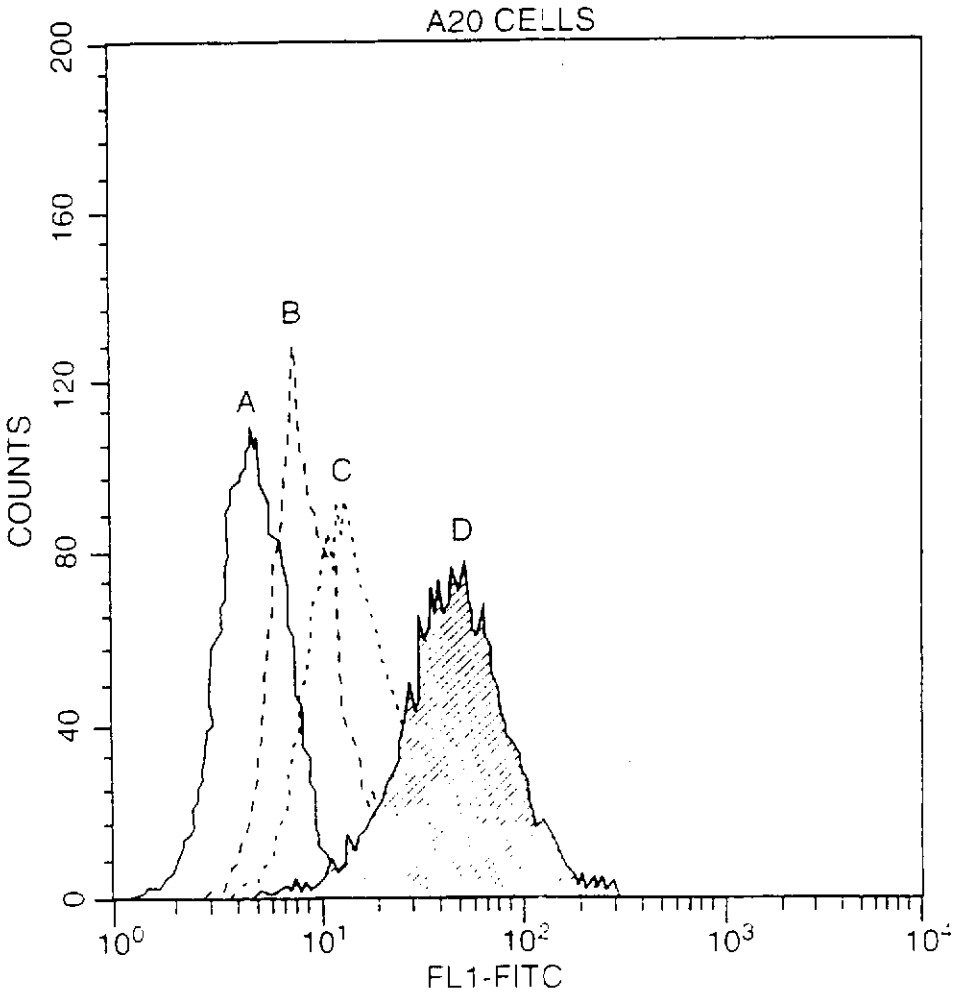
FIG. 3B-3

SUBSTITUTE SHEET (RULE 26)



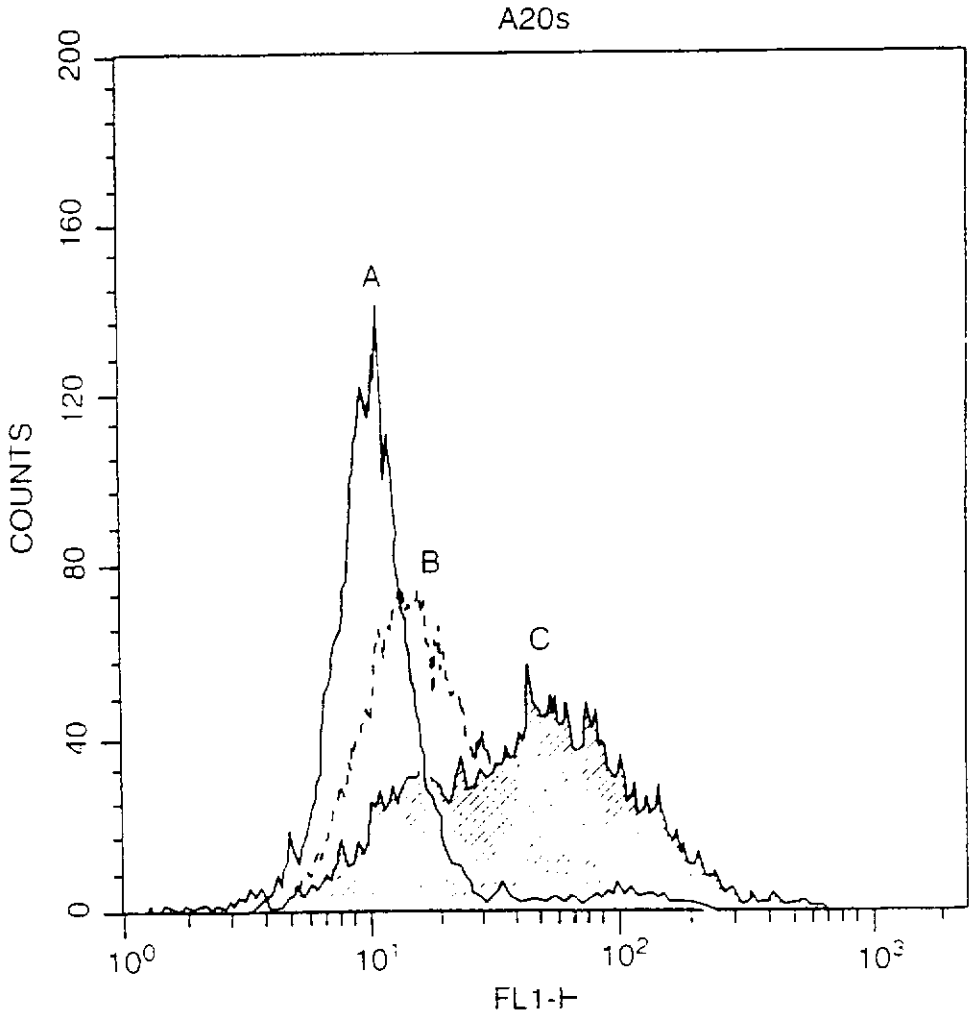
A: UNSTAINED CELLS
B: RABBIT SERA 1532 CONTROL
C: 450ng APRIL + R1532

FIG. 4A



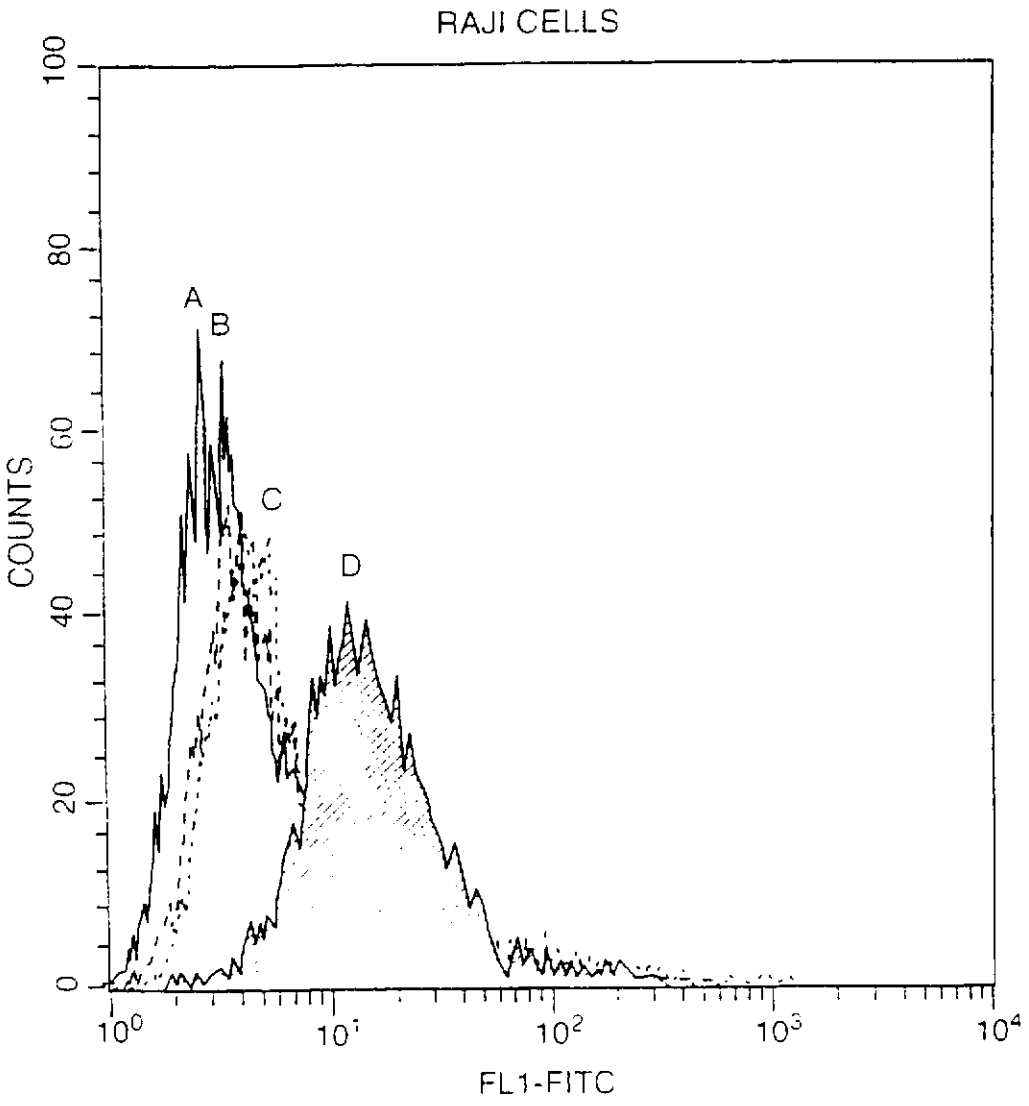
A: UNSTAINED CELLS
B: RABBIT SERA 1532 CONTROL
C: 1 ug RANK-L + R1532
D: 450ng mAPRIL – R1532

FIG. 4B



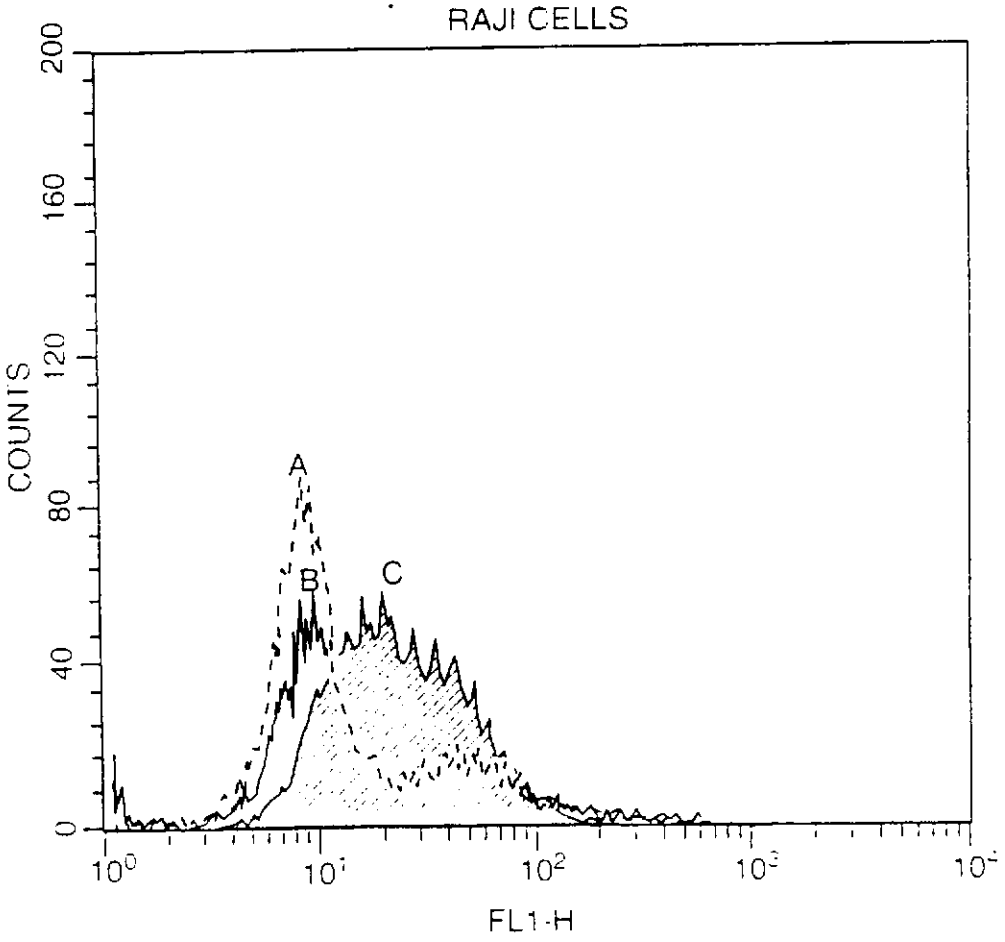
A: 450ngs APRIL + IRRELEVANT RABBIT SERA
B: RABBIT SERA 1532 CONTROL
C: 450ngs APRIL + R1532

FIG. 4C



A: UNSTAINED CELLS
B: RABBIT SERA 1532 CONTROL
C: 1ug RANK-L + R1532
D: 450ngs APRIL + R1532

FIG. 5A



A: 450ngs APRIL + IRRELEVANT RABBIT SERA
B: RABBIT SERA 1532 CONTROL
C: 450ngs APRIL + R1532

FIG. 5B

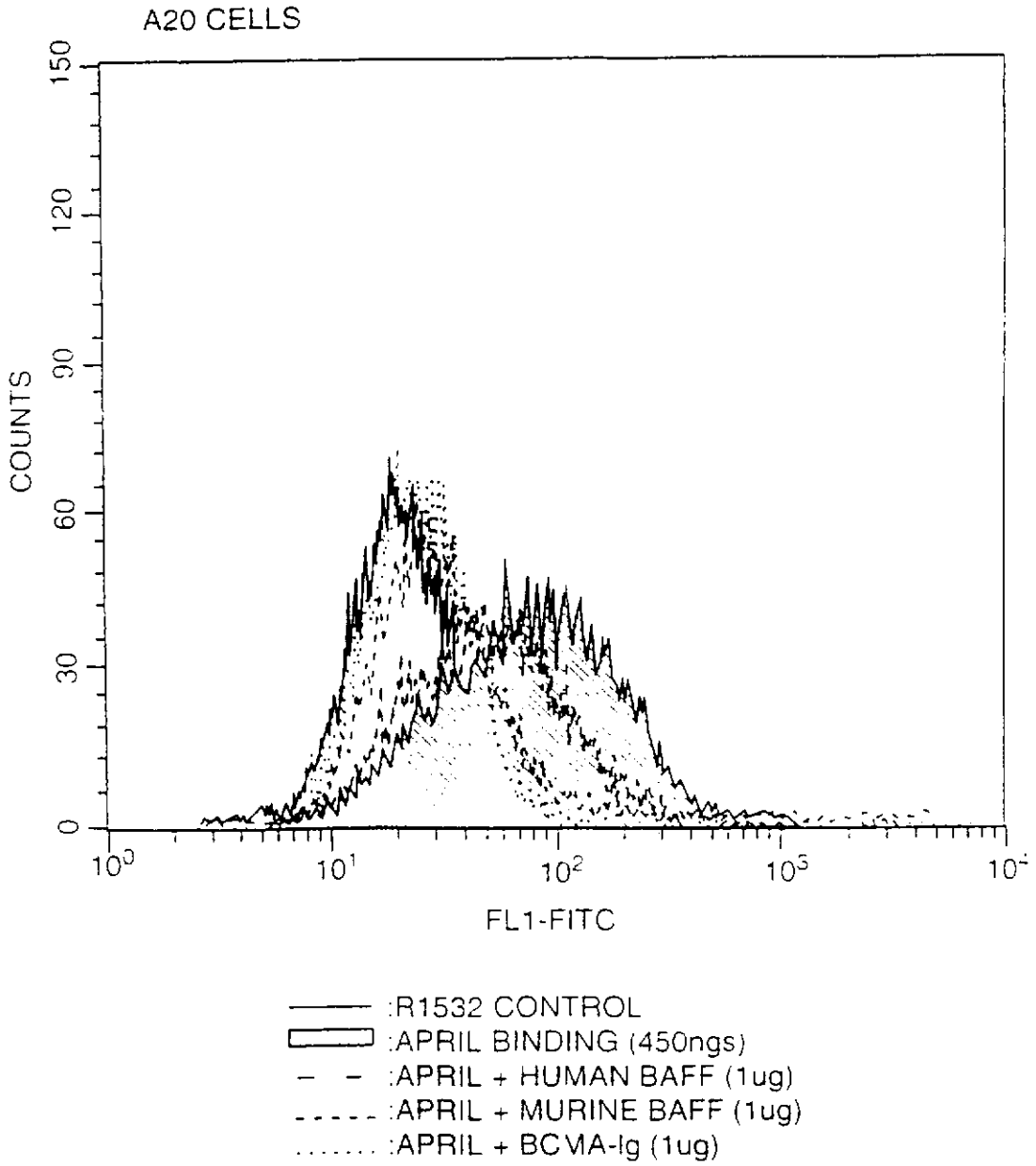

A20 CELLS
COUNTS
0
30
60
90
120
150
10^0
10^1
10^2
10^3
10^4
FL1-FITC
:R1532 CONTROL
:APRIL BINDING (450ngs)
:APRIL + HUMAN BAFF (1ug)
:APRIL + MURINE BAFF (1ug)
:APRIL + BCMA-Ig (1ug)

FIG. 6A

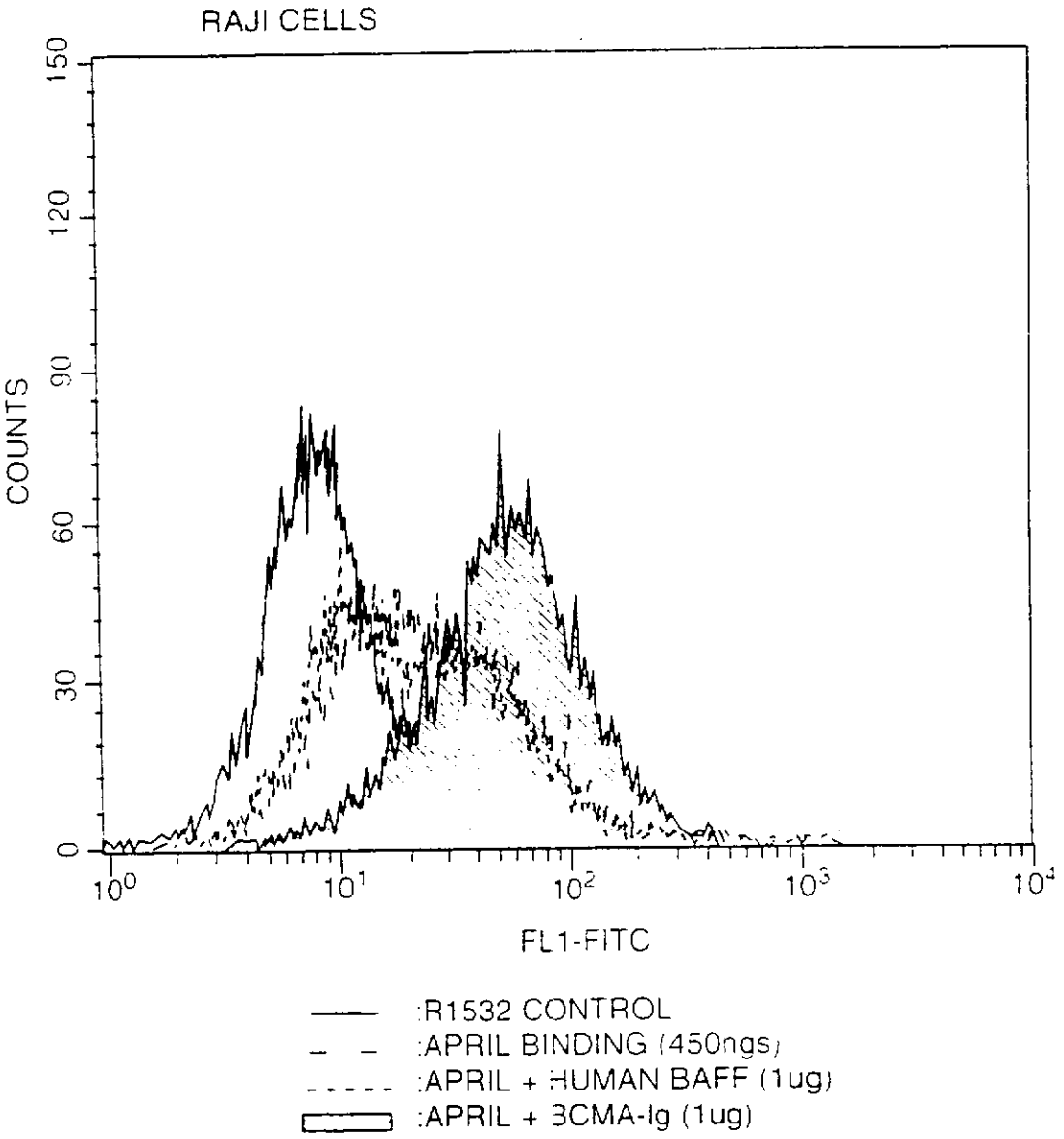

RAJI CELLS
COUNTS
0
30
60
90
120
150
10^0
10^1
10^2
10^3
10^4
FL1-FITC
:R1532 CONTROL
:APRIL BINDING (450ngs)
:APRIL + HUMAN BAFF (1ug)
:APRIL + BCMA-Ig (1ug)

FIG. 6B

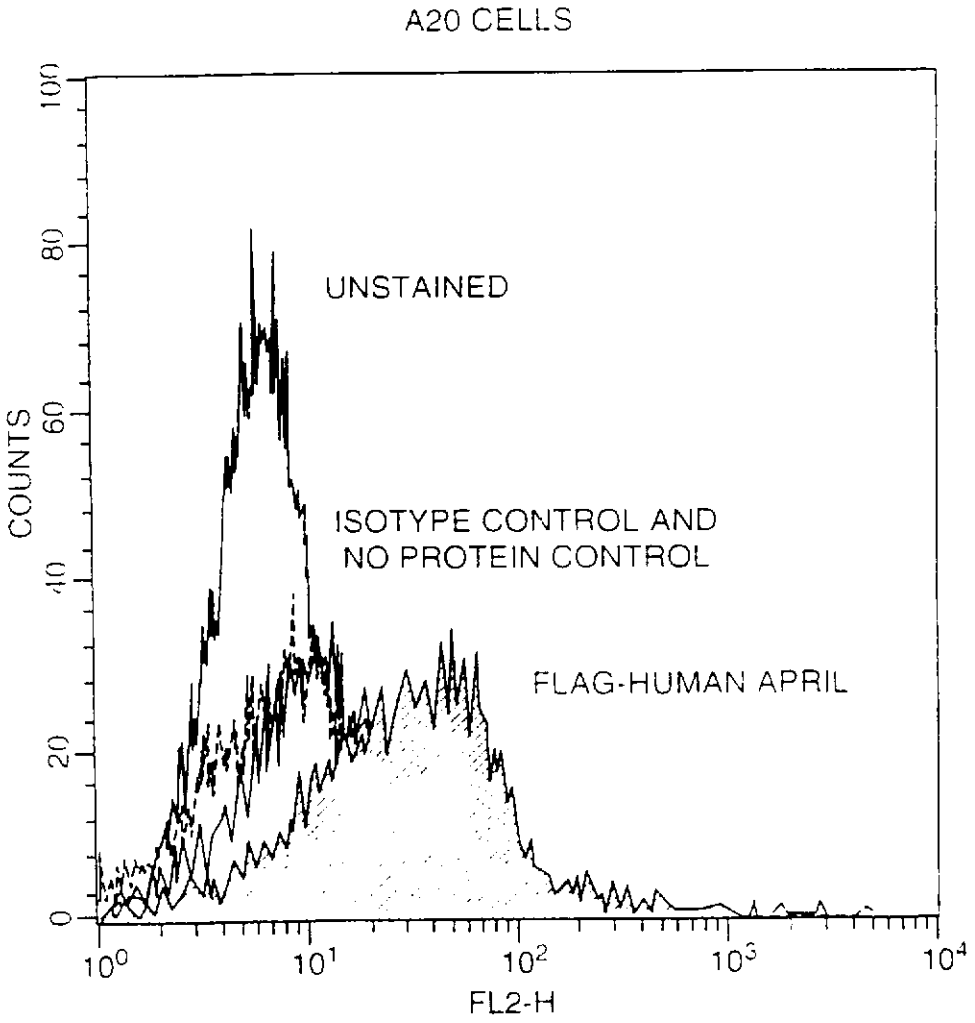
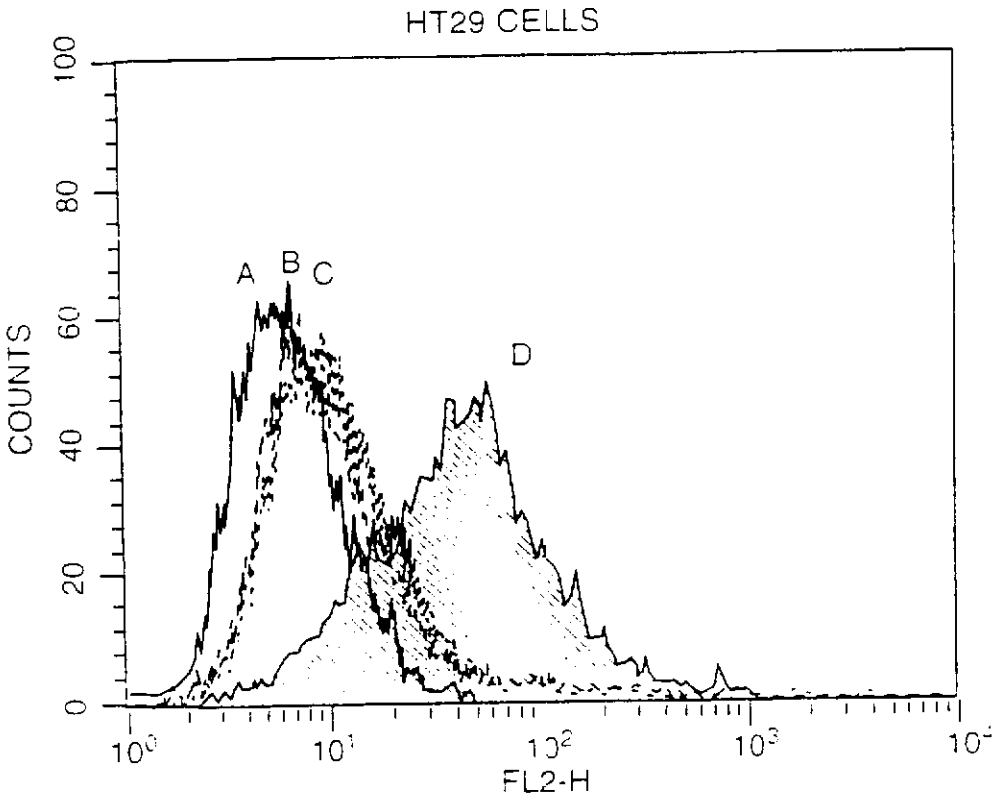
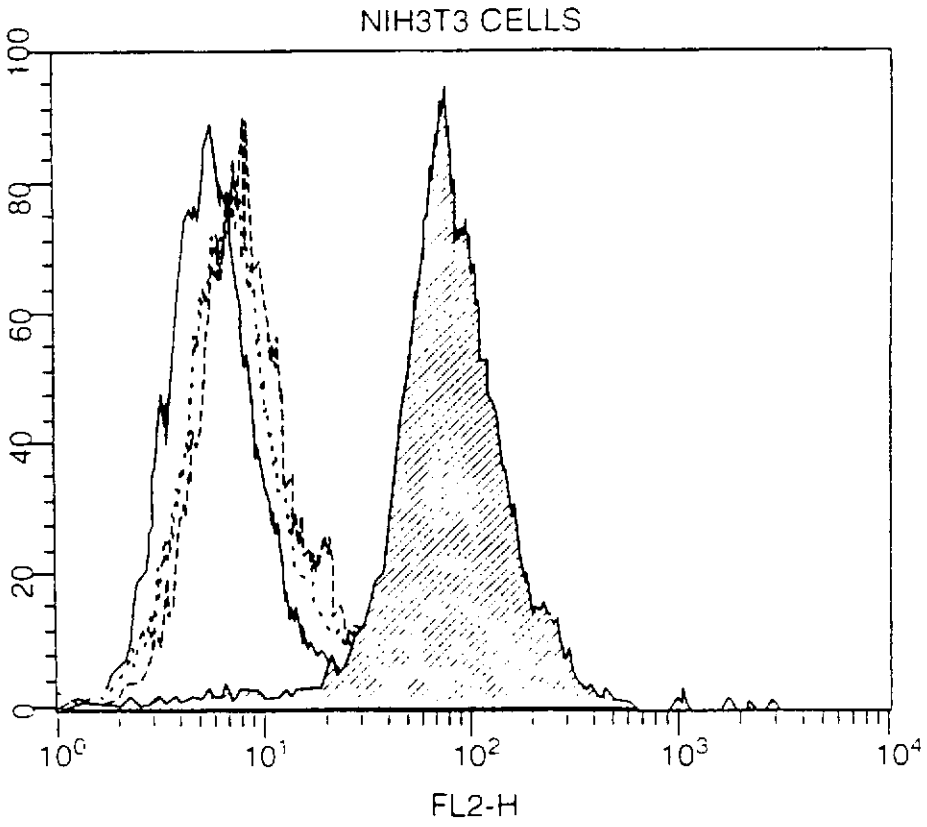

A20 CELLS
UNSTAINED
ISOTYPE CONTROL AND
NO PROTEIN CONTROL
FLAG-HUMAN APRIL
COUNTS
0
20
40
60
80
100
10^0
10^1
10^2
10^3
10^4
FL2-H

FIG. 7A



A: UNSTAINED CELLS
B: NO PROTEIN CONTROL + BIOTINYLATED M2 ANTI-FLAG mAb
C: 1ug/ml FLAG-APRIL + BIOTINYLATED ISOTYPE CONTROL mAb
D: 1ug/ml FLAG-APRIL + BIOTINYLATED MW ANTI-FLAG mAb

FIG. 7B



A: UNSTAINED CELLS
B: NO PROTEIN CONTROL + BIOTINYLATED M2 ANTI-FLAG mAb
C: 1ug/ml FLAG-APRIL + BIOTINYLATED ISOTYPE CONTROL mAb
D: 1ug/ml FLAG-APRIL + BIOTINYLATED MW ANTI-FLAG mAb

FIG. 7C

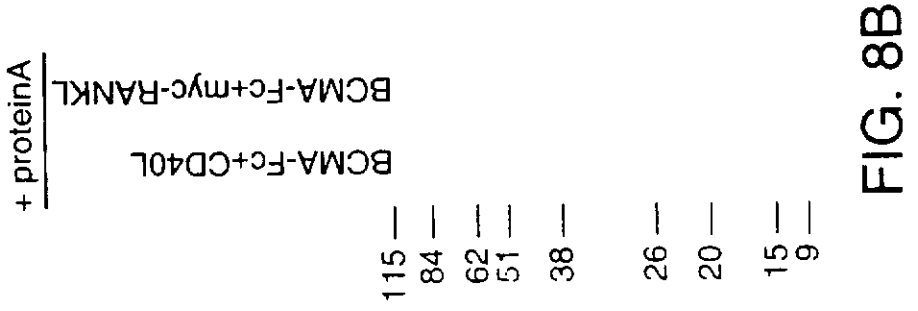

+ proteinA
BCMA-Fc+myc-RANKL
BCMA-Fc+CD40L
115
84
62
51
38
26
20
15
9

FIG. 8B

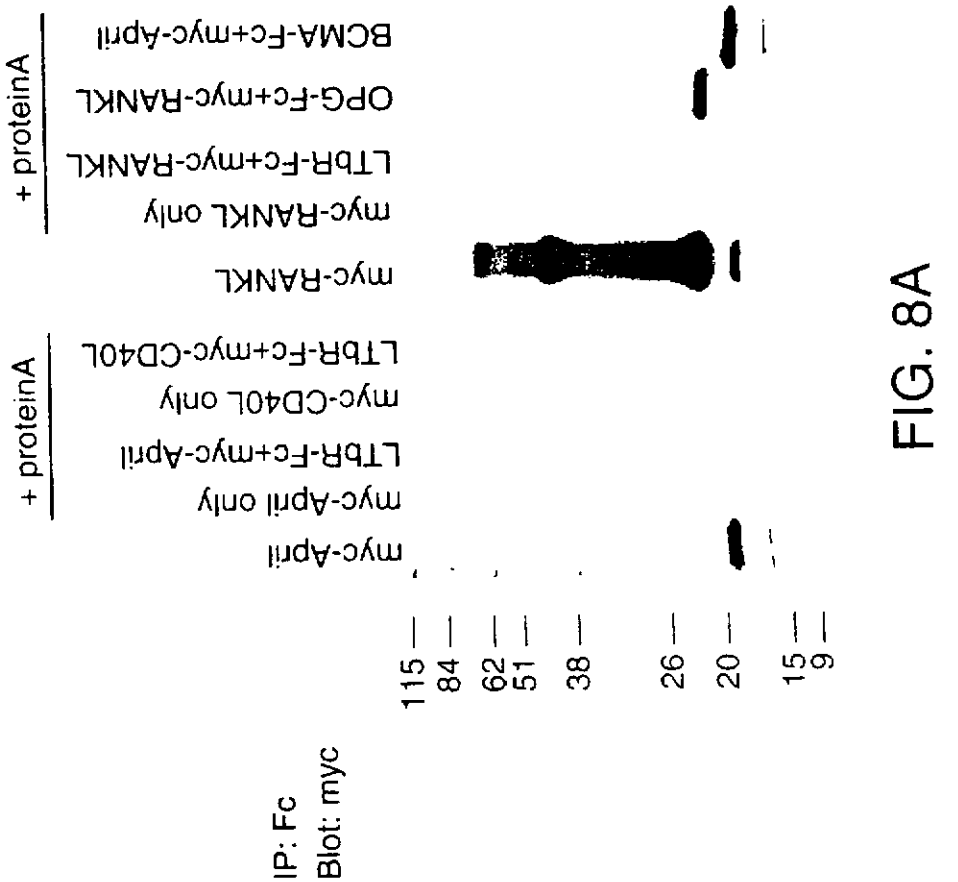

+ proteinA
BCMA-Fc+myc-April
OPG-Fc+myc-RANKL
LTbR-Fc+myc-RANKL
myc-RANKL only
myc-RANKL
+ proteinA
LTbR-Fc+myc-CD40L
myc-CD40L only
LTbR-Fc+myc-April
myc-April only
myc-April
115
84
62
51
38
26
20
15
9
IP: Fc
Blot: myc

FIG. 8A

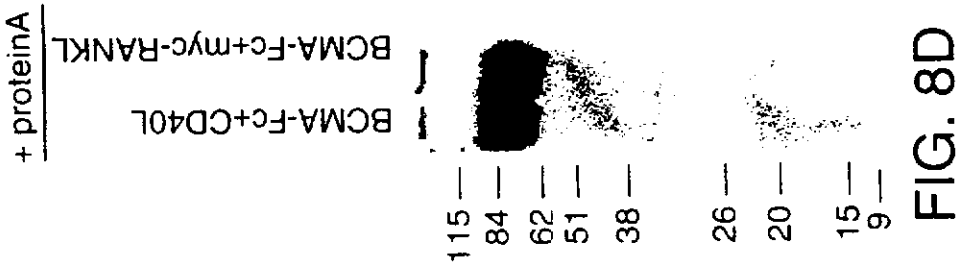

+ proteinA
BCMA-Fc+myc-RANKL
BCMA-Fc+CD40L
115
84
62
51
38
26
20
15
9

FIG. 8D

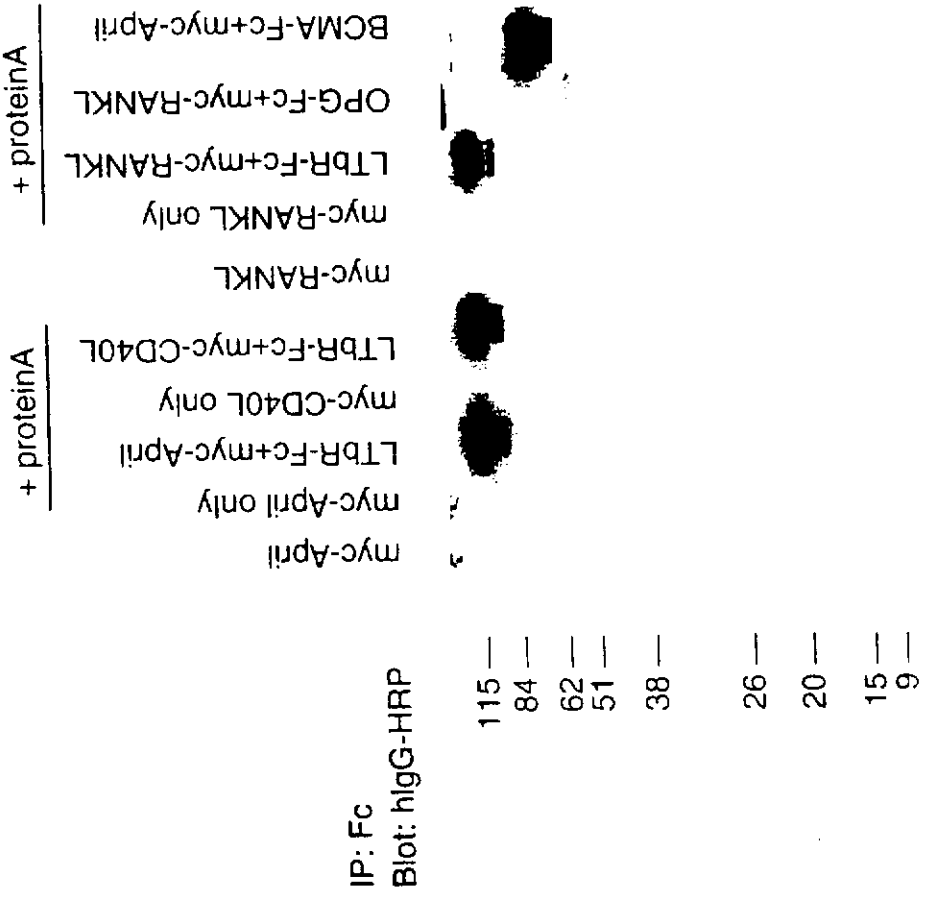

IP: Fc
Blot: hIgG-HRP
+ proteinA
BCMA-Fc+myc-April
OPG-Fc+myc-RANKL
LTbR-Fc+myc-RANKL
myc-RANKL only
myc-RANKL
+ proteinA
LTbR-Fc+myc-CD40L
myc-CD40L only
LTbR-Fc+myc-April
myc-April only
myc-April
115
84
62
51
38
26
20
15
9

FIG. 8C

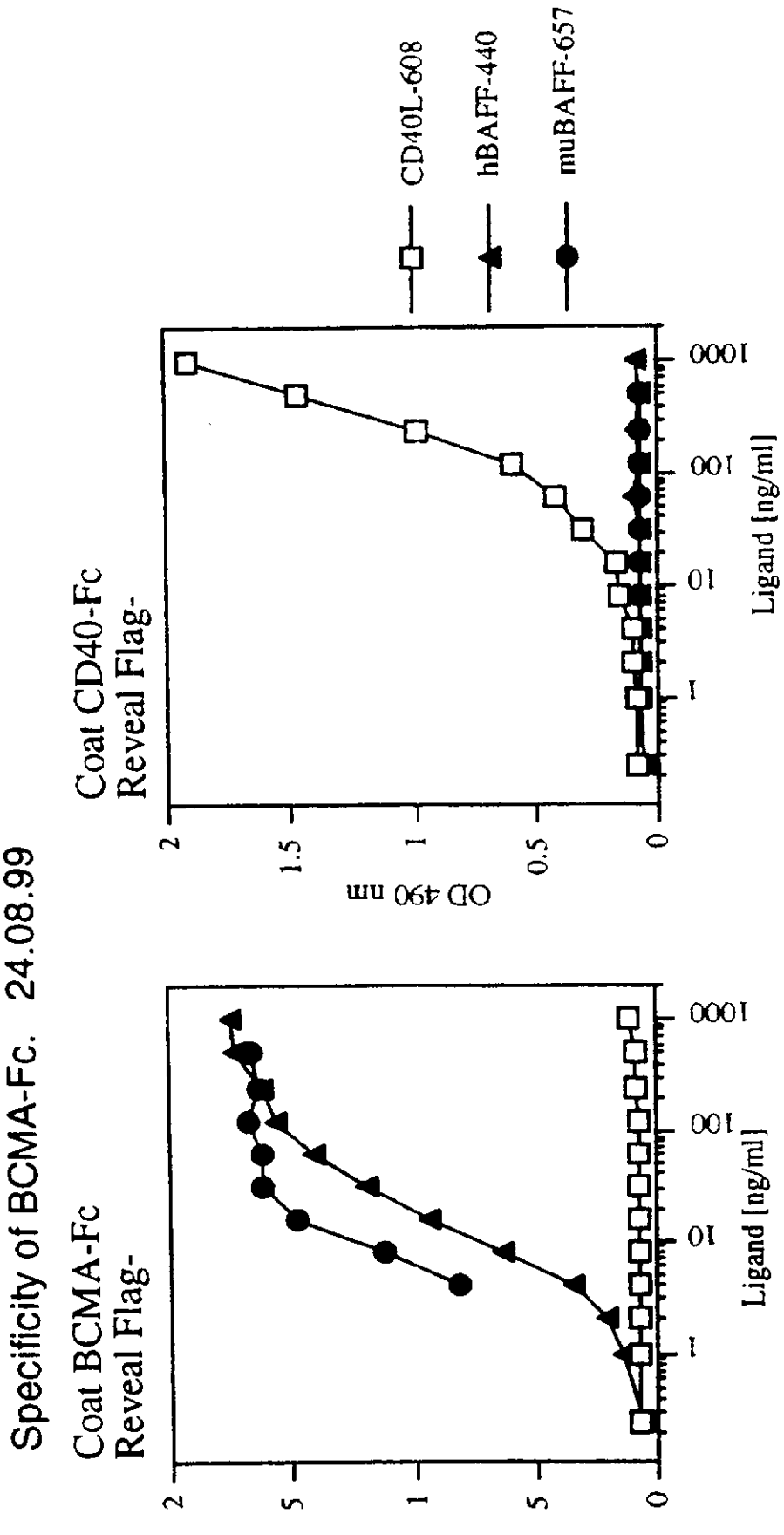

Specificity of BCMA-Fc. 24.08.99
Coat BCMA-Fc
Reveal Flag-
Ligand [ng/ml]
Coat CD40-Fc
Reveal Flag-
OD 490 nm
CD40L-608
hBAFF-440
muBAFF-657
Ligand [ng/ml]

FIG. 9A-1

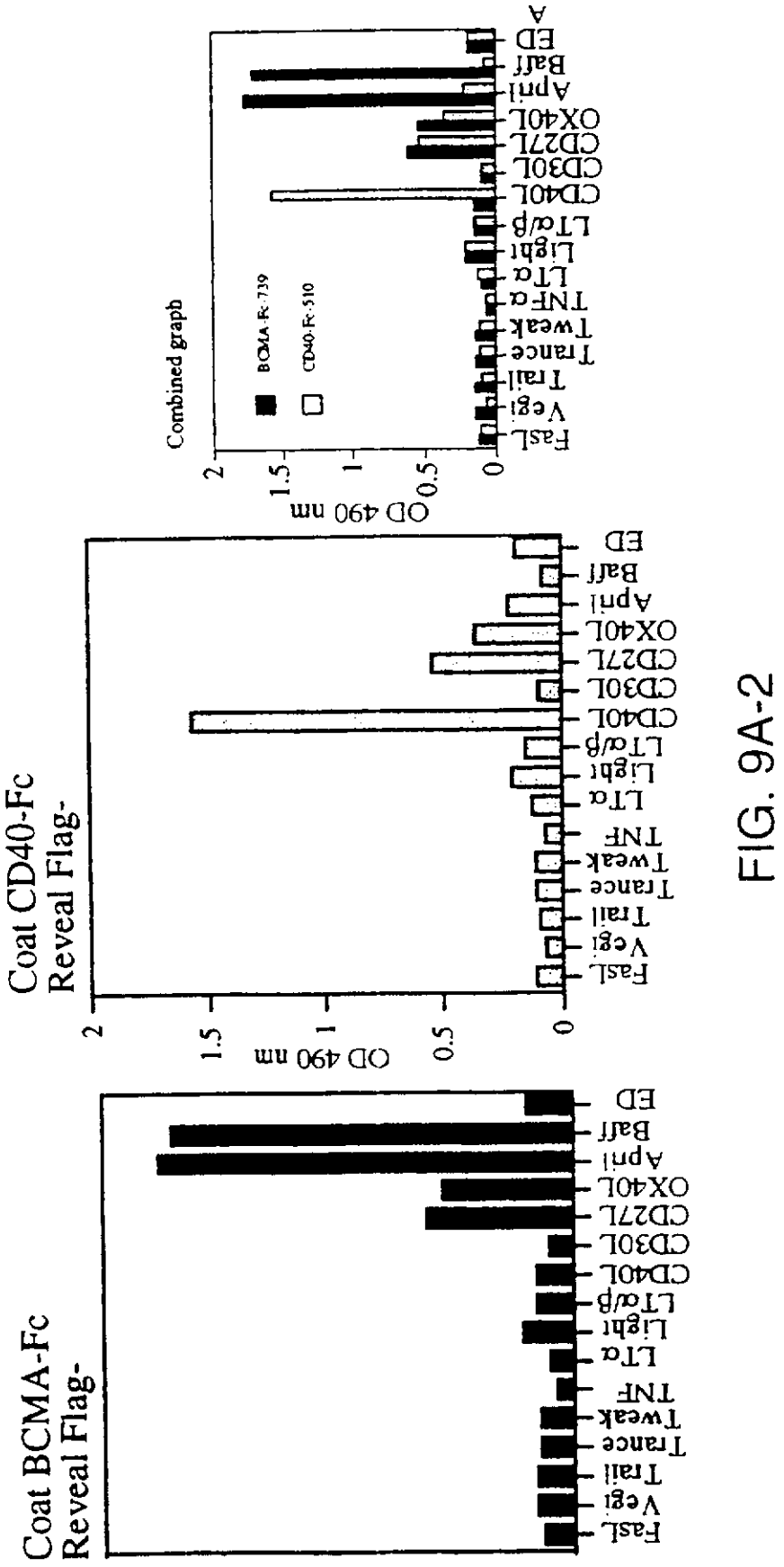

Coat BCMA-Fc
Reveal Flag-
OD 490 nm
0
0.5
1
1.5
2
FasL
Vegi
Trail
Trance
Tweak
TNF
LTα
Light
LTα/β
CD40L
CD30L
CD27L
OX40L
April
BAFF
ED
Coat CD40-Fc
Reveal Flag-
OD 490 nm
0
0.5
1
1.5
2
FasL
Vegi
Trail
Trance
Tweak
TNF
LTα
Light
LTα/β
CD40L
CD30L
CD27L
OX40L
April
BAFF
ED
Combined graph
BCMA-Fc 739
CD40-Fc 510
OD 490 nm
0
0.5
1
1.5
2
FasL
Vegi
Trail
Trance
Tweak
TNF
LTα
Light
LTα/β
CD40L
CD30L
CD27L
OX40L
April
BAFF
ED
A

FIG. 9A-2

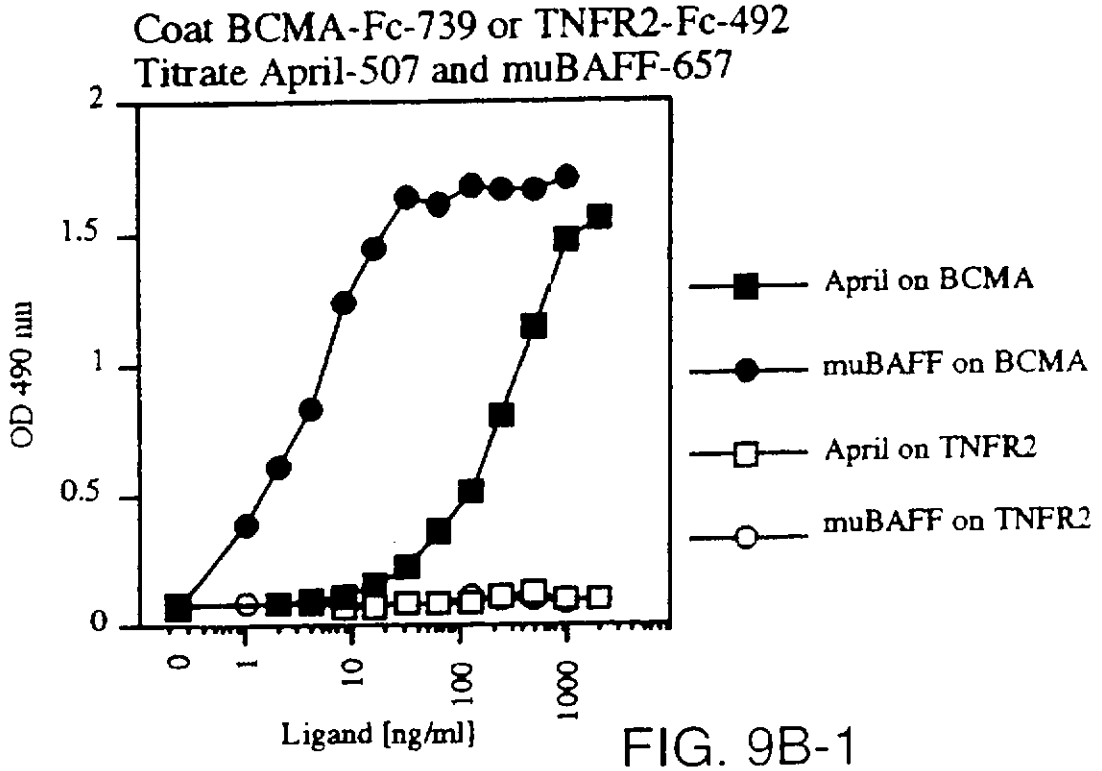
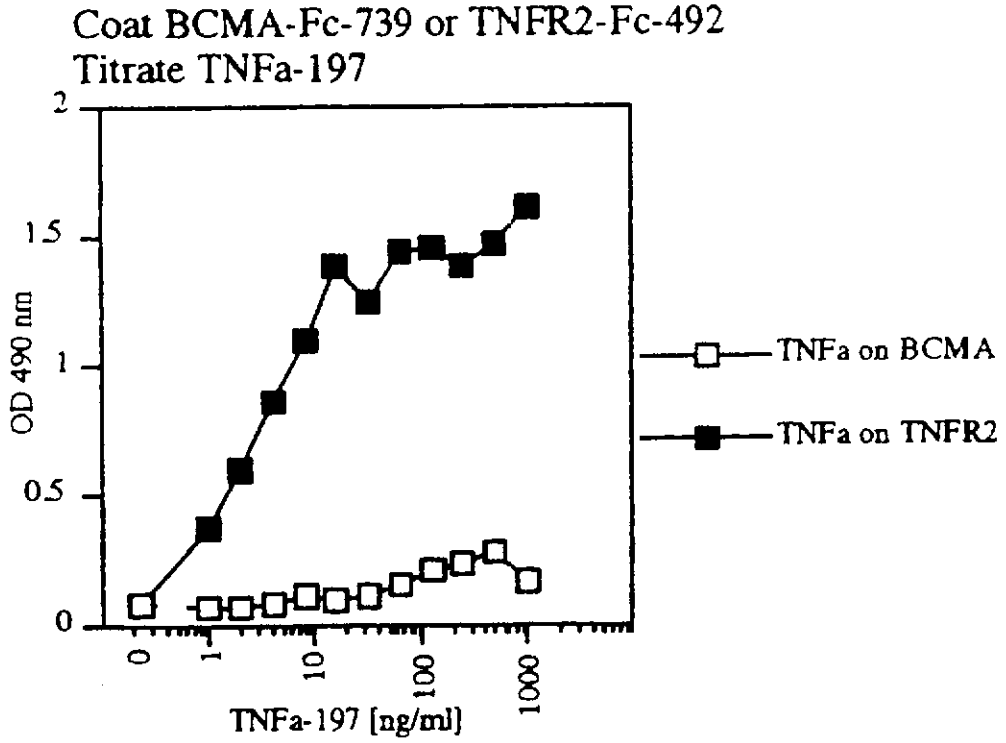

Coat BCMA-Fc-739 or TNFR2-Fc-492
Titrate April-507 and muBAFF-657
OD 490 nm
2
1.5
1
0.5
0
0
1
10
100
1000
Ligand [ng/ml]
April on BCMA
muBAFF on BCMA
April on TNFR2
muBAFF on TNFR2

FIG. 9B-1


Coat BCMA-Fc-739 or TNFR2-Fc-492
Titrate TNFa-197
OD 490 nm
2
1.5
1
0.5
0
0
1
10
100
1000
TNFa-197 [ng/ml]
TNFa on BCMA
TNFa on TNFR2

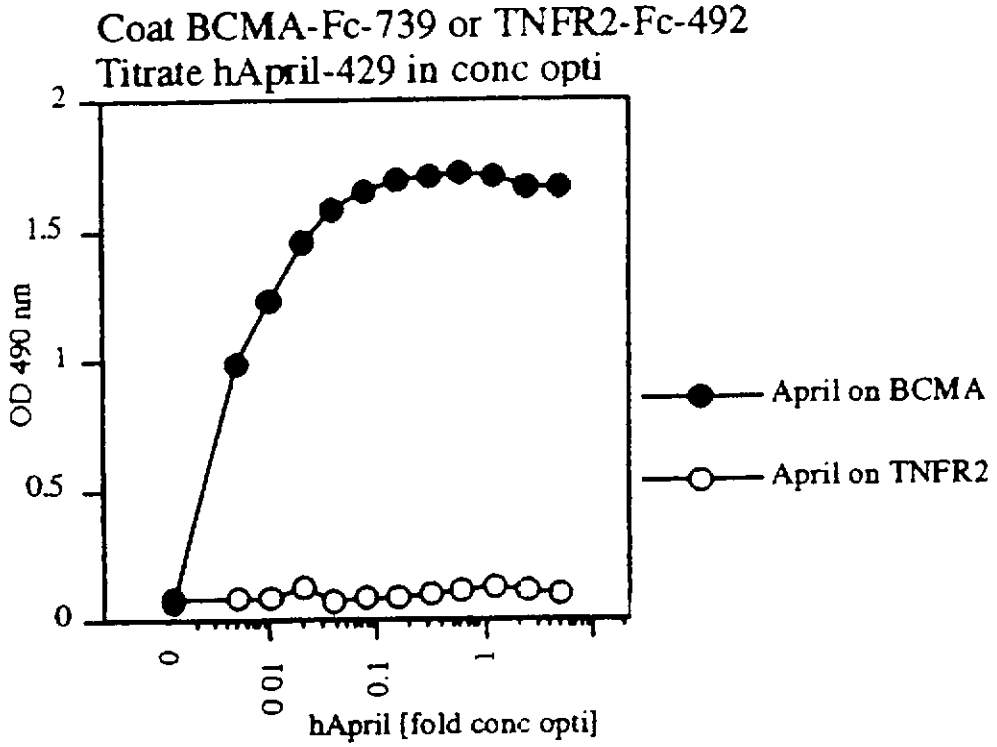
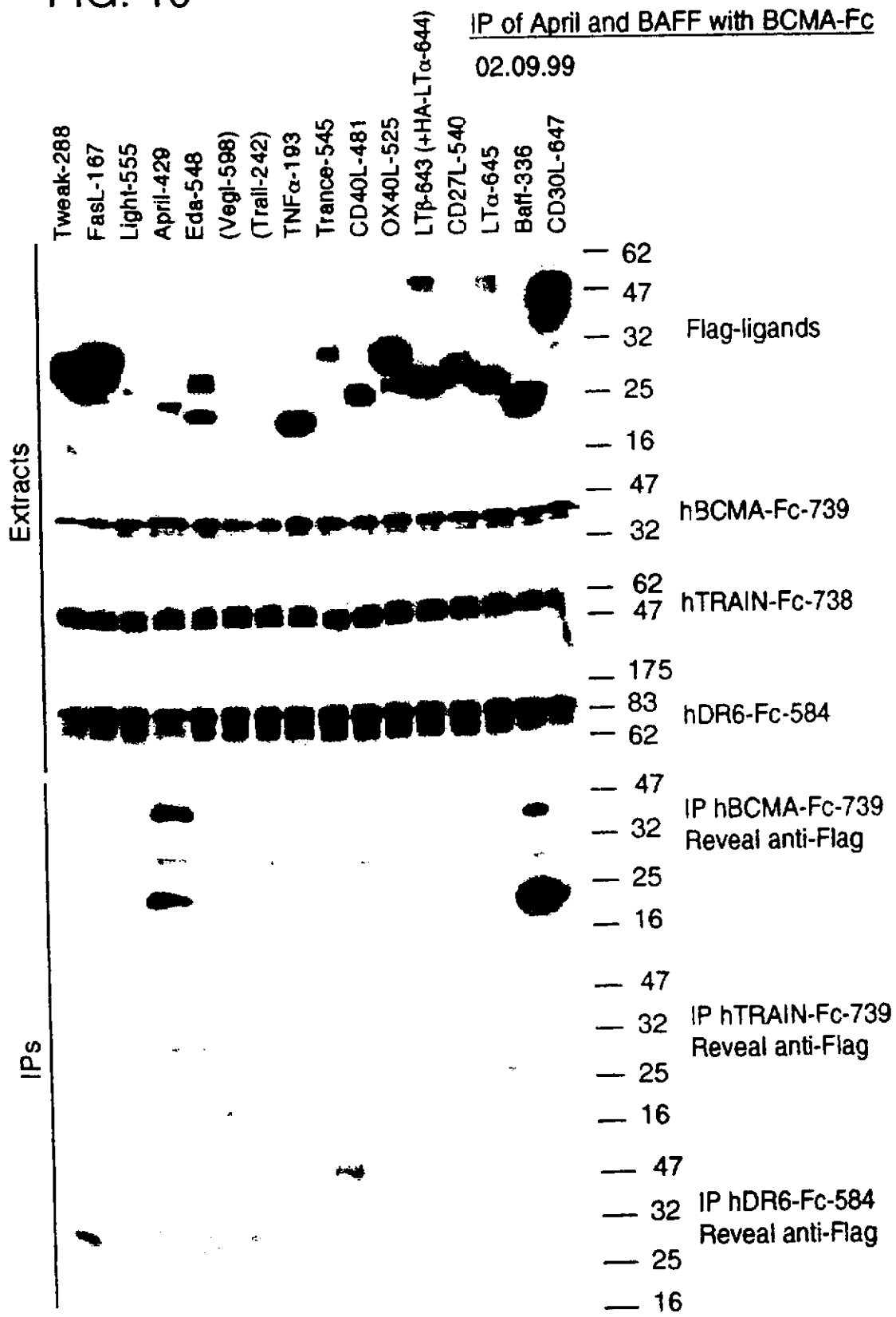

Coat BCMA-Fc-739 or TNFR2-Fc-492
Titrate hApril-429 in conc opti
OD 490 nm
2
1.5
1
0.5
0
0
0.01
0.1
1
hApril [fold conc opti]
April on BCMA
April on TNFR2

FIG. 9B-3

FIG. 10



Transfect 293T with indicated expression plasmid (Rec-Fc or Flag ligand) in 9 cm plate.

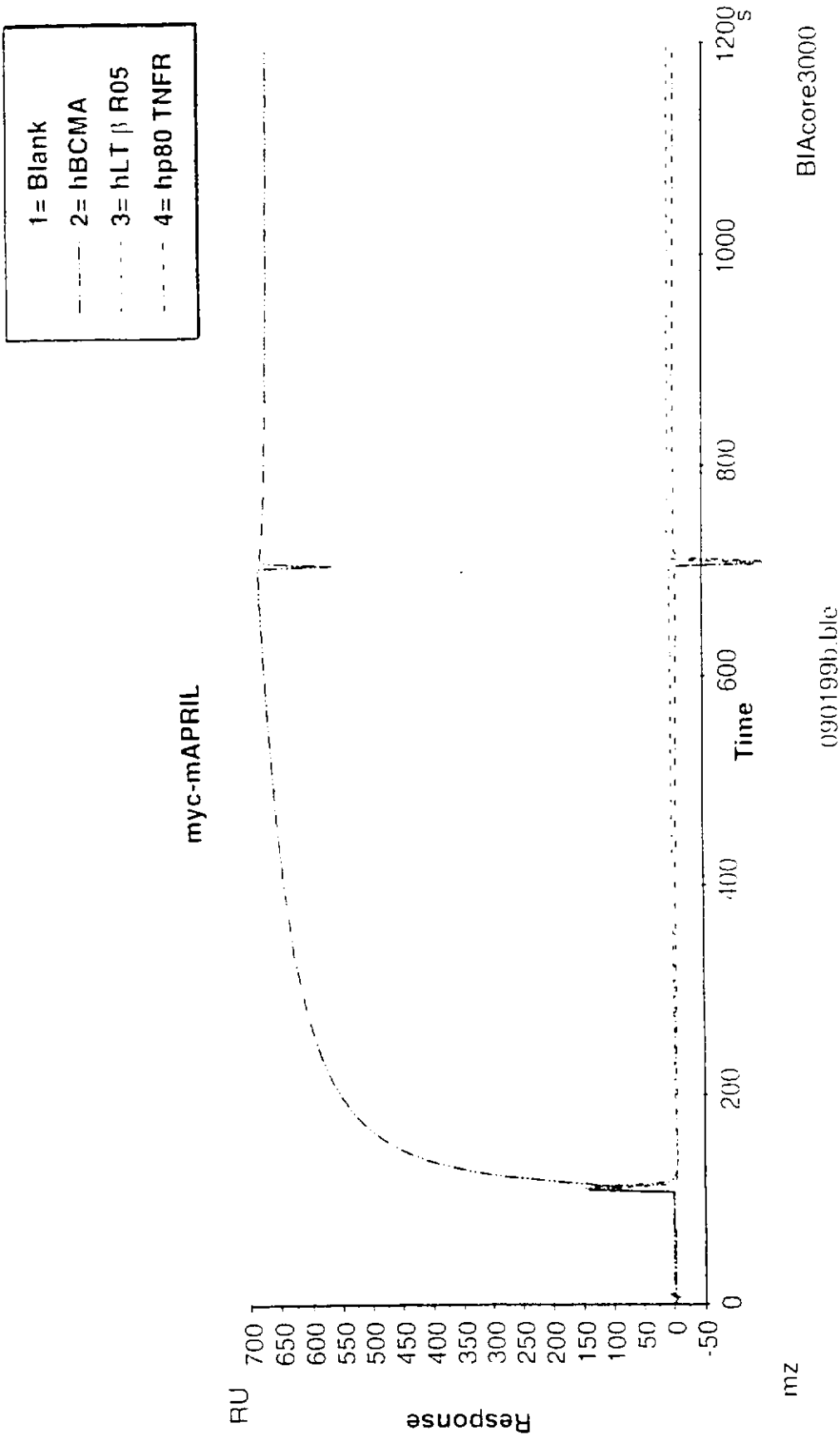

1= Blank
2= hBCMA
3= hLT β R05
4= hp80 TNFR
myc-mAPRIL
BIAcore3000
Response
RU
Time
S
mz
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
-50
0
200
400
600
800
1000
1200

FIG. 11A

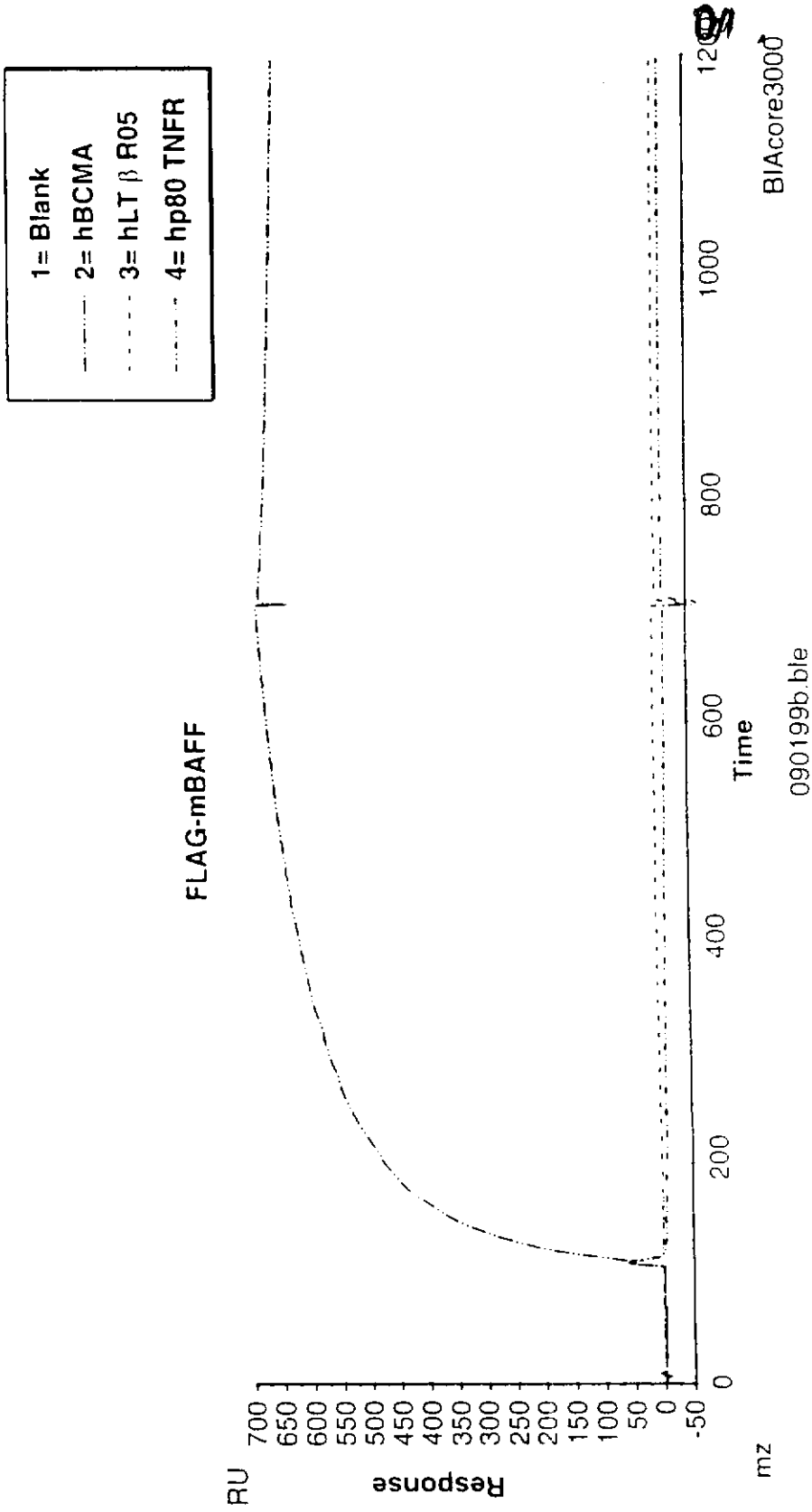

1= Blank
2= hBCMA
3= hLT β R05
4= hp80 TNFR
FLAG-mBAFF
BIAcore3000
090199b.ble
Time
Response
RU
mz
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
-50
0
200
400
600
800
1000

FIG. 11C

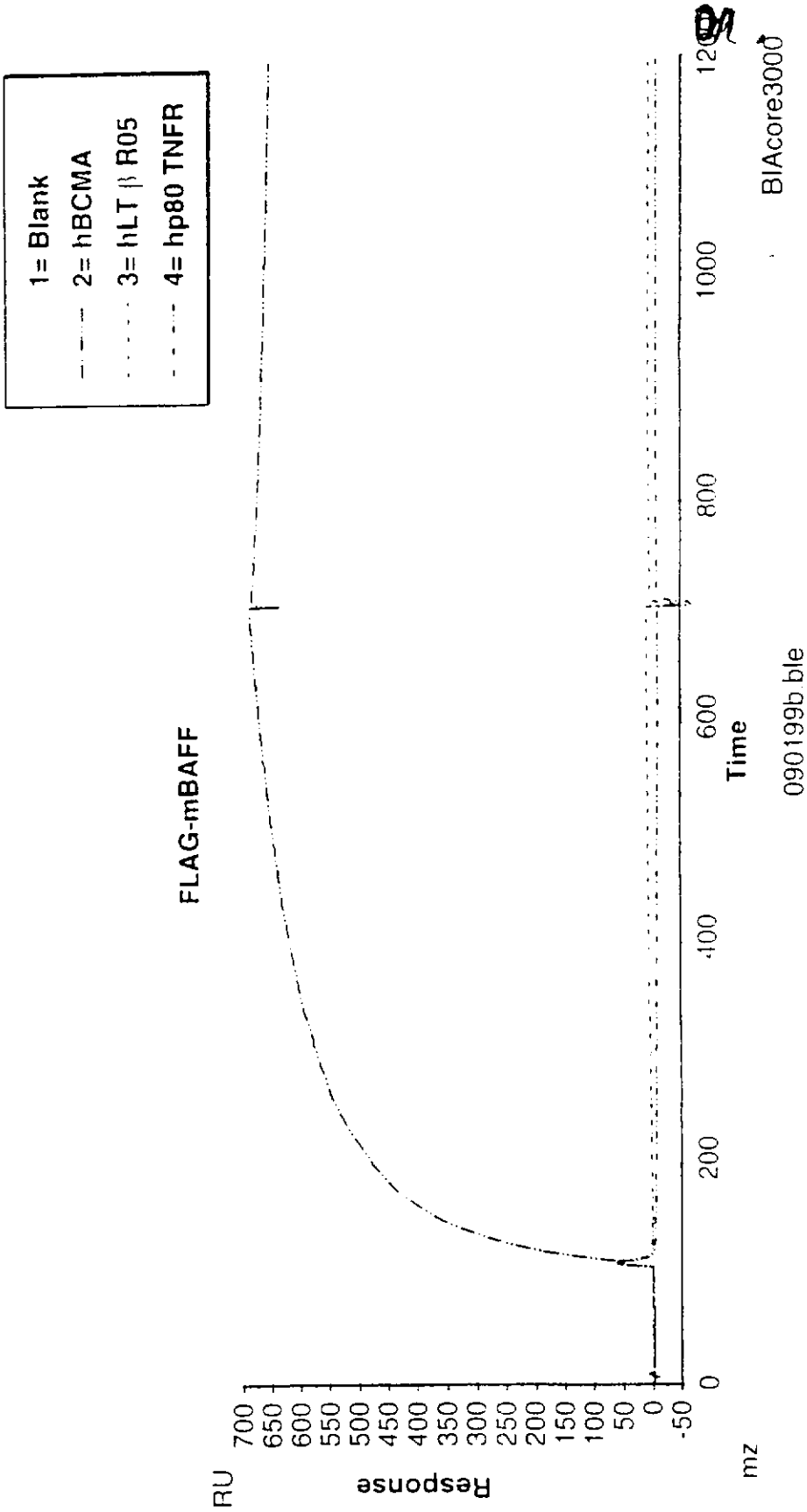
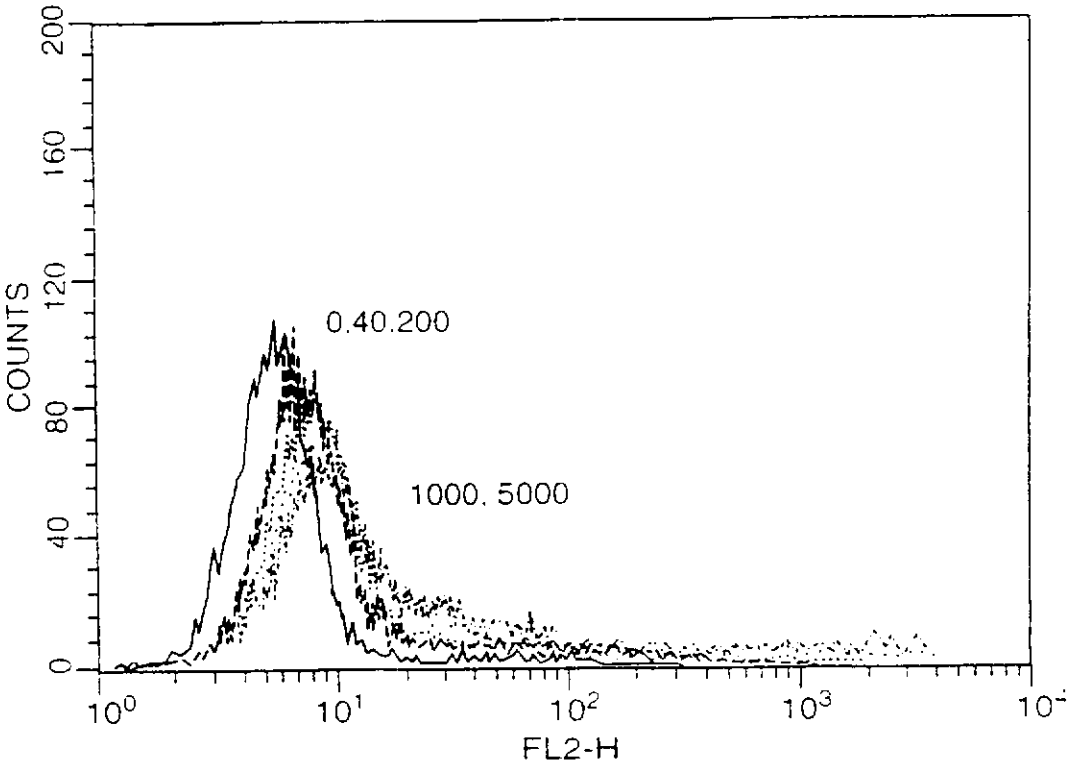
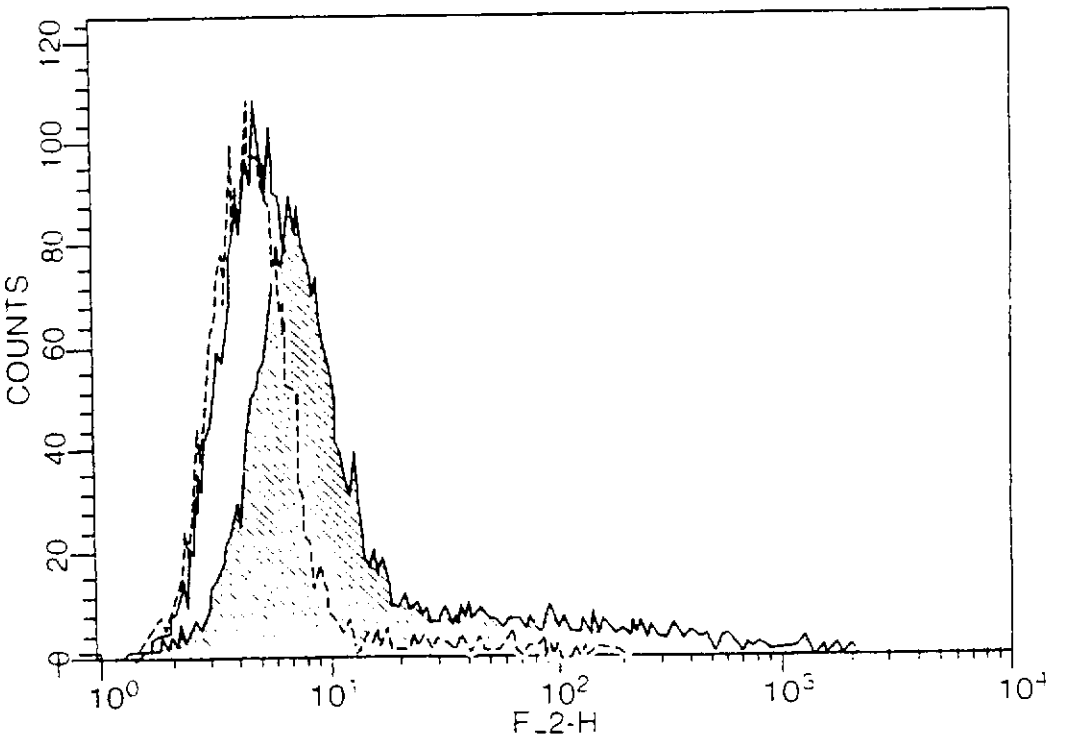

1= Blank
2= hBCMA
3= hLT β R05
4= hp80 TNFR
FLAG-mBAFF
BIAcore3000
090199b.ble
Response
RU
Time
mz
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
-50
0
200
400
600
800
1000

FIG. 11C



293EBNA CELLS TRANSFECTED WITH FULL LENGHT hBCMA WERE STAINED WITH 0. 40. 200, 1000, OR 5000 mg/ml myc-mAPRIL. RABBIT ANTI-APRIL ANTISERA (R1532). AND PE-LABELLED DONKEY ANTI-RABBIT IgG.

FIG. 12A



293EBNA CELLS TRANSFECTED WITH FULL LENGHT hBCMA WERE

A: RABBIT SERA (R1532) CONTROL
B: 200 ng/ml myc-mAPRIL + 1ug/ml DCMA-Ig
C: 200 ng/ml myc-mAPRIL

FIG. 12B

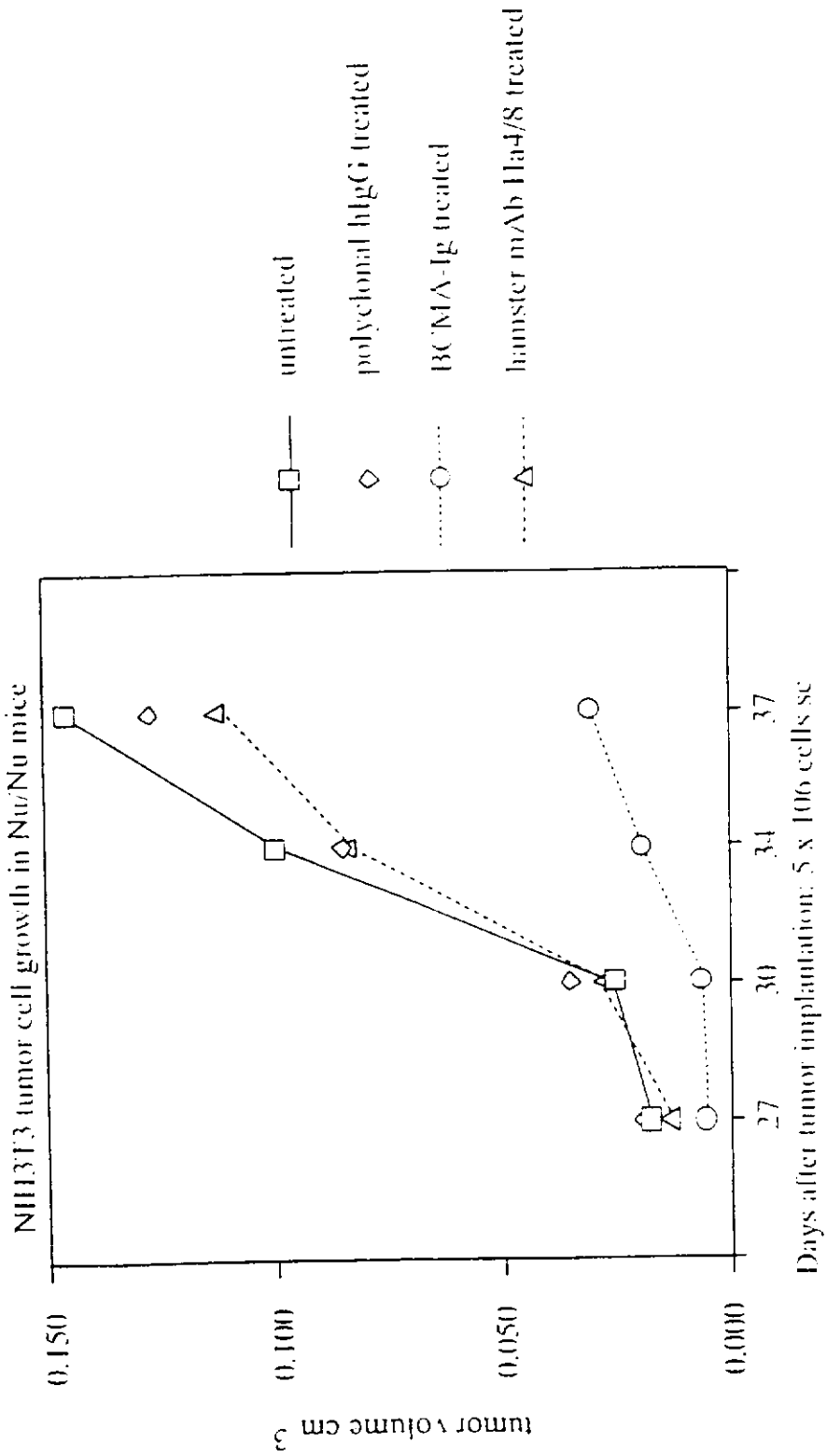

untreated
polyclonal hIgG treated
BCMA-Ig treated
hamster mAb Ha4/8 treated
NIH3T3 tumor cell growth in Nu/Nu mice
tumor volume cm 3
0.150
0.100
0.050
0.000
27
30
34
37
Days after tumor implantation: 5 x 106 cells sc

FIG. 13

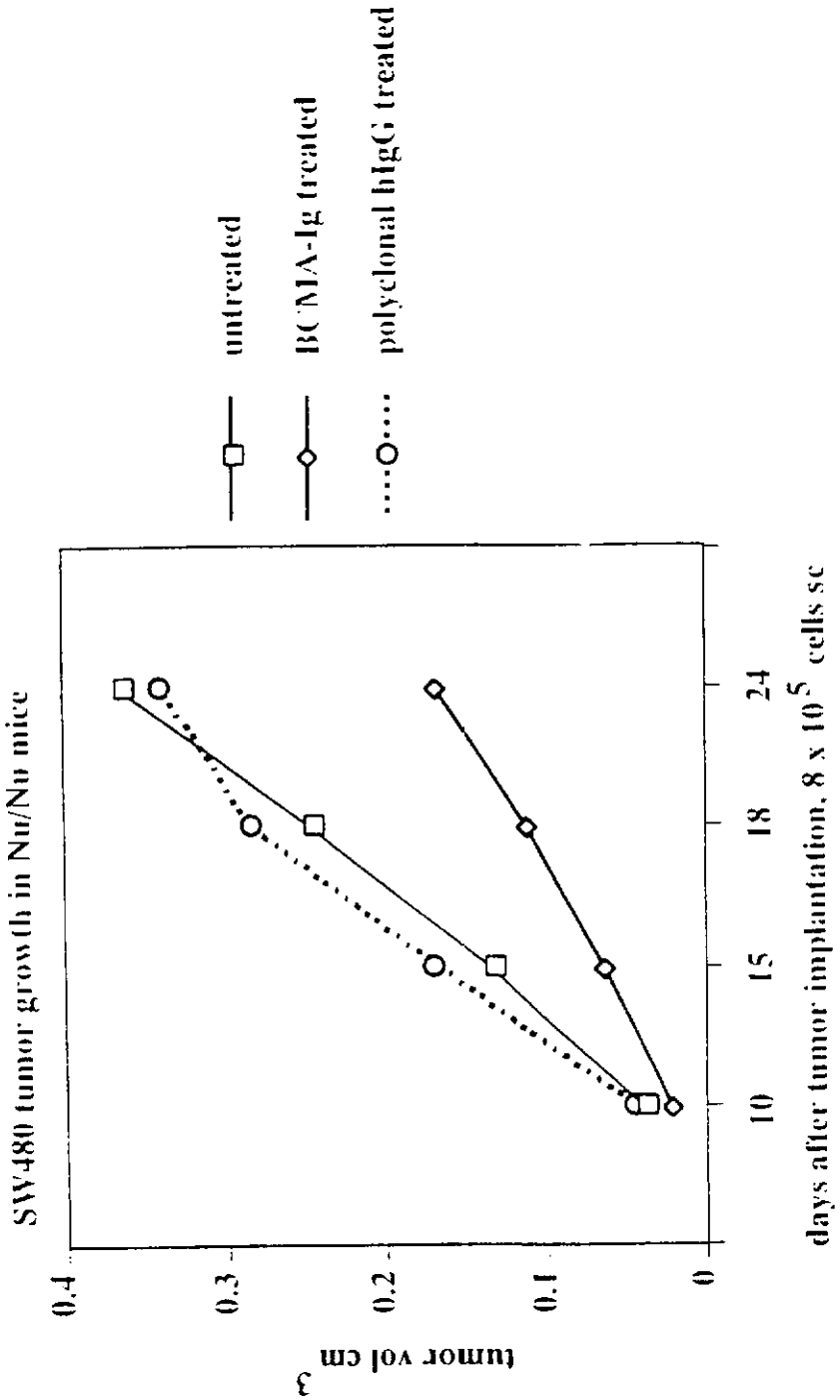

SW480 tumor growth in Nu/Nu mice
tumor vol cm 3
0.4
0.3
0.2
0.1
0
10
15
18
24
days after tumor implantation, 8 x 10 5 cells sc
untreated
BCMA-Ig treated
polyclonal hIgG treated

FIG. 14

FIG. 15

FIG. 15A	FIG. 15B

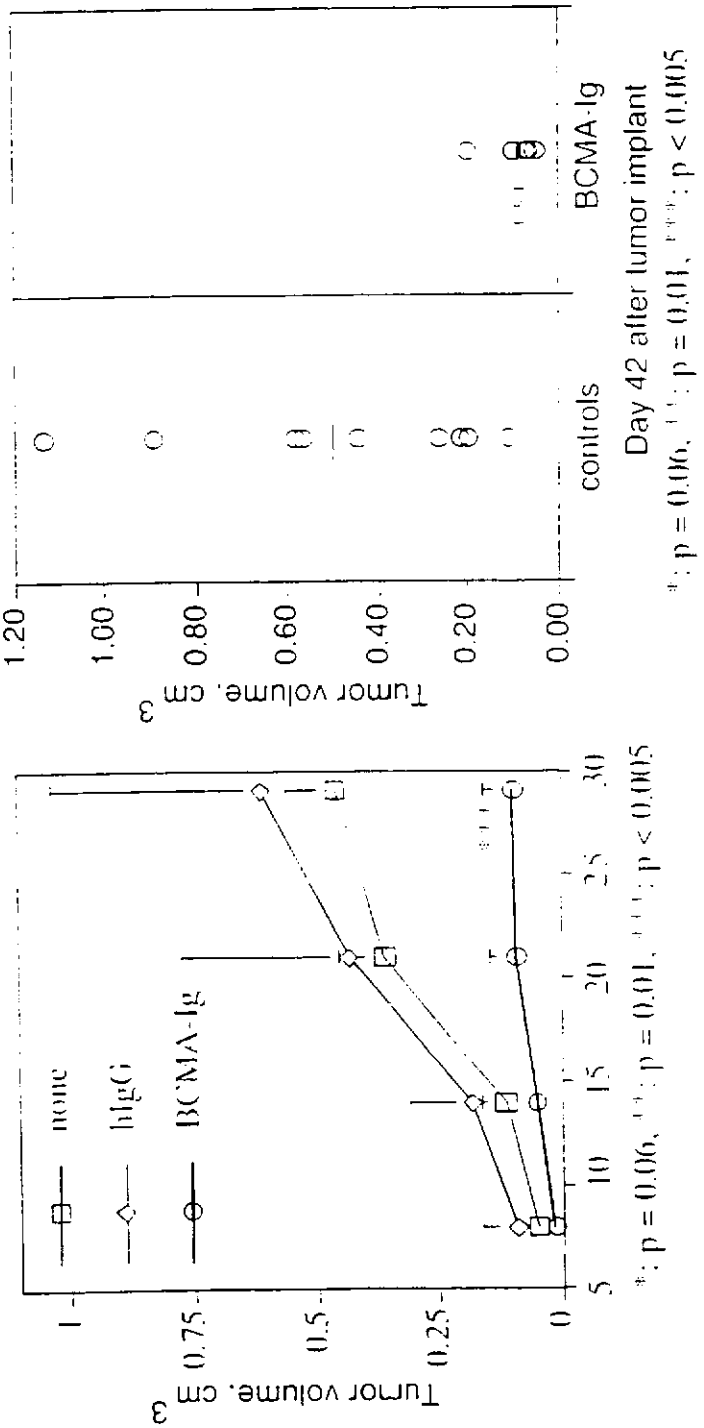


FIG. 15A

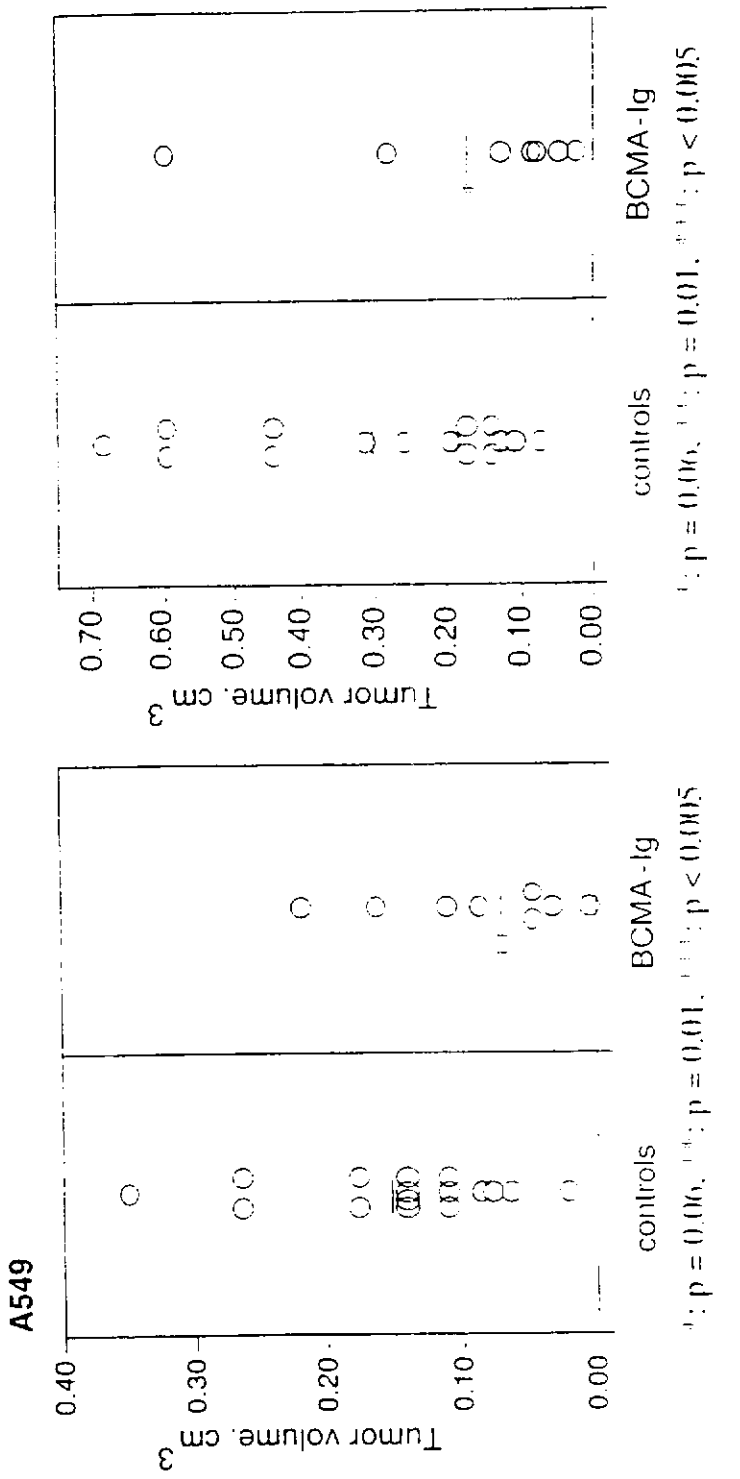

A549
Tumor volume, cm 3
0.40
0.30
0.20
0.10
0.00
controls
BCMA-Ig
Tumor volume, cm 3
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
controls
BCMA-Ig

FIG. 15B