GCTTCCCGAGGCTCCGCACCAGCCGCGCTTCTGTCCGCCTGCAGGGCATTCCA
GAAAGATGAGGATATTTGCTGTCTTTATATTCATGACCTACTGGCATTTGCTG
AACGCATTTACTGTCACGGTTCCCAAGGACCTATATGTGGTAGAGTATGGTA
GCAATATGACAATTGAATGCAAATTCCCAGTAGAAAAACAATTAGACCTGGC
TGCACTAATTGTCTATTGGGAAATGGAGGATAAGAACATTATTCAATTTGTGC
ATGGAGAGGAAGACCTGAAGGTTCAGCATAGTAGCTACAGACAGAGGGCCC
GGCTGTTGAAGGACCAGCTCTCCCTGGGAAATGCTGCACTTCAGATCACAGA
TGTGAAATTGCAGGATGCAGGGGTGTACCGCTGCATGATCAGCTATGGTGCT
GCCGACTACAAGCGAATTACTGTGAAAGTCAATGCCCCATACAACAAAATCA
ACCAAAGAATTTTGGTTGTGGATCCAGTCACCTCTGAACATGAACTGACATGT
CAGGCTGAGGGCTACCCCAAGGCCGAAGTCATCTGGACAAGCAGTGACCATC
AAGTCCTGAGTGGTAAGACCACCACCACCAATTCCAAGAGAGAGGAGAAGC
TTTTCAATGTGACCAGCACACTGAGAATCAACACAACAACTAATGAGATTTT
CTACTGCACTTTTAGGAGATTAGATCCTGAGGAAAACCATACAGCTGAATTG
GTCATCCCAGGTAATATTCTGAATGTGTCCATTAAAATATGTCTAACACTGTC
CCCTAGCACCTAGCATGATGTCTGCCTATCATAGTCATTCAGTGATTGTTGAA
TAAATGAATGAATGAATAACACTATGTTTACAAAATATATCCTAATTCCTCAC
CTCCATTCATCCAAACCATATTGTTACTTAATAAACATTCAGCAGATATTTAT
GGAATAAAAAAAAAAAAAAAAAAAAA

FIGURE 1

CGAGGCTCCGCACCAGCCGCGCTTCTGTCCGCCTGCAGGGCATTCCAGAAAGA
TGAGGATATTTGCTGTCTTTATATTCATGACCTACTGGCATTTGCTGAACGCATT
TACTGTCACGGTTCCCAAGGACCTATATGTGGTAGAGTATGGTAGCAATATGAC
AATTGAATGCAAATTCCCAGTAGAAAAACAATTAGACCTGGCTGCACTAATTGT
CTATTGGGAAATGGAGGATAAGAACATTATTCAATTTGTGCATGGAGAGGAAG
ACCTGAAGGTTCAGCATAGTAGCTACAGACAGAGGGCCCGGCTGTTGAAGGAC
CAGCTCTCCCTGGGAAATGCTGCACTTCAGATCACAGATGTGAAATTGCAGGAT
GCAGGGGTGTACCGCTGCATGATCAGCTATGGTGGTGCCGACTACAAGCGAAT
TACTGTGAAAGTCAATGCCCCATACAACAAAATCAACCAAAGAATTTTGGTTGT
GGATCCAGTCACCTCTGAACATGAACTGACATGTCAGGCTGAGGGCTACCCCA
AGGCCGAAGTCATCTGGACAAGCAGTGACCATCAAGTCCTGAGTGGTAAGACC
ACCACCACCAATTCCAAGAGAGAGGAGAAGCTTTTCAATGTGACCAGCACACT
GAGAATCAACACAACAACTAATGAGATTTTCTACTGCACTTTTAGGAGATTAGA
TCCTGAGGAAAACCATACAGCTGAATTGGTCATCCCAGAACTACCTCTGGCACA
TCCTCCAAATGAAAGGACTCACTTGGTAATTCTGGGAGCCATCTTATTATGCCTT
GGTGTAGCACTGACATTCATCTTCCGTTTAAGAAAAGGGAGAATGATGGATGT
GAAAAAATGTGGCATCCAAGATACAAACTCAAAGAAGCAAAGTGATACACATTT
GGAGGAGACGTAATCCAGCATTGGAACTTCTGATCTTCAAGCAGGGATTCTCA
ACCTGTGGTTTAGGGGTTCATCGGGGCTGAGCGTGACAAGAGGAAGGAATGG
GCCCGTGGGATGCAGGCAATGTGGGACTTAAAAGGCCCAAGCACTGAAAATG
GAACCTGGCGAAAGCAGAGGAGGAGAATGAAGAAAGATGGAGTCAAACAGGG
AGCCTGGAGGGAGACCTTGATACTTTCAAATGCCTGAGGGGCTCATCGACGCC
TGTGACAGGGAGAAAGGATACTTCTGAACAAGGAGCCTCCAAGCAAATCATCC
ATTGCTCATCCTAGGAAGACGGGTTGAGAATCCCTAATTTGAGGGTCAGTTCCT
GCAGAAGTGCCCTTTGCCTCCACTCAATGCCTCAATTTGTTTTCTGCATGACTGA
GAGTCTCAGTGTTGGAACGGGACAGTATTTATGTATGAGTTTTTCCTATTTATTT
TGAGTCTGTGAGGTCTTCTTGTCATGTGAGTGTGGTTGTGAATGATTTCTTTTGA
AGATATATTGTAGTAGATGTTACAATTTTGTCGCCAAACTAAACTTGCTGCTTAA
TGATTTGCTCACATCTAGTAAAACATGGAGTATTTGTAAAAAAAAAAAAAAA

FIGURE 2

292 secreted (245 amino acids)

Signal/IgV/IgC/hydrophilic tail
(a) (b) (c) (d)

Ig cysteines in large bold

MRIFAVFIFMTYWHLLNA (signal)

FTVTVPKDLYVVEYGSNMTIE**C**KFPVEKQLDLAALIVYWEMEDKN
IIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQD
AGVYR**C**MISYGGADYKRITVKVNAPY (IgV)

NKINQRILVVDPVTSEHELT**C**QAEGYPKAEVIWTSSDHQVLSGKT
TTTNSKREEKLFNVTSTLRINTTTNEIFY**C**TFRRLDPEENHTAEL
VIP (IgC)

GNILNVSIKICLTLSPST (hydrophilic tail)

FIGURE 3

292 membrane (290 amino acids)

Signal/IgV/IgC/transmembrane (underlined)
plus cytoplasmic

Ig cysteines in large bold

MRIFAVFIFMTYWHLLNA (signal)

FTVTVPKDLYVVEYGSNMTIE**C**KFPVEKQLDLAALIVYWEMEDKN
IIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQD
AGVYR**C**MISYGGADYKRITVKVNAPY (IgV)

NKINQRILVVDPVTSEHELT**C**QAEGYPKAEVIWTSSDHQVLSGKT
TTTNSKREEKLFNVTSTLRINTTTNEIFY**C**TFRRLDPEENHTAEL
VIP (IgC)

ELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKC
GIQDTNSKKQSDTHLEET (transmembrane plus cytoplasmic)

FIGURE 4

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AGATAGTTCCCAAAACATGAGGATATTTGCTGGCATTATATTCACAGCCTGC
TGTCACTTGCTACGGGCGTTTACTATCACGGCTCCAAAGGACTTGTACGTG
GTGGAGTATGGCAGCAACGTCACGATGGAGTGCAGATTCCCTGTAGAACG
GGAGCTGGACCTGCTTGCGTTAGTGGTGTACTGGGAAAAGGAAGATGAGC
AAGTGATTCAGTTTGTGGCAGGAGAGGAGGACCTTAAGCCTCAGCACAGCA
ACTTCAGGGGGAGAGCCTCGCTGCCAAAGGACCAGCTTTTGAAGGGAAAT
GCTGCCCTTCAGATCACAGACGTCAAGCTGCAGGACGCAGGCGTTTACTGC
TGCATAATCAGCTACGGTGGTGCGGACTACAAGCGAATCACGCTGAAAGTC
AATGCCCCATACCGCAAAATCAACCAGAGAATTTCCGTGGATCCAGCCACTT
CTGAGCATGAACTAATATGTCAGGCCGAGGGTTATCCAGAAGCTGAGGTAA
TCTGGACAAACAGTGACCACCAACCCGTGAGTGGGAAGAGAAGTGTCACCA
CTTCCCGGACAGAGGGGATGCTTCTCAATGTGACCAGCAGTCTGAGGGTCA
ACGCCACAGCGAATGATGTTTTCTACTGTACGTTTTGGAGATCACAGCCAG
GGCAAAACCACACAGCGGAGCTGATCATCCCAGAACTGCCTGCAACACATC
CTCCACAGAACAGGACTCACTGGGTGCTTCTGGGATCCATCCTGTTGTTCC
TCATTGTAGTGTCCACGGTCCTCCTCTTCTTGAGAAAACAAGTGAGAATGCT
AGATGTGGAGAAATGTGGCGTTGAAGATACAAGCTCAAAAAACCGAAATGA
TACACAATTCGAGGAGACGTAAGCAGTGTTGAACCCTCTGATCGTCGATTG
GCAGCTTGTGGTCTGTGAAAGAAAGGGCCCATGGGACATGAGTCCAAAGAC
TCAAGATGGAACCTGAGGGAGAGAACCAAGAAAGTGTTGGGAGAGGAGCC
TGGAACAACGGACATTTTTTCCAGGGAGACACTGCTAAGCAAGTTGCCCAT
CAGTCGTCTTGGGAAATGGATTGAGGGTTCCTGGCTTAGCAGCTGGTCCTT
GCACAGTGACCTTTTCCTCTGCTCAGTGCCGGGATGAGAGATGGAGTCATG
AGTGTTGAAGAATAAGTGCCTTCTATTTATTTTGAGTCTGTGTGTTCTCACTT
TGGGCATGTAATTATGACTGGTGAATTCTGACGACATGATAGATCTTAAGAT
GTAGTCACCAAACTCAACTGCTGCTTAGCATCCTCCGTAACTACTGATACAA
GCAGGGAACACAGAGGTCACCTGCTTGGTTTGACAGGCTCTTGCTGTCTGA
CTCAAATAATCTTTATTTTTCAGTCCTCAAGGCTCTTCGATAGCAGTTGTTCT
GTATCAGCCTTATAGGTGTCAGGTATAGCACTCAACATCTCATCTCATTACA
ATAGCAACCCTCATCACCATAGCAACAGCTAACCTCTGTTATCCTCACTTCA
TAGCCAGGAAGCTGAGCGACTAAGTCACTTGCCCACAGAGTATCAGCTCTC
AGATTTCTGTTCTTCAGCCACTGTCCTTTCAGGATAGAATTTGTCGTTAAGAA
ATTAATTTAAAAACTGATTATTGAGTAGCATTGTATATCAATCACAACATGCC
TTGTGCACTGTGCTGGCCTCTGAGCATAAAGATGTACGCCGGAGTACCGGT
CGGACATGTTTATGTGTGTTAAATACTCAGAGAAATGTTCATTAACAAGGAG
CTTGCATTTTAGAGACACTGGAAAGTAACTCCAGTTCATTGTCTAGCATTAC
ATTTACCTCATTTGCTATCCTTGCCATACAGTCTCTTGTTCTCCATGAAGTGT
CATGAATCTTGTTGAATAGTTCTTTTATTTTTTAAATGTTTCTATTTAAATGATA
TTGACATCTGAGGCGATAGCTCAGTTGGTAAAACCCTTTCCTCACAAGTGTG
AAACCCTGAGTCTTATCCCTAGAACCCACATAAAAAACAGTTGCGTATGTTT
GTGCATGCTTTTGATCCCAGCACTAGGGAGGCAGAGGCAGGCAGATCCTG
AGCTCTCATTGACCACCCAGCCTAGCCTACATGGTTAGCTCCAGGCCTACA
GGAGCTGGCAGAGCCTGAAAAACGATGCCTAGACACACACACACACACACA
CACACACACACACACACACACACACACCATGTACTCATAGACCTAAGTGCACC
CTCCTACACATGCACACACATACAATTCAAACACAAATCAACAGGGAATTGT

Figure 5

CTCAGAATGGTCCCCAAGACAAAGAAGAAGAAAAACACCAAACCAGCTCTA
TTCCCTCAGCCTATCCTCTCTACTCCTTCCTAGAAGCAACTACTATTGTTTTT
GTATATAAATTTACCCAACGACAGTTAATATGTAGAATATATATTAAAGTGTC
TGTCAATATATATTATCTCTTTCTTTCTTTCTTCCTTTCTTTCTTTCTTTCTTTC
TTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTCCTTCCTTCCTTCCTTCCTTC
CTTCCTTCCTTCCTTTCTTTCTTTCTTTCTTTTTTTCTGTCTATCTGTACCTAAA
TGGTTGCTCACTATGCATTTTCTGTGCTCTTCGCCCTTTTTATTTAATGTATG
GATATTTATGCTGCTTCCAGAATGGATCTAAAGCTCTTTGTTTCTAGGTTTTC
TCCCCCATCCTTCTAGGCATCTCTCACACTGTCTAGGCCAGACACCATGTCT
GCTGCCTGAATCTGTAGACACCATTTATAAAGCACGTACTCACCGAGTTTGT
ATTTGGCTTGTTCTGTGTCTGATTAAAGGGAGACCATGAGTCCCCAGGGTA
CACTGAGTTACCCCAGTACCAAGGGGGAGCCTTGTTTGTGTCTCCATGGCA
GAAGCAGGCCTGGAGCCATTTTGGTTTCTTCCTTGACTTCTCTCAAACACAG
ACGCCTCACTTGCTCATTACAGGTTCTCCTTTGGGAATGTCAGCATTGCTCC
TTGACTGCTGGCTGCCCTGGAAGGAGCCCATTAGCTCTGTGTGAGCCCTTG
ACAGCTACTGCCTCTCCTTACCACAGGGGCCTCTAAGATACTGTTACCTAGA
GGTCTTGAGGATCTGTGTTCTCTGGGGGGAGGAAAGGAGGAGGAACCCAG
AACTTTCTTACAGTTTTCCTTGTTCTGTCACATGTCAAGACTGAAGGAACAG
GCTGGGCTACGTAGTGAGATCCTGTCTCAAAGGAAAGACGAGCATAGCCGA
ACCCCCGGTGGAACCCCCTCTGTTACCTGTTCACACAAGCTTATTGATGAGT
CTCATGTTAATGTCTTGTTTGTATGAAGTTTAAGAAAATATCGGGTTGGGCAA
CACATTCTATTTATTCATTTTATTTGAAATCTTAATGCCATCTCATGGTGTTGG
ATTGGTGTGGCACTTTATTCTTTTGTGTTGTGTATAACCATAAATTTTATTTTG
CATCAGATTGTCAATGTATTGCATTAATTTAATAAATATTTTTATTTATTAAAAA
AAAAAAAAAAAAAAA

Figure 5
(continued)

MRIFAGIIFTACCHLLRAFTITAPKDLYVVEYGSNVTMECRFPVERELDLLALVVYWEKEDEQVIQFVAGEE
DLKPQHSNFRGRASLPKDQLLKGNAALQITDVKLQDAGVYCCIISYGGADYKRITLKVNAPYRKINQRISV
DPATSEHELICQAEGYPEAEVIWTNSDHQPVSGKRSVTTSRTEGMLLNVTSSLRVNATANDVFYCTFWR
SQPGQNHTAELIIPELPATHPPQNRTHWVLLGSILLFLIVVSTVLLFLRKQVRMLDVEKCGVEDTSSKNRN
DTQFEET.

Figure 6

mB7-4 vs. hB7-4

69% identity

```
mB7-4   1   MRIFAGIIFTACCHLLRAFTITAPKDLYVVEYGSNVTMECRFPVERELDLLALVVYWEKE 60
            MRIFA  IF    HLL AFT+T PKDLYVVEYGSN+T+EC+FPVE++LDL AL+VYWE E
hB7-4   1   MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEME 60

mB7-4  61   DEQVIQFVAGEEDLKPQHSNFRGRASLPKDQLLKGNAALQITDVKLQDAGVYCCIISYGG 120
            D+ +IQFV GEEDLK QHS++R RA L KDQL  GNAALQITDVKLQDAGVY C+ISYGG
hB7-4  61   DKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGG 120

mB7-4 121   ADYKRITLKVNAPYRKINQRI-SVDPATSEHELICQAEGYPEAEVIWTNSDHQPVSGKRS 179
            ADYKRIT+KVNAPY KINQRI  VDP TSEHEL CQAEGYP+AEVIWT+SDHQ +SGK +
hB7-4  121   ADYKRITVKVNAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTT 180

mB7-4  180   VTTSRTEGMLLNVTSSLRVNATANDVFYCTFWRSQPGQNHTAELIIPELPATHPPQNRTH 239
             T S+ E  L NVTS+LR+N T N++FYCTF R  P +NHTAEL+IPELP  HPP  RTH
hB7-4  181   TTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTH 240

mB7-4  240   WVLLGSILLFLIVVSTVLLFLRKQVRMLDVEKCGVEDTSSKNRNDTQFEET 290
             V+LG+ILL L V  T +  LRK  RM+DV+KCG++DT+SK ++DT  EET
hB7-4  241   LVILGAILLCLGVALTFIFRLRKG-RMMDVKKCGIQDTNSKKQSDTHLEET 290
```

Figure 7

23 FEB 2004

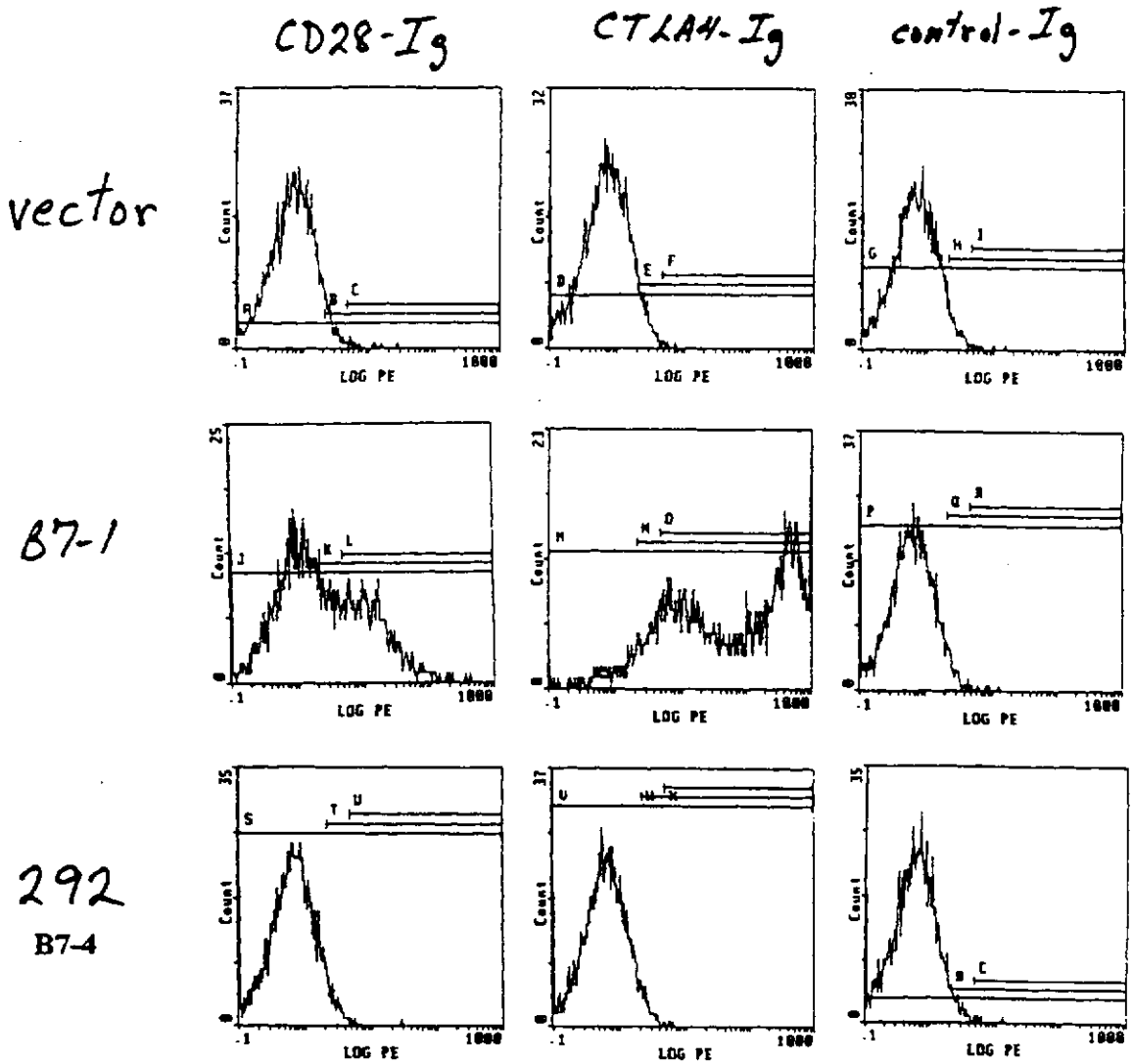
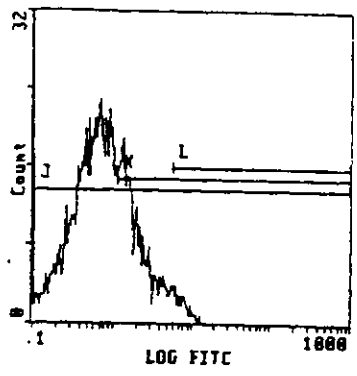
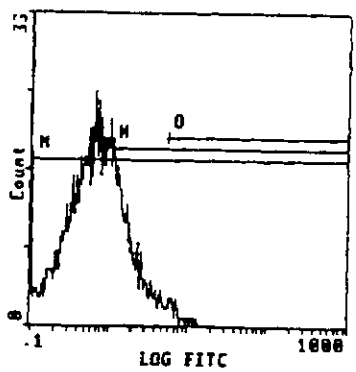


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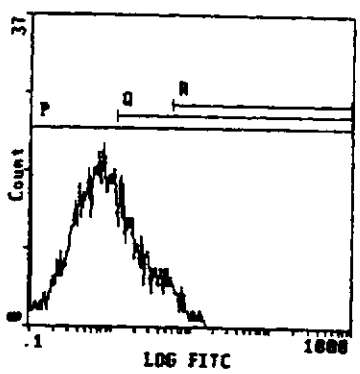
IgG mICOS-his

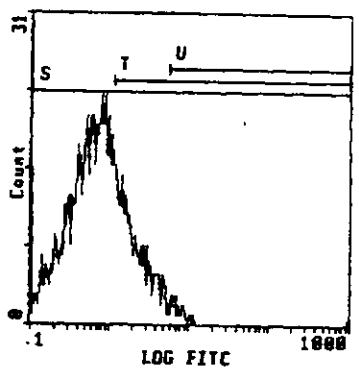
Vector





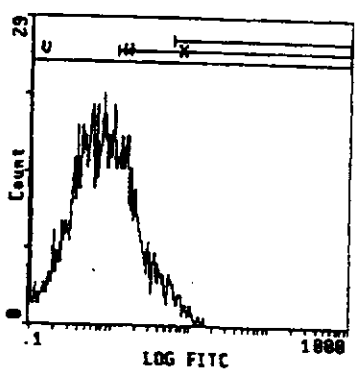
B7-1





292

B7-4



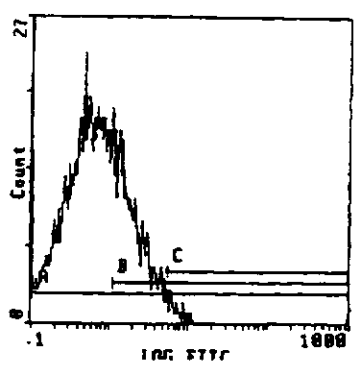


Figure 9

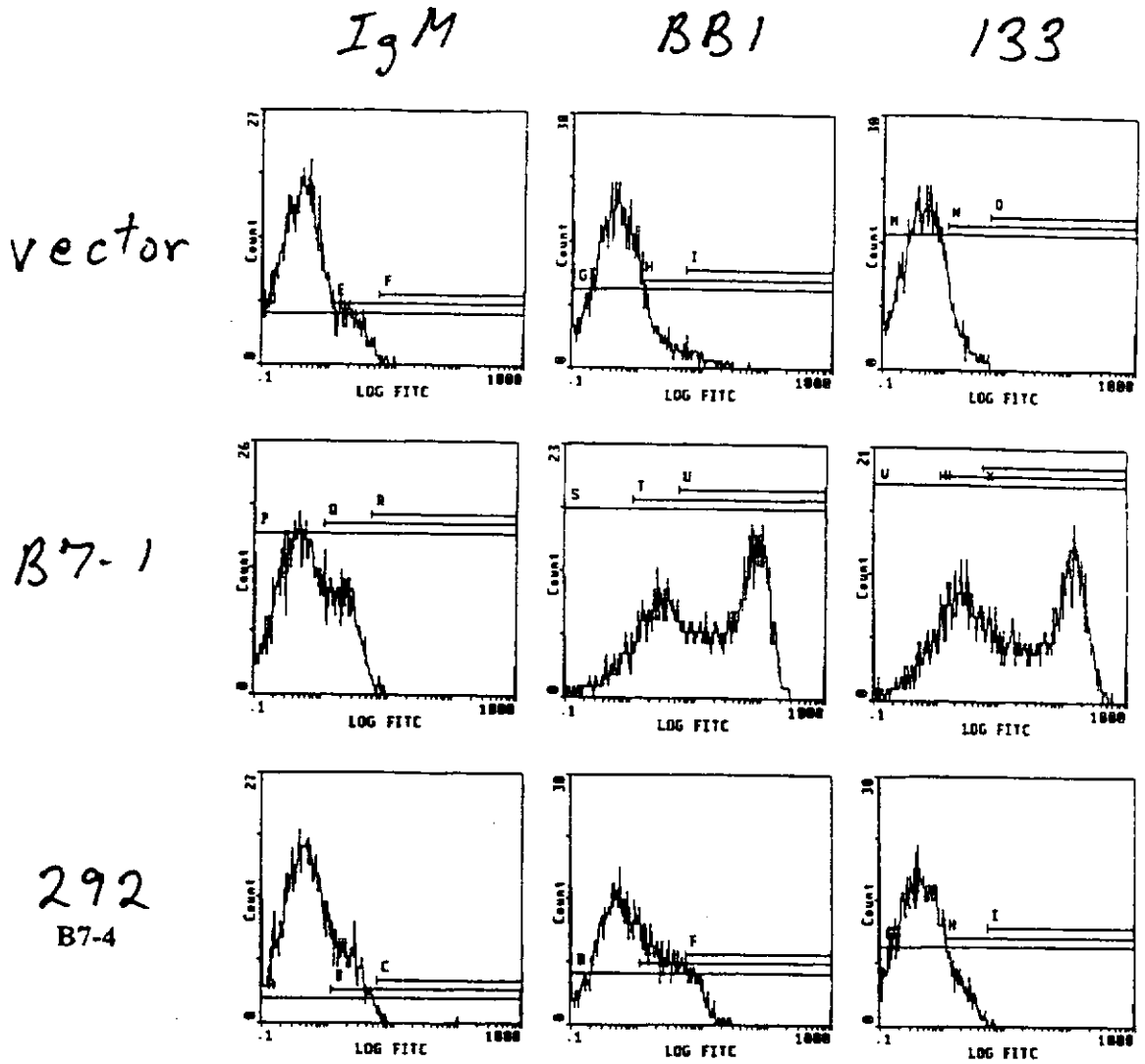
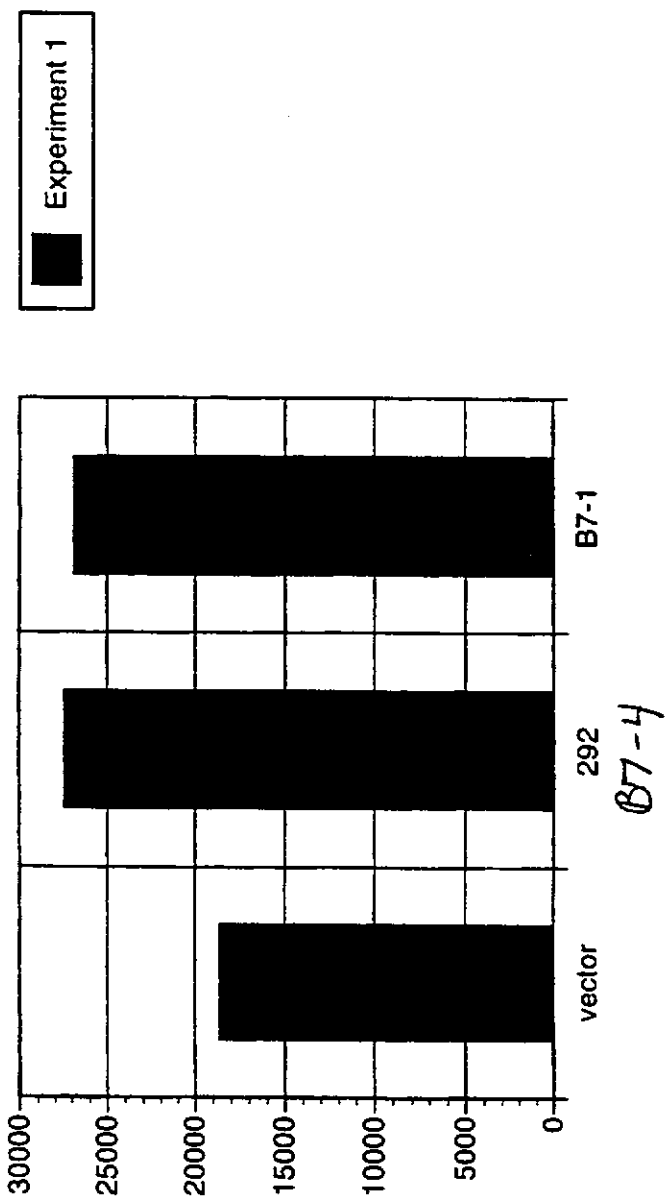


Figure 10



B7-4

Figure 11

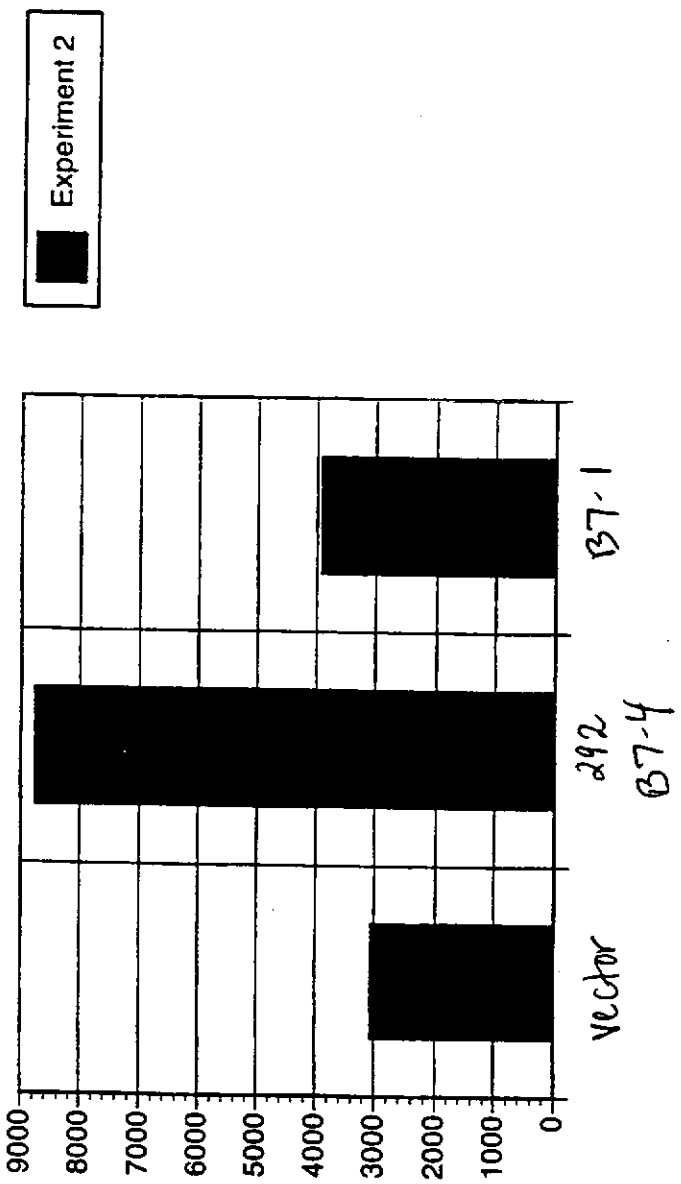
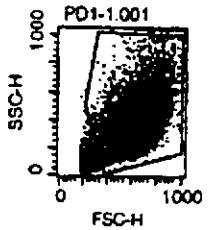
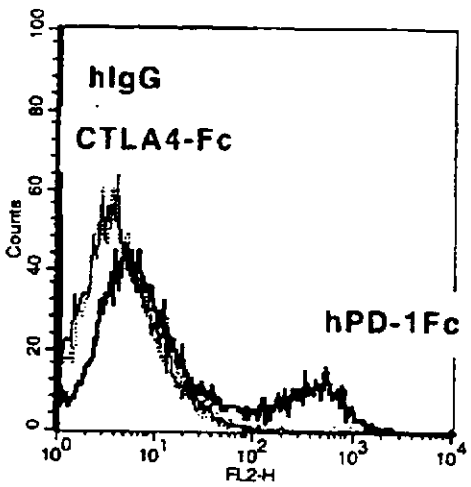
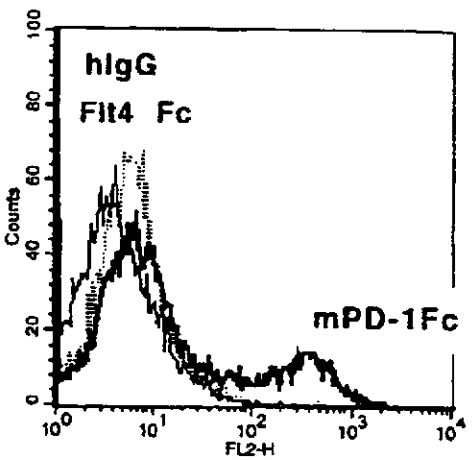

Experiment 2
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Vector
292
B7-4
B7-1

Figure 12

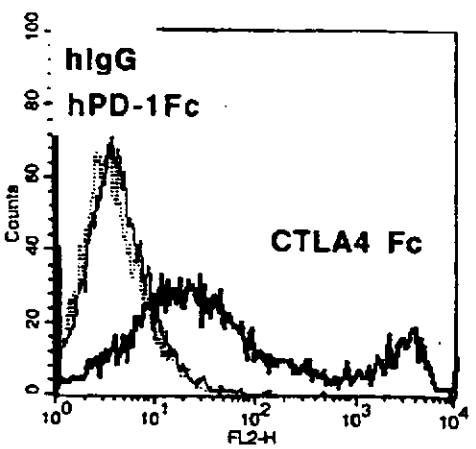




292
B7-4



292
B7-4



B7.1

Figure 13

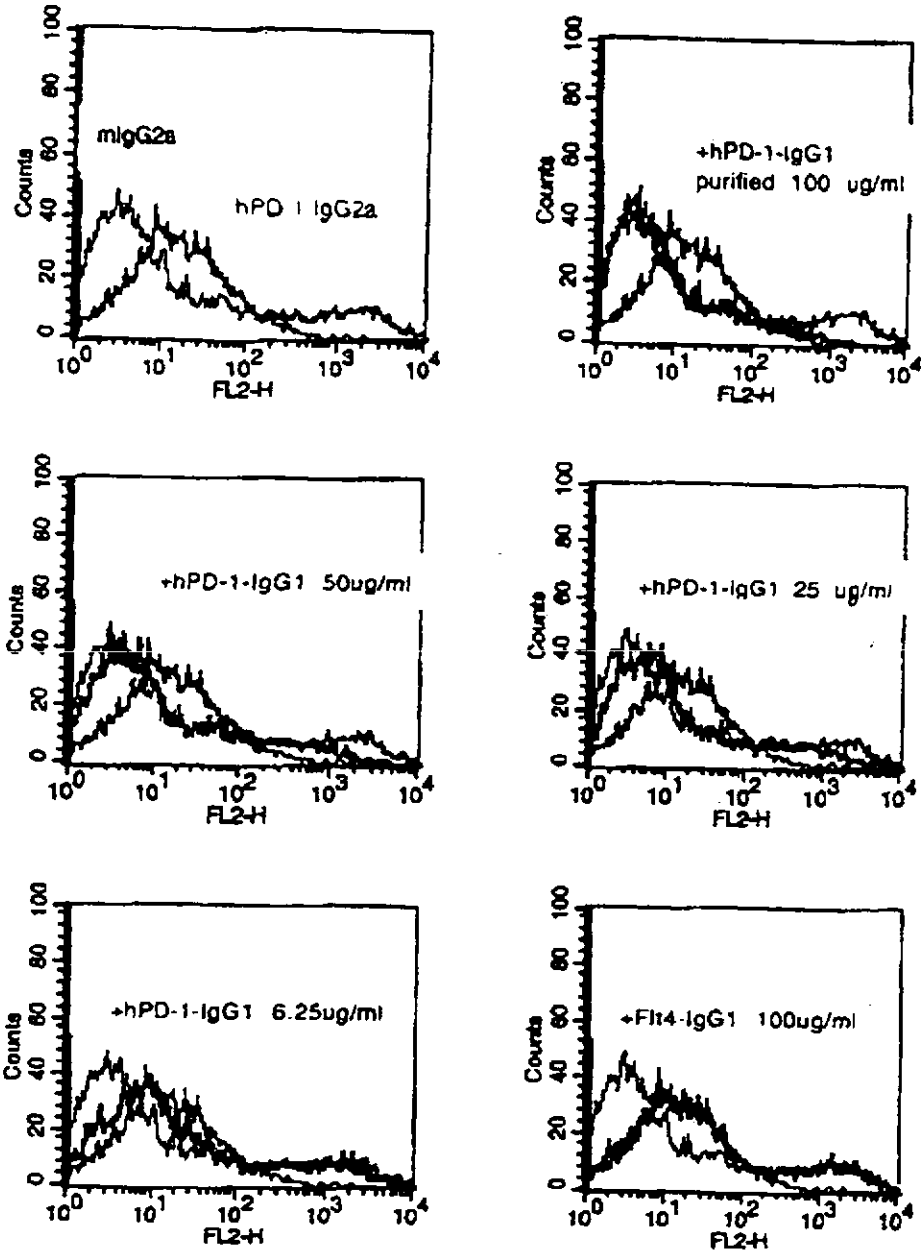

mIgG2a
hPD I IgG2a
+hPD-1-IgG1
purified 100 ug/ml
+hPD-1-IgG1 50ug/ml
+hPD-1-IgG1 25 ug/ml
+hPD-1-IgG1 6.25ug/ml
+Flt4-IgG1 100ug/ml
Counts
FL2-H

Figure 14

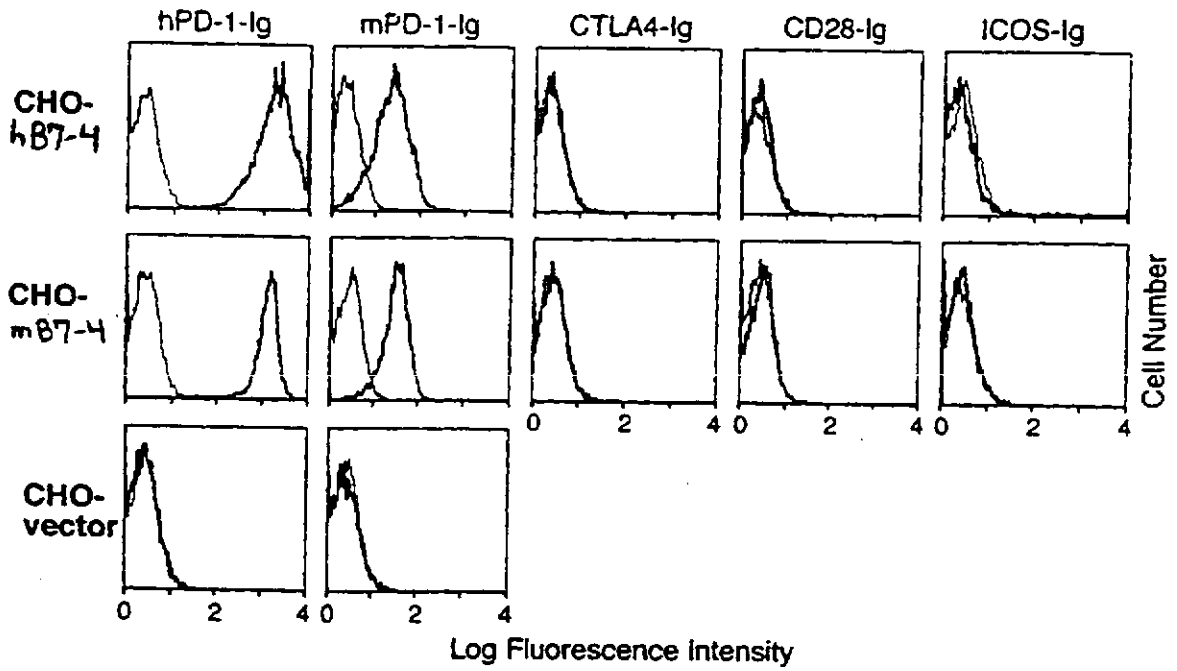

hPD-1-Ig
mPD-1-Ig
CTLA4-Ig
CD28-Ig
ICOS-Ig
CHO-hB7-4
CHO-mB7-4
CHO-vector
Cell Number
0
2
4
Log Fluorescence Intensity

Figure 15

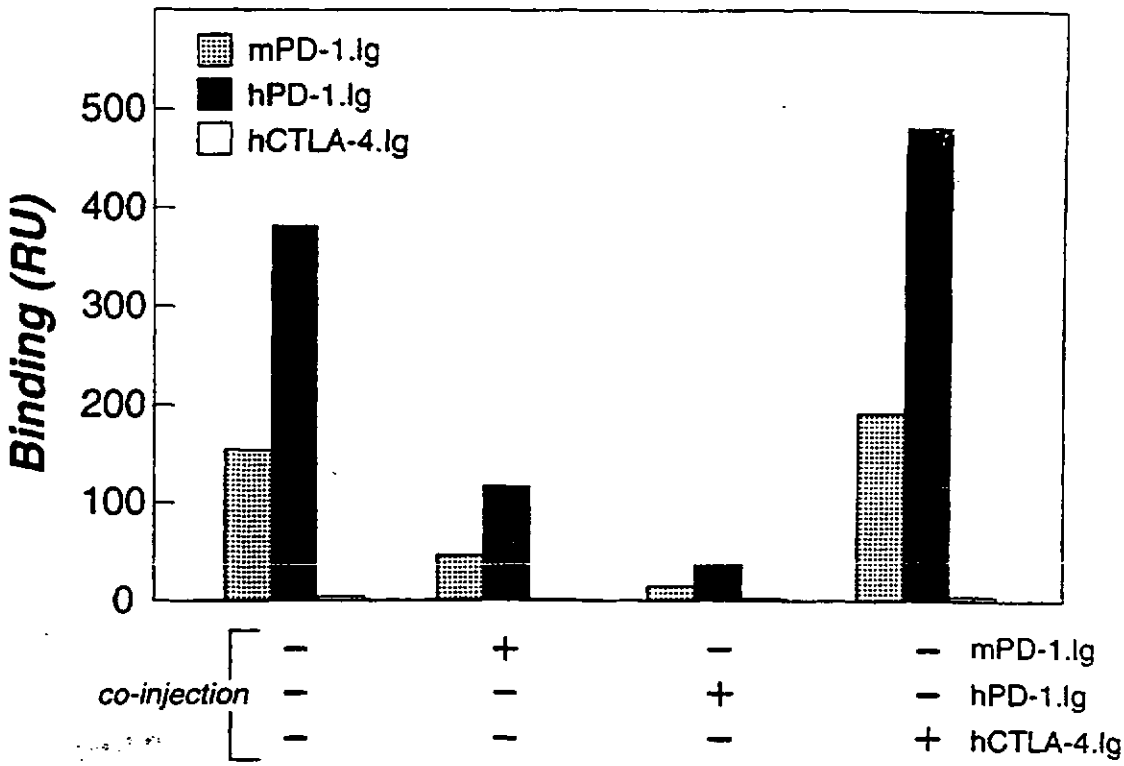

mPD-1.lg
hPD-1.lg
hCTLA-4.lg
Binding (RU)
500
400
300
200
100
0
co-injection
- + - - mPD-1.lg
- - + - hPD-1.lg
- - - + hCTLA-4.lg

Figure 16

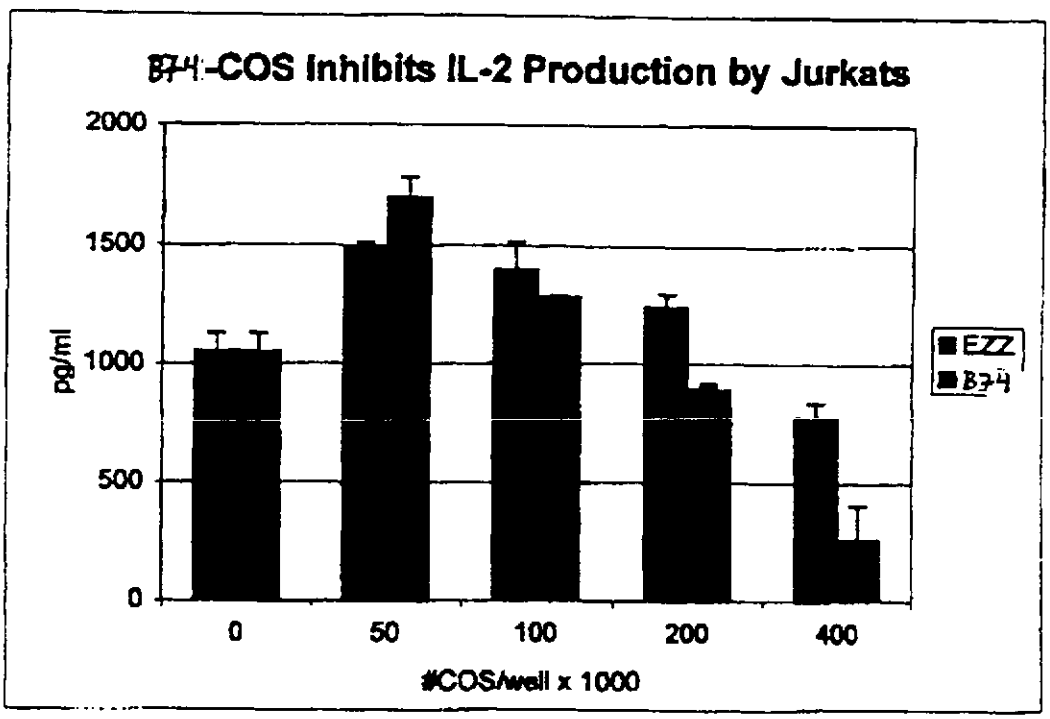

B7-COS Inhibits IL-2 Production by Jurkats
2000
1500
1000
500
0
pg/ml
0
50
100
200
400
#COS/well x 1000
EZZ
B7

Figure 17

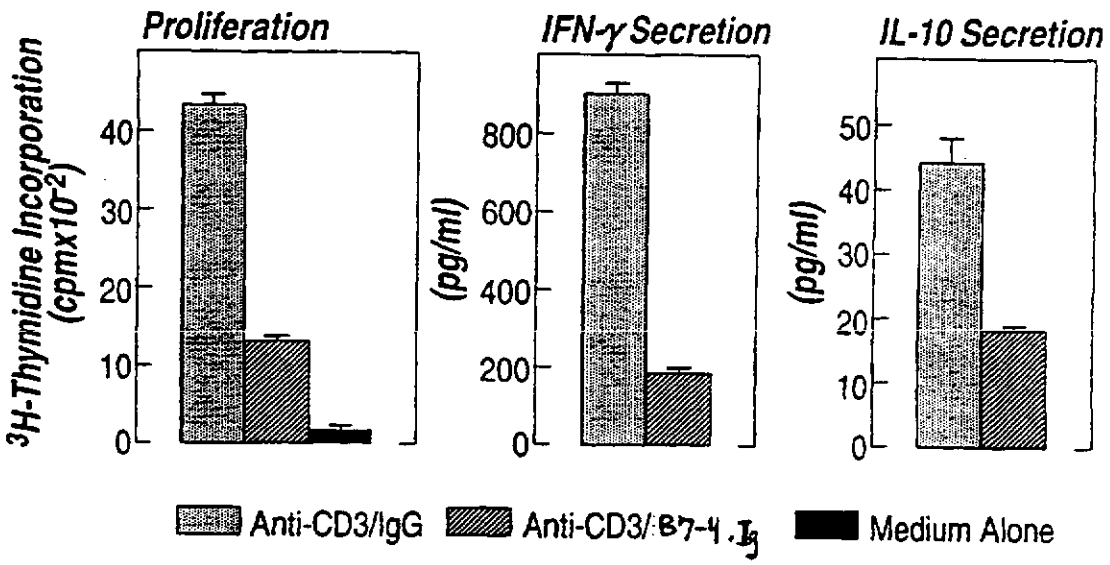

Proliferation
IFN-γ Secretion
IL-10 Secretion
^{3}H-Thymidine Incorporation (cpmx10^{-2})
(pg/ml)
(pg/ml)
0
10
20
30
40
200
400
600
800
50
Anti-CD3/IgG
Anti-CD3/B7-4.Ig
Medium Alone

Figure 18

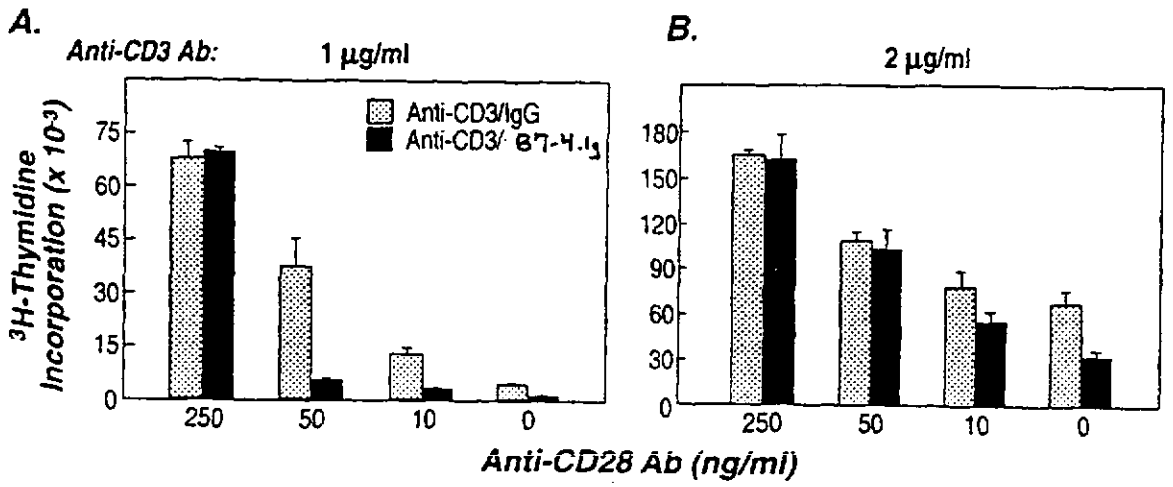

A.
Anti-CD3 Ab:
1 µg/ml
Anti-CD3/IgG
Anti-CD3/ 87-4.1
3H-Thymidine Incorporation (x 10-3)
75
60
45
30
15
0
250
50
10
0
B.
2 µg/ml
180
150
120
90
60
30
0
250
50
10
0
Anti-CD28 Ab (ng/ml)

Figure 19

Anti B7.2
Anti IA^d
hPD1-Ig
CHO-IA^d
CHO-IA^d/B7.2
CHO-IA^d/B7.2/B7-4
Cell Number
Log Flourescence

FIGURE 20

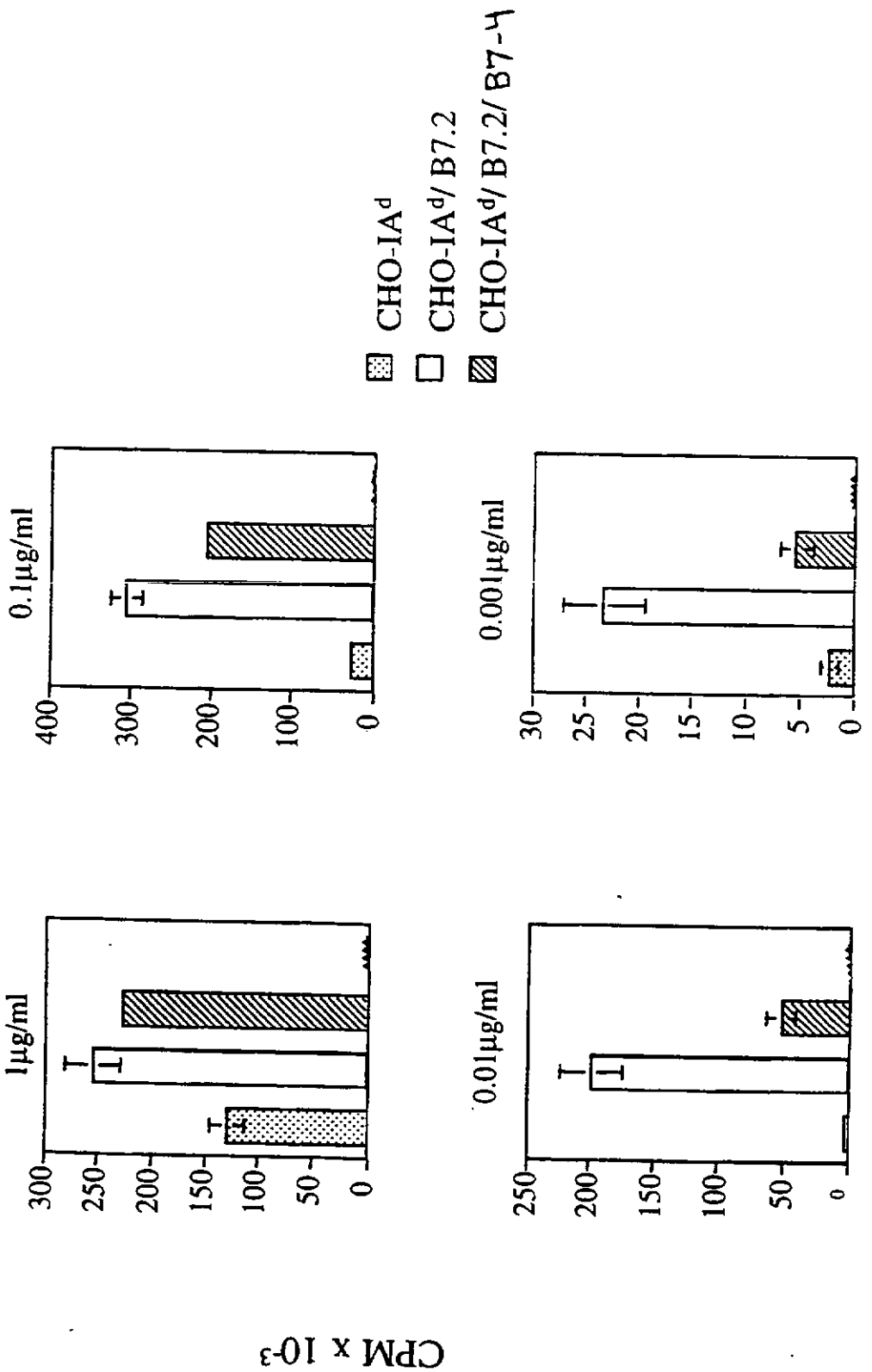

CHO-IA^d
CHO-IA^d/ B7.2
CHO-IA^d/ B7.2/ B7-4
1µg/ml
0.1µg/ml
0.01µg/ml
0.001µg/ml
CPM x 10-3

FIGURE 21

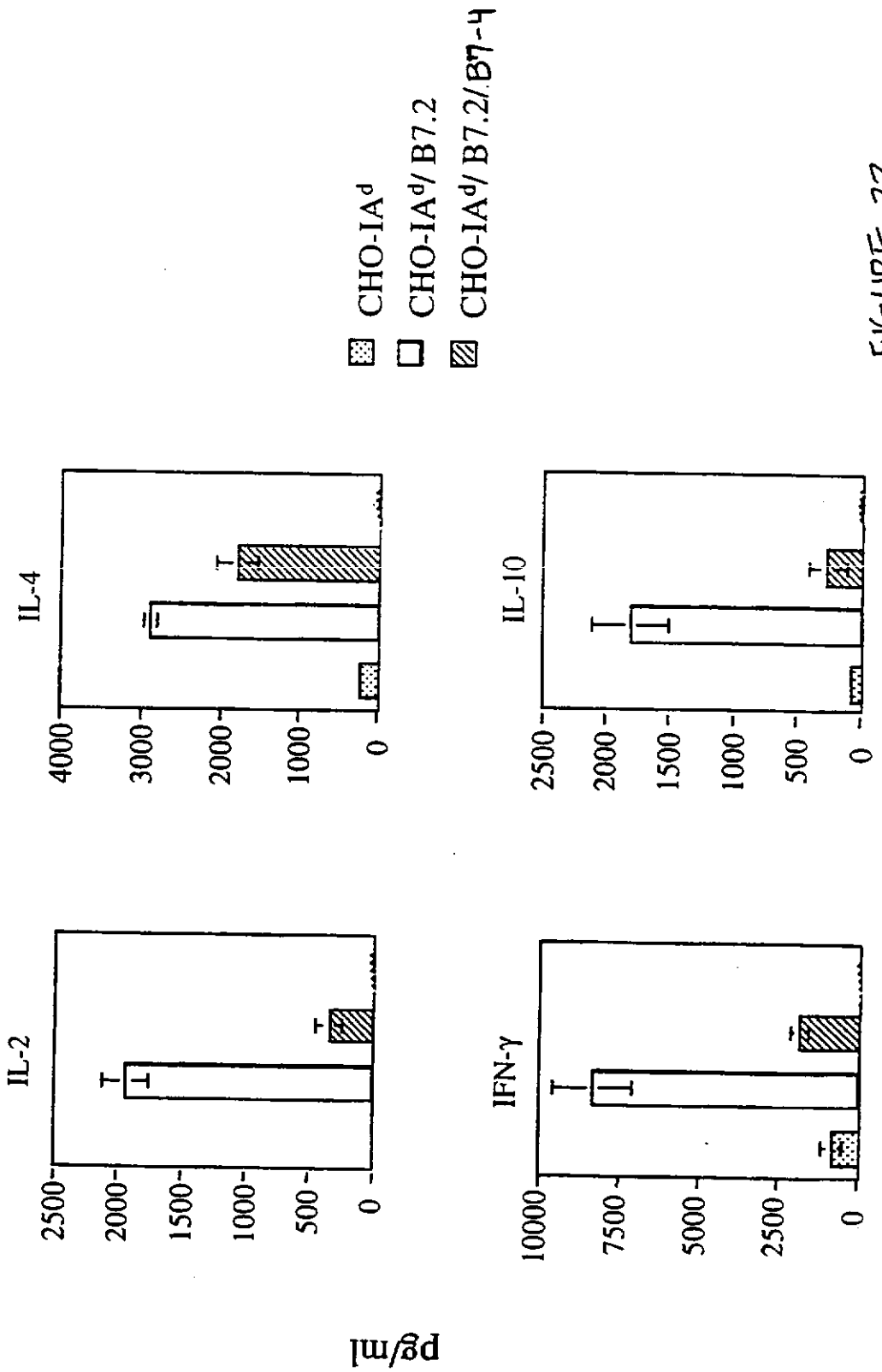

CHO-IA^d
CHO-IA^d/ B7.2
CHO-IA^d/ B7.2/B7-4
IL-2
IL-4
IL-10
IFN-γ
pg/ml
0
500
1000
1500
2000
2500
1000
2000
3000
4000
500
1000
1500
2000
2500
2500
5000
7500
10000

FIGURE 22

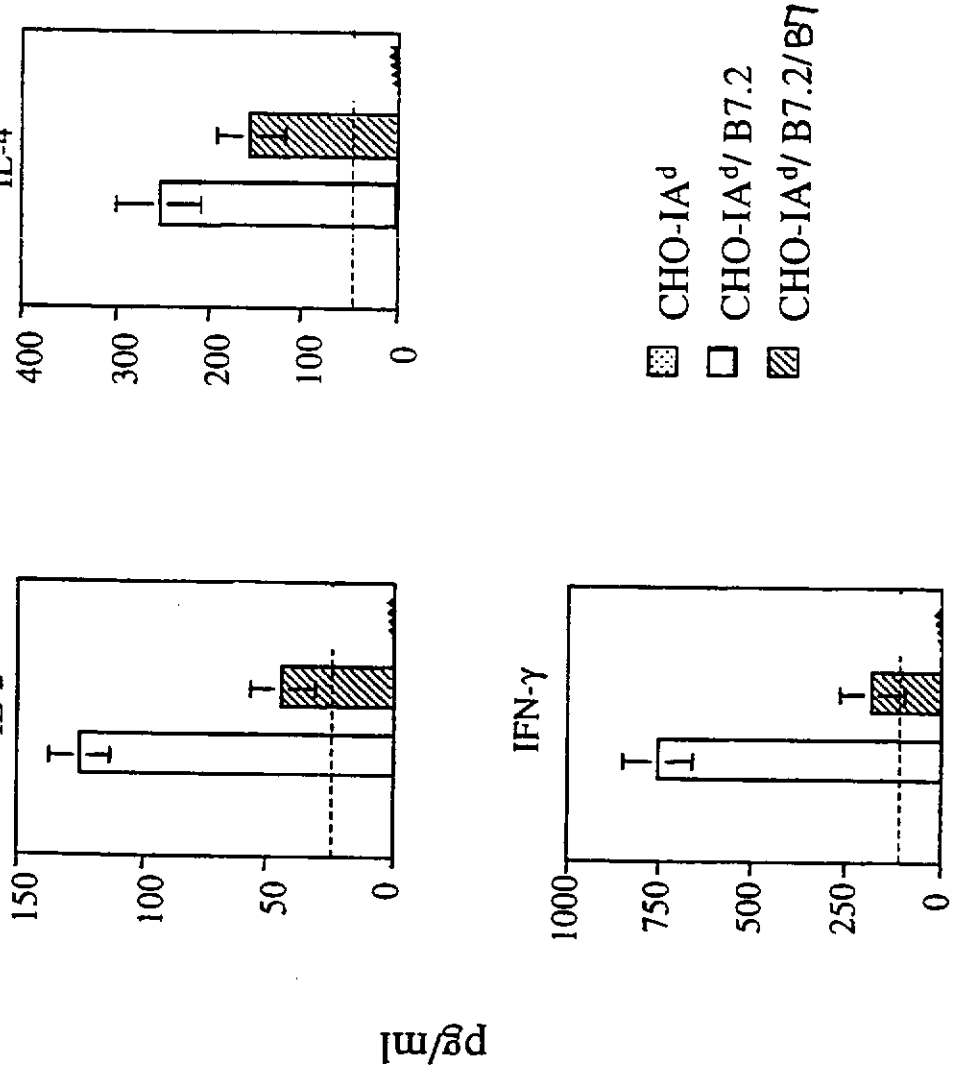

IL-4
400
300
200
100
0
IL-2
150
100
50
0
IFN-γ
1000
750
500
250
0
pg/ml
CHO-IA^d
CHO-IA^d/ B7.2
CHO-IA^d/ B7.2/B7-4

FIGURE 23

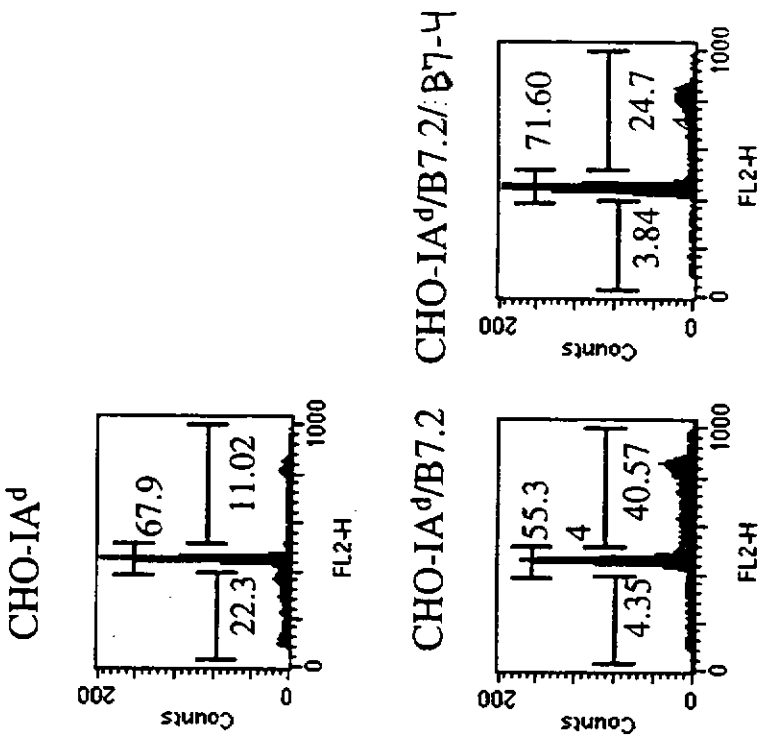
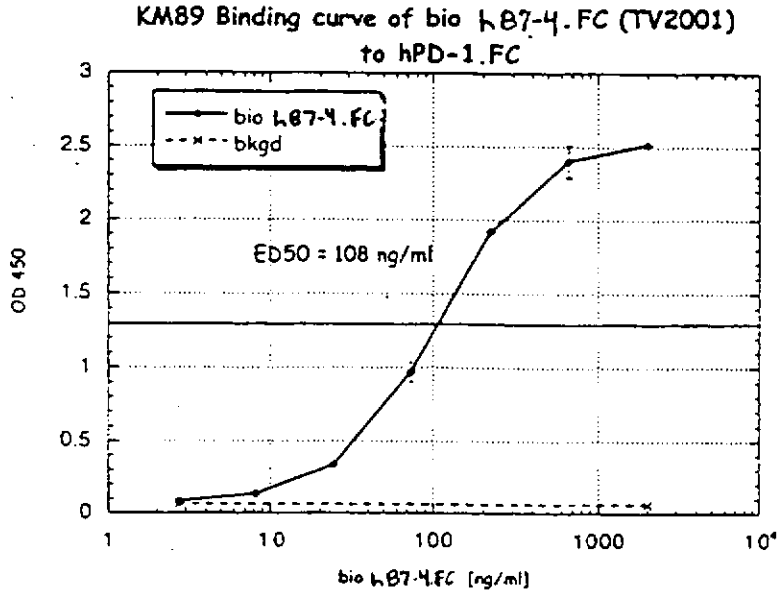
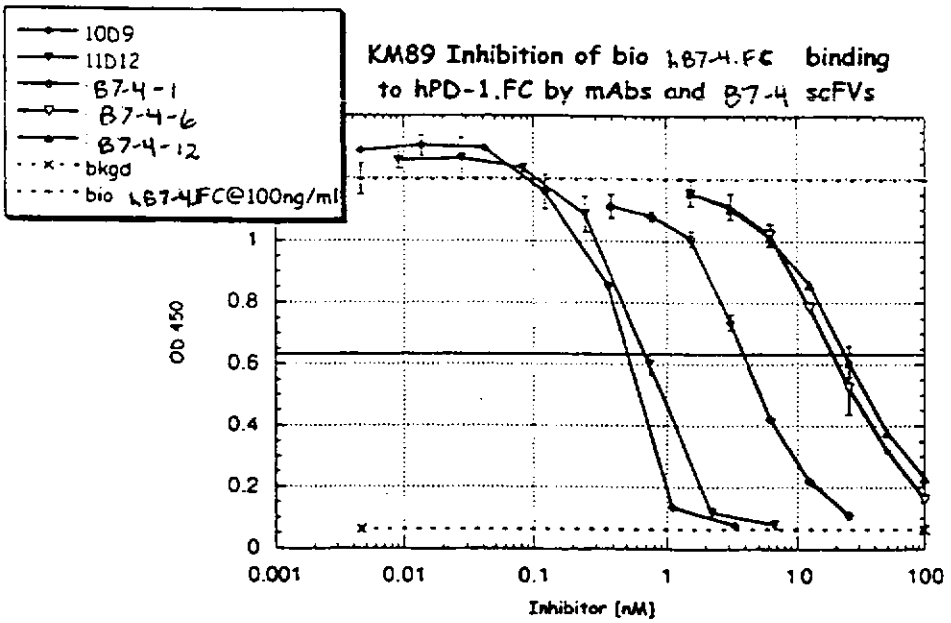

CHO-IA^d
67.9
22.3
11.02
Counts
FL2-H
CHO-IA^d/B7.2
55.3
4
4.35
40.57
CHO-IA^d/B7.2/B7-4
71.60
3.84
24.7

FIGURE 24





Inhibitor	IC50
10D9	0.5
11D12	0.7
B7-4-1	4
B7-4-6	19
B7-4-12	24

Figure 25

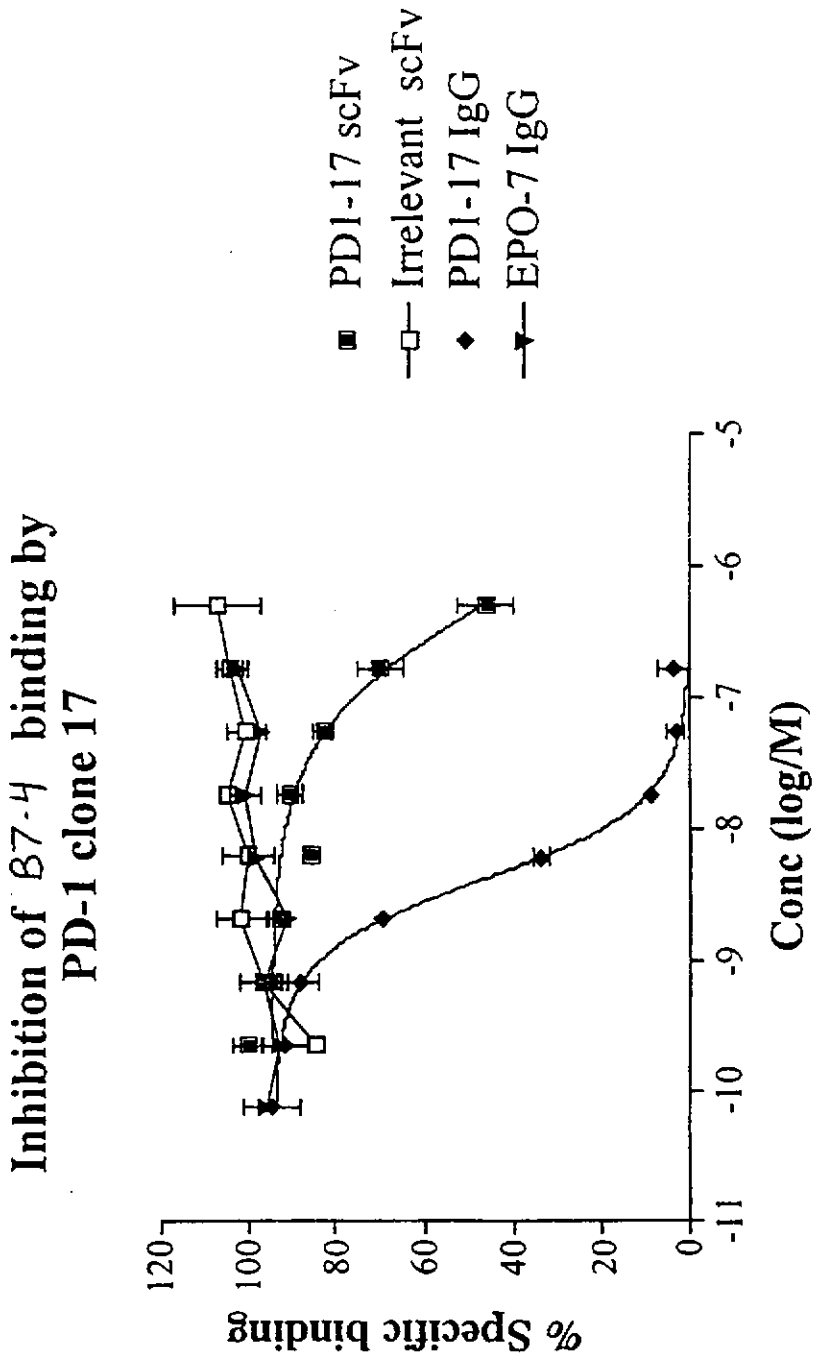
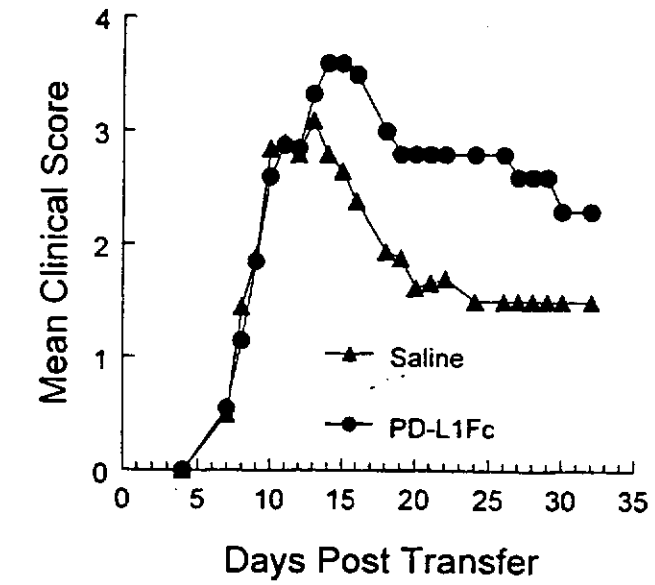

Inhibition of B7-4 binding by PD-1 clone 17
PD1-17 scFv
Irrelevant scFv
PD1-17 IgG
EPO-7 IgG
% Specific binding
120
100
80
60
40
20
0
-11
-10
-9
-8
-7
-6
-5
Conc (log/M)

FIGURE 2c


Mean Clinical Score
4
3
2
1
0
0
5
10
15
20
25
30
35
Saline
PD-L1Fc
Days Post Transfer

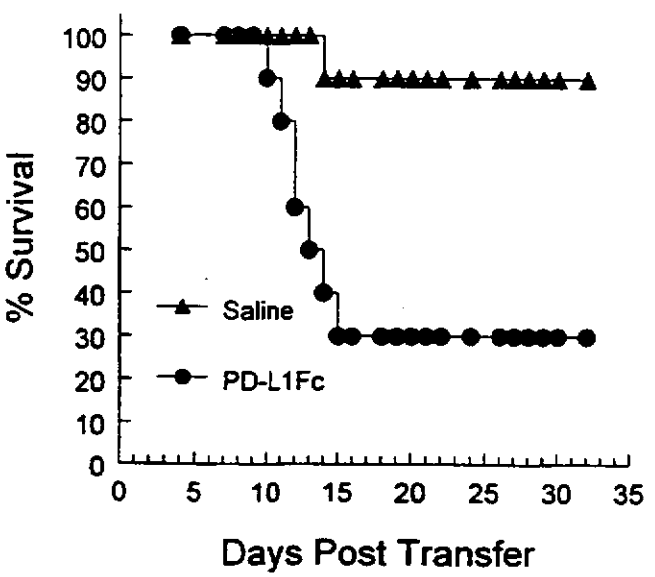

% Survival
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
Saline
PD-L1Fc
Days Post Transfer

Figure 27