Sequences for Variable Domains in Humanized Murine Anti-CD20 mAb (H1286 + L373)

Sequence of variable domain (H1286; SEQ ID NO:29) for humanized murine anti-CD20 mAb heavy chain (CDR1, CDR2, and CDR3, respectively, are underlined):

QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYNMHWVRQAPGQGLEWIGAIY
PGNGDTSYNQKFKGKATITADKSTSTAYMELSSLRSDDTAVYYCARSTYYGGD
WYFNVWGQGTLVTVSS

Sequence of variable domain (L373; SEQ ID NO:10) for humanized murine anti-CD20 mAb light chain (CDR1, CDR2, and CDR3, respectively, are underlined)*:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYIHWFQQKPGKAPKPLIYAASSLQS
GVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQWTSNPPTFGGGTKLEIK

*Note: CDR2 of variable light domain is derived from a human V_K and not a murine V_K

FIG. 1

CDR3 Optimization

Pos. #	1	2	3	4	5	6	7	8	9	10	11	12
271:	S	T	Y	Y	G	G	D	W	Y	F	N	V
1236:	S	T	Y	Y	G	G	D	W	**N**	F	N	**N**
1237:	S	T	Y	Y	**A**	G	D	W	**N**	F	N	**N**
1238:	S	T	Y	Y	**A**	G	D	W	**N**	F	N	**D**

FIG. 2

Amino Acid Sequences of V_H Domains in Optimized Humanized mAbs

H1569 (SEQ ID NO:12) pairs with L373 to produce optimized mAb 1236:

QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYNMHWVRQAPGQGLEWIGAIYP
GNGDTSYNQKFKGKATITADKSTSTAYMELSSLRSDDTAVYYCARSTYYGGDW
NFNNWGQGTLVTVSS

H1570 (SEQ ID NO:13) pairs with L373 to produce optimized mAb 1237:

QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYNMHWVRQAPGQGLEWIGAIYP
GNGDTSYNQKFKGKATITADKSTSTAYMELSSLRSDDTAVYYCARSTYYAGDWN
FNNWGQGTLVTVSS

H1571 (SEQ ID NO:14) pairs with L373 to produce optimized mAb 1238:

QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYNMHWVRQAPGQGLEWIGAIYP
GNGDTSYNQKFKGKATITADKSTSTAYMELSSLRSDDTAVYYCARSTYYAGDWN
FNDWGQGTLVTVSS

FIG. 3

Coding Sequences for V_H Domains in Optimized Humanized mAbs

H1569 (SEQ ID NO:19):

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCAGTGAAGGTTTCCTGCAAG
GCATCTGGGTACACCTTCACCAGTTACAATATGCACTGGGTGCGACAGGCCCCTGGACAAGGGCTAGAG
TGGATTGGAGCTATTTATCCCGGAAATGGTGATACTTCCTACAATCAGAAGTTCAAAGGCAAGGCCACAAT
AACTGCAGACAAATCCACGAGCACAGCCTACATGGAGCTCAGCAGCCTGAGATCTGACGACACGGCCGT
GTATTACTGTGCAAGATCGACTTACTACGGCGGTGACTGGAACTTCAATAACTGGGGCCAGGGAACCCTG
GTCACCGTCTCCTCA

H1570 (SEQ ID NO:20):

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCAGTGAAGGTTTCCTGCAAG
GCATCTGGGTACACCTTCACCAGTTACAATATGCACTGGGTGCGACAGGCCCCTGGACAAGGGCTAGAG
TGGATTGGAGCTATTTATCCCGGAAATGGTGATACTTCCTACAATCAGAAGTTCAAAGGCAAGGCCACAAT
AACTGCAGACAAATCCACGAGCACAGCCTACATGGAGCTCAGCAGCCTGAGATCTGACGACACGGCCGT
GTATTACTGTGCAAGATCGACTTACTACGCCGGTGACTGGAACTTCAATAACTGGGGCCAGGGAACCCTG
GTCACCGTCTCCTCA

H1571 (SEQ ID NO:21):

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCAGTGAAGGTTTCCTGCAAG
GCATCTGGGTACACCTTCACCAGTTACAATATGCACTGGGTGCGACAGGCCCCTGGACAAGGGCTAGAG
TGGATTGGAGCTATTTATCCCGGAAATGGTGATACTTCCTACAATCAGAAGTTCAAAGGCAAGGCCACAAT
AACTGCAGACAAATCCACGAGCACAGCCTACATGGAGCTCAGCAGCCTGAGATCTGACGACACGGCCGT
GTATTACTGTGCAAGATCGACTTACTACGCCGGTGACTGGAACTTCAATGACTGGGGCCAGGGAACCCTG
GTCACCGTCTCCTCA

FIG. 4

Sequences for L373 Variable Domain

Sequence of variable domain (L373; SEQ ID NO:10) for humanized murine anti-CD20 mAb light chain (CDR1, CDR2, and CDR3, respectively, are underlined)*:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYIHWFQQKPGKAPKPLIYAASSLQSGVPSR
FSGSGSGTDYTLTISSLQPEDFATYYCQQWTSNPPTFGGGTKLEIK

Respective coding sequence:

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCA
CCATCACTTGCCGGGCAAGTTCGAGCGTTAGTTATATACATTGGTTTCAGCAGAAACC
AGGGAAAGCCCCTAAACCCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCC
ATCAAGGTTCAGTGGCAGTGGATCTGGGACAGATTACACTCTCACCATCAGCAGTCTG
CAACCTGAGGATTTCGCAACTTACTACTGTCAACAGTGGACTTCCAACCCGCCCACTT
TCGGCGGAGGGACCAAGCTCGAGATCAAA

*Note: CDR2 of variable light domain is derived from a human V_K and not a murine V_K

FIG. 5

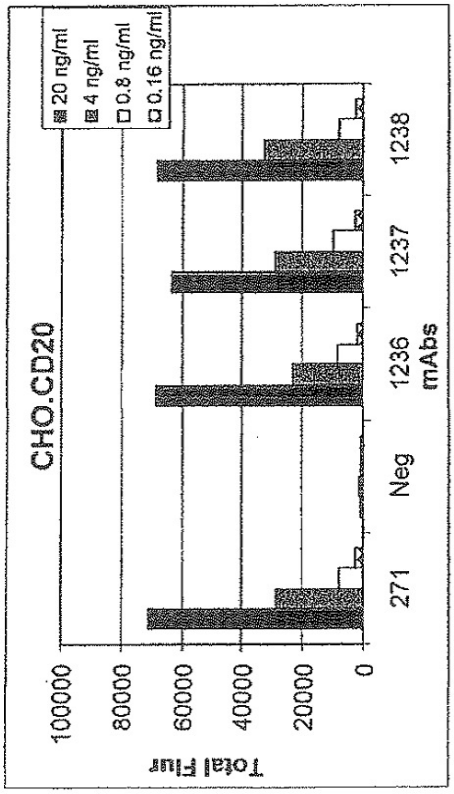


FIG. 6A

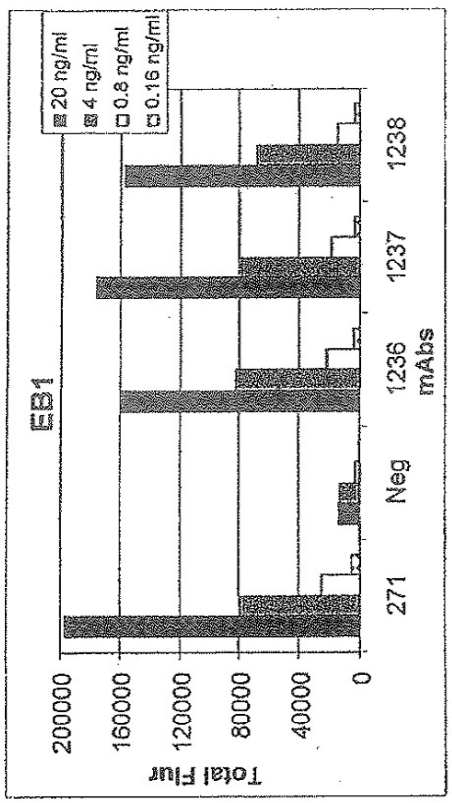


FIG. 6B

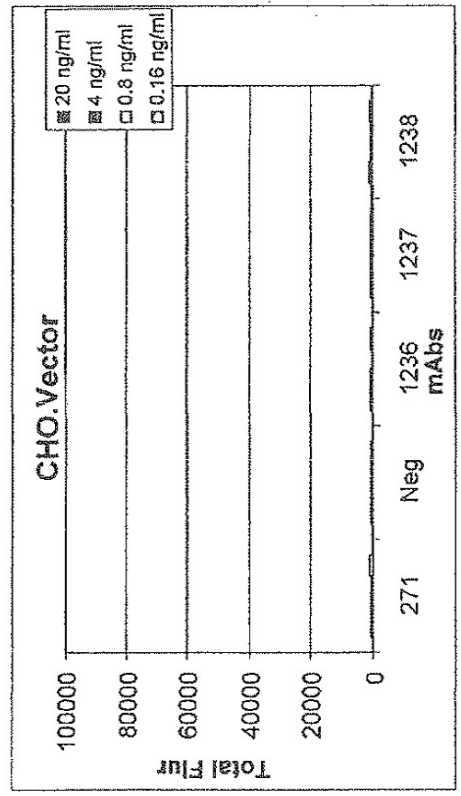


FIG. 6C

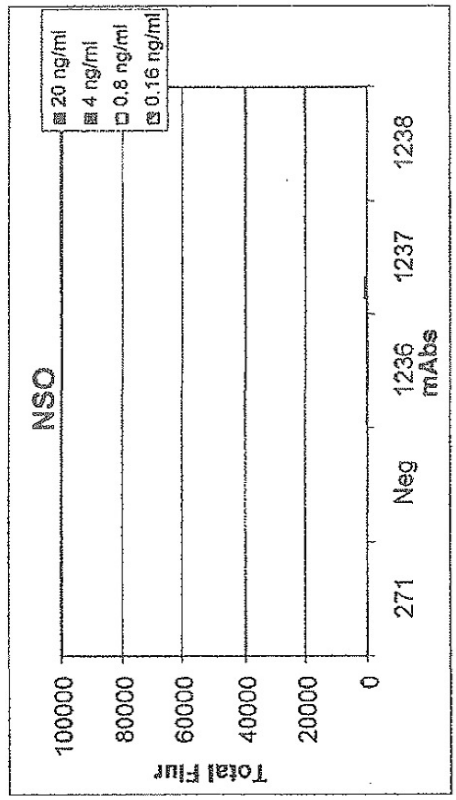


FIG. 6D

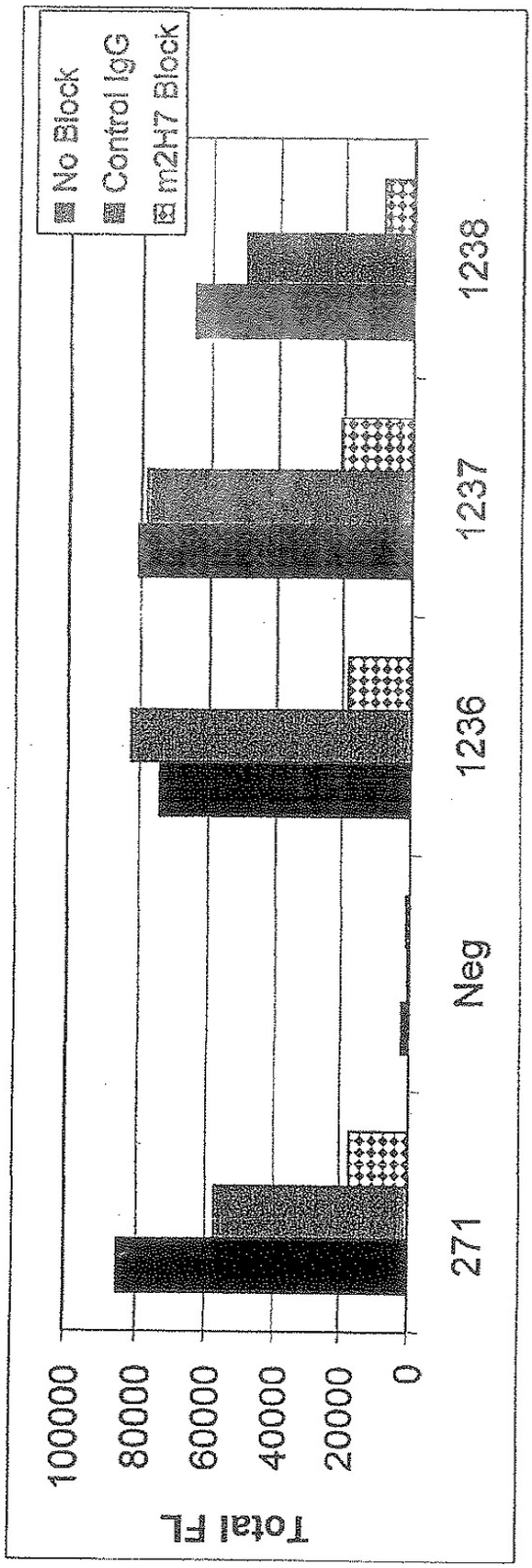

No Block
Control IgG
m2H7 Block
Total FL
100000
80000
60000
40000
20000
0
271
Neg
1236
1237
1238

FIG. 7

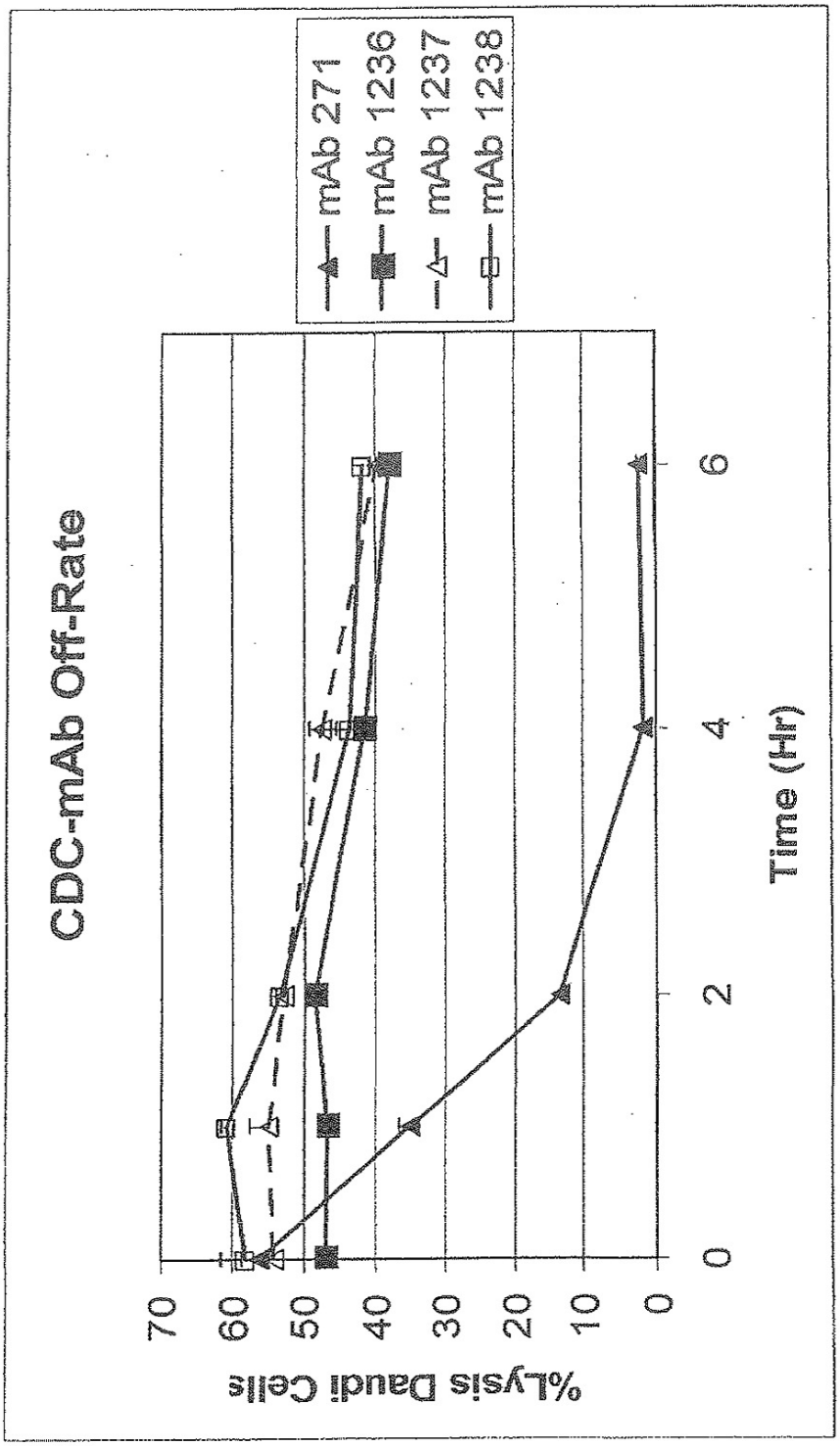
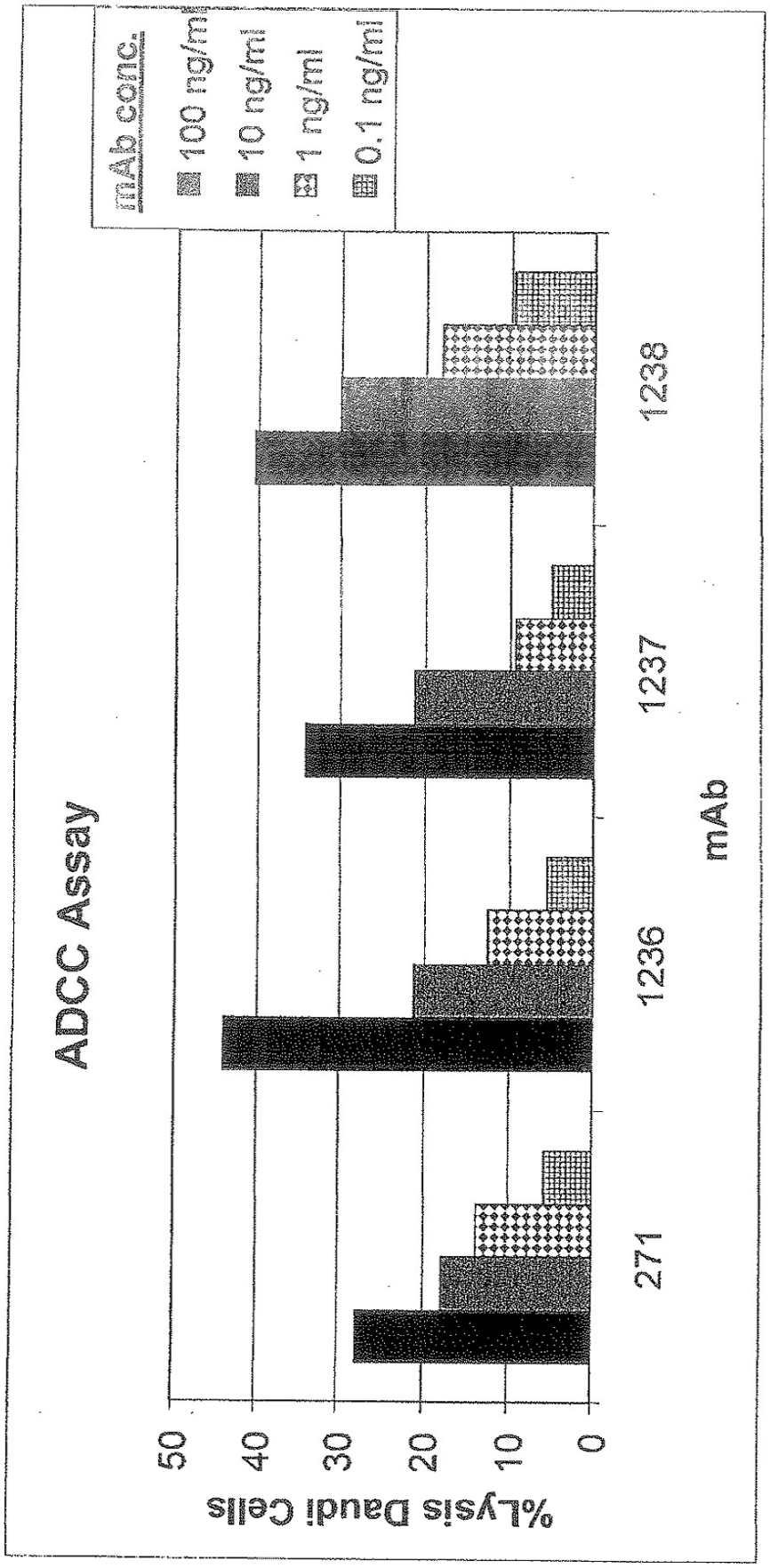
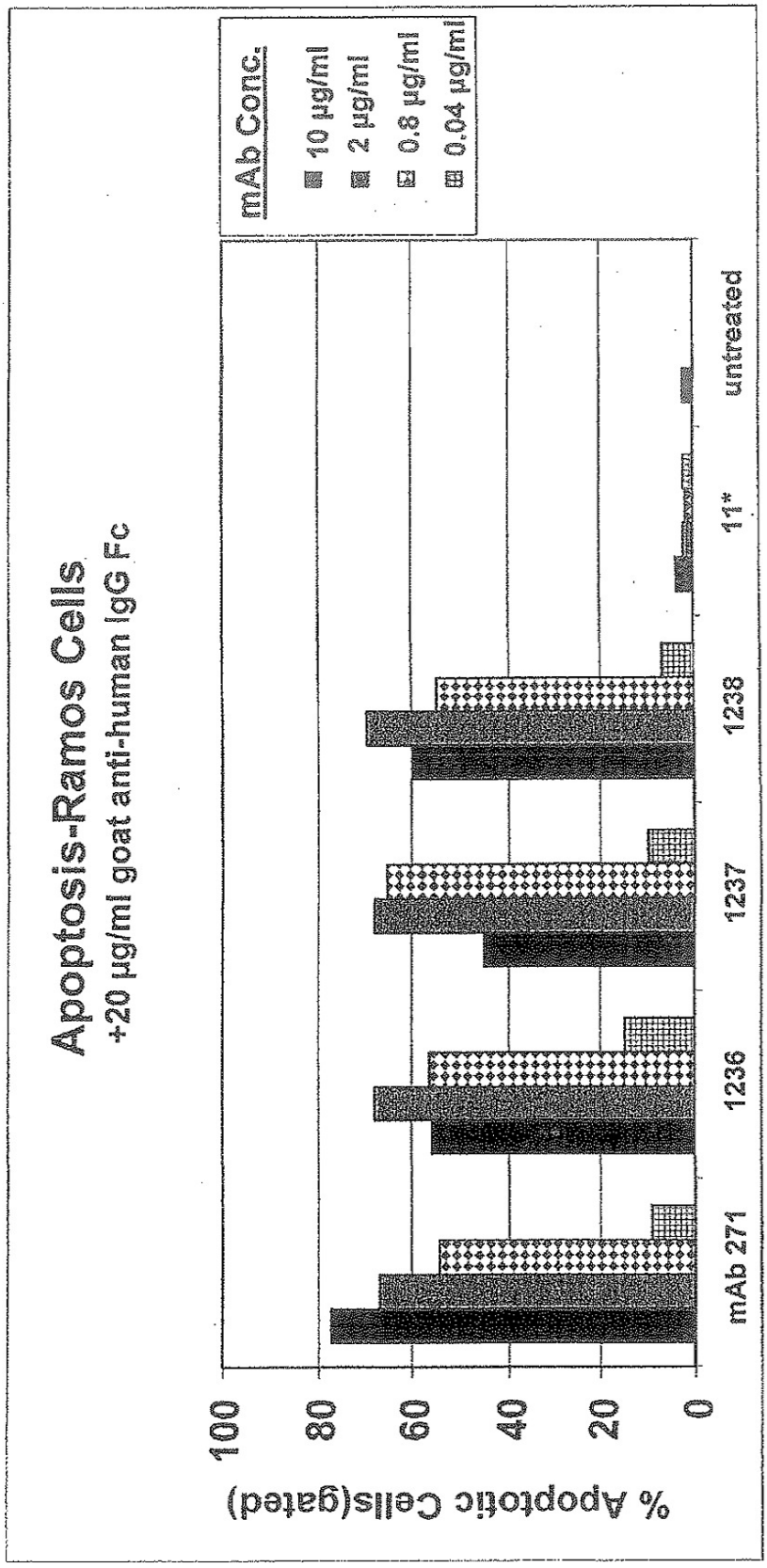

CDC-mAb Off-Rate
%Lysis Daudi Cells
70
60
50
40
30
20
10
0
0
2
4
6
Time (Hr)
mAb 271
mAb 1236
mAb 1237
mAb 1238

FIG. 8



* Negative Control Ab at 1 µg/ml gave 4 % background lysis

FIG. 9



* mAb 11 is an isotype control mAb

FIG. 10

Sequences for Variable Domains in Optimized mAb 1589 (H1639 + L373)

Sequence of variable domain (H1639; SEQ ID NO:16) for optimized mAb 1589 heavy chain (CDR1, CDR2, and CDR3, respectively, are underlined):

QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYNMHWVRQAPGQGLEWIGAIYPG
NGATSYNQKFKGKATITADKSTSTAYMELSSLRSDDTAVYYCARSTYYAGDWNFN
NWGQGTLVTVSS

Sequence of variable domain (L373; SEQ ID NO:10) for optimized mAb 1589 light chain (CDR1, CDR2, and CDR3, respectively, are underlined):

DIQMTQSPSSLSASVGDRVTITCRASSSVSYIHWFQQKPGKAPKPLIYAASSLQSG
VPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQWTSNPPTFGGGTKLEIK

FIG. 11

Coding Sequences for Variable Domains in Optimized mAb 1589

H1639 Variable Heavy Domain (SEQ ID NO:22)*:

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCAG
TGAAGGTTTCCTGCAAGGCATCTGGGTACACCTTCACCAGTTACAATATGCACT
GGGTGCGACAGGCCCCTGGACAAGGGCTAGAGTGGATTGGAGCTATTTATCC
CGGAAATGGTGCTACTTCCTACAATCAGAAGTTCAAAGGCAAGGCCACAATAA
CTGCAGACAAATCCACGAGCACAGCCTACATGGAGCTCAGCAGCCTGAGATCT
GACGACACGGCCGTGTATTACTGTGCAAGATCGACTTACTACGCCGGTGACTG
GAACTTCAATAACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA

L373 Variable Light Domain (SEQ ID NO:23)*:

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACA
GAGTCACCATCACTTGCCGGGCAAGTTCGAGCGTTAGTTATATACATTGGTT
TCAGCAGAAACCAGGGAAAGCCCCTAAACCCCTGATCTATGCTGCATCCAGT
TTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACAGAT
TACACTCTCACCATCAGCAGTCTGCAACCTGAGGATTTCGCAACTTACTACT
GTCAACAGTGGACTTCCAACCCGCCCACTTTCGGCGGAGGGACCAAGCTCG
AGATCAAA

*Codons for CDR1, CDR2, and CDR3, respectively, are underlined

FIG. 12

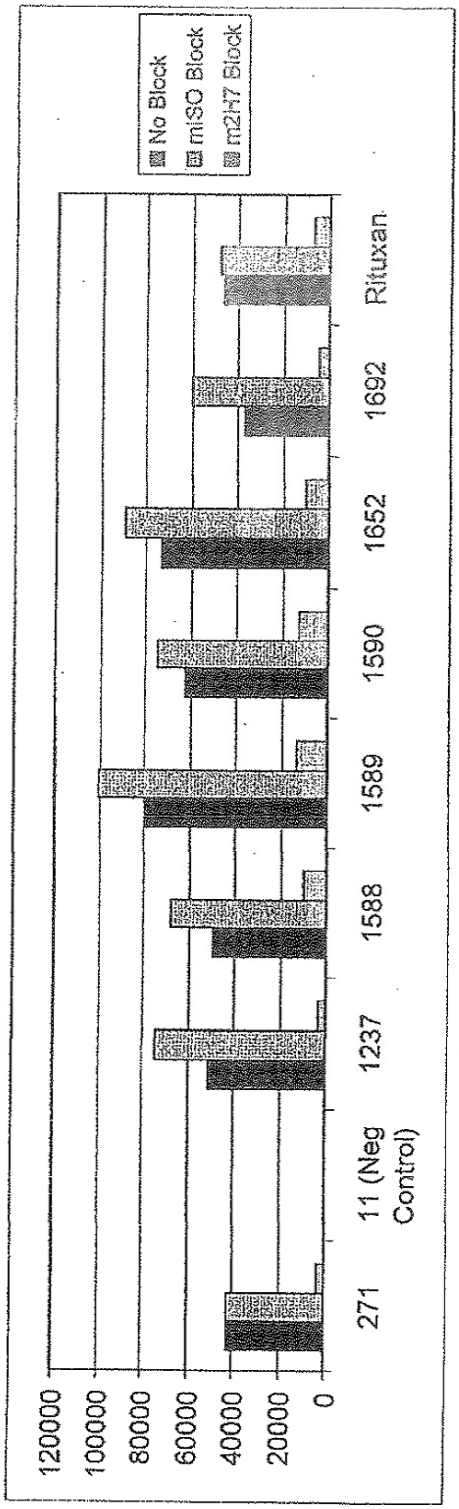

No Block
miSO Block
m2H7 Block
120000
100000
80000
60000
40000
20000
0
271
11 (Neg Control)
1237
1588
1589
1590
1652
1692
Rituxan

FIG. 13

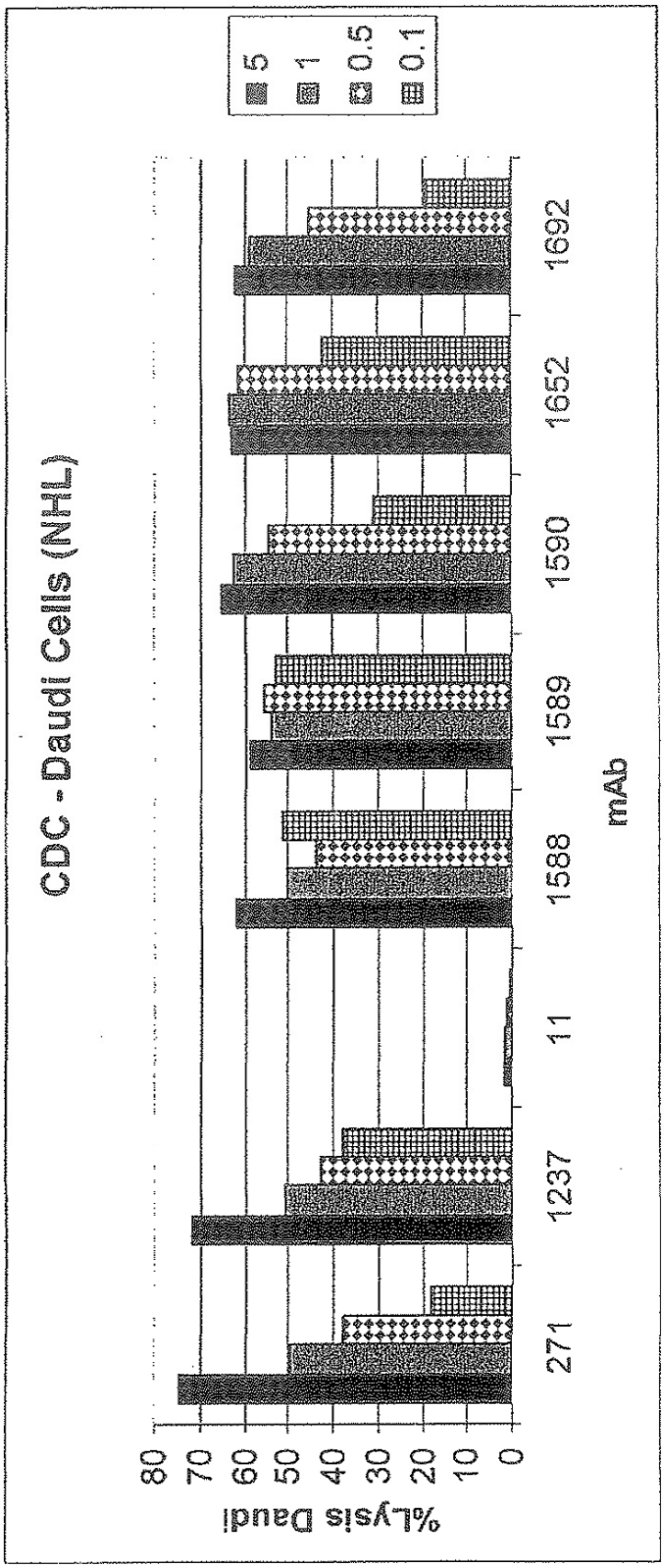

CDC - Daudi Cells (NHL)
5
1
0.5
0.1
%Lysis Daudi
0
10
20
30
40
50
60
70
80
271
1237
11
1588
1589
1590
1652
1692
mAb

FIG. 14

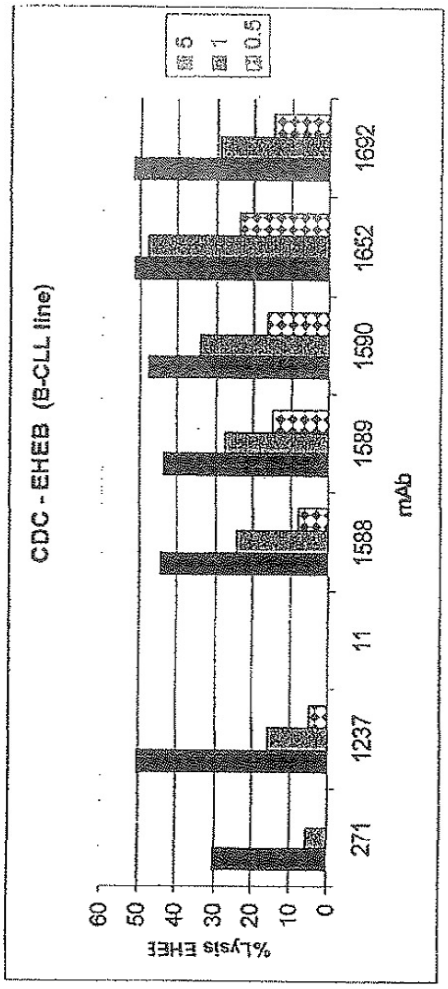


FIG. 15A

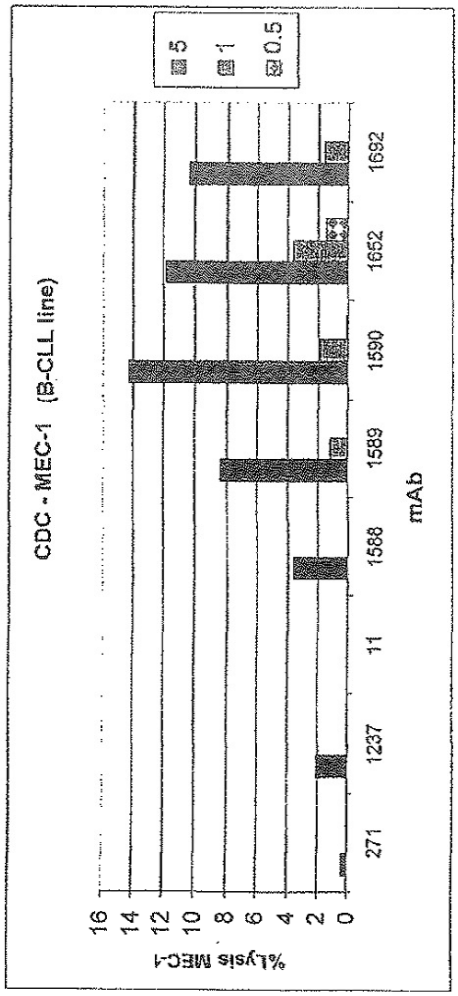


FIG. 15B

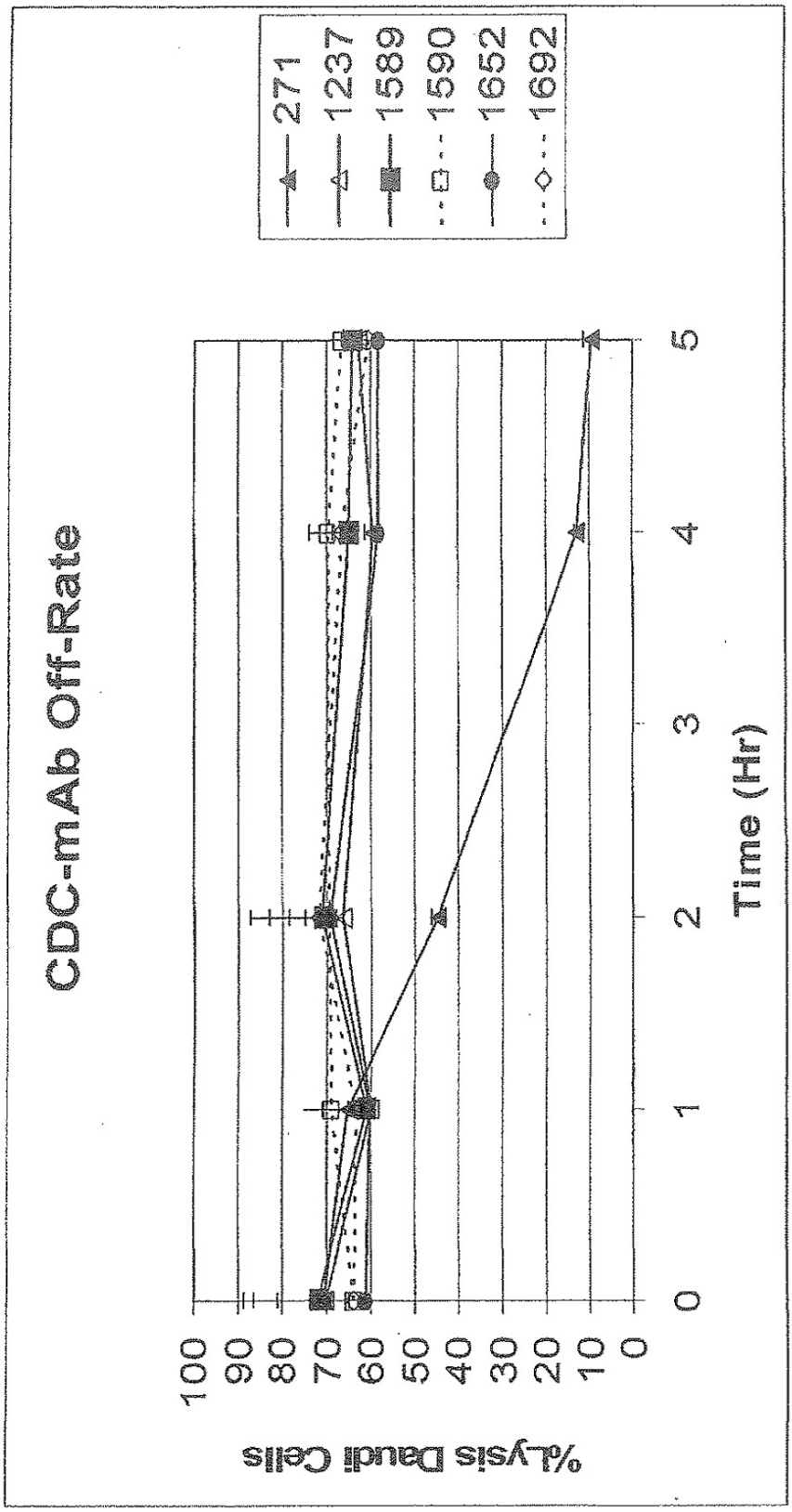

CDC-mAb Off-Rate
%Lysis Daudi Cells
100
90
80
70
60
50
40
30
20
10
0
Time (Hr)
0
1
2
3
4
5
271
1237
1589
1590
1652
1692

FIG. 16

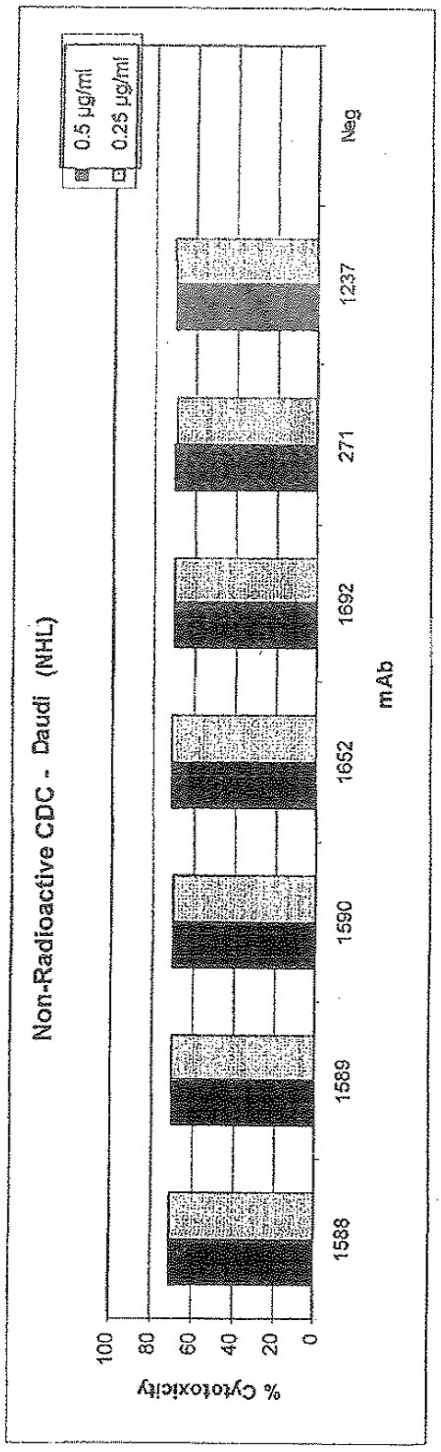


FIG. 17A

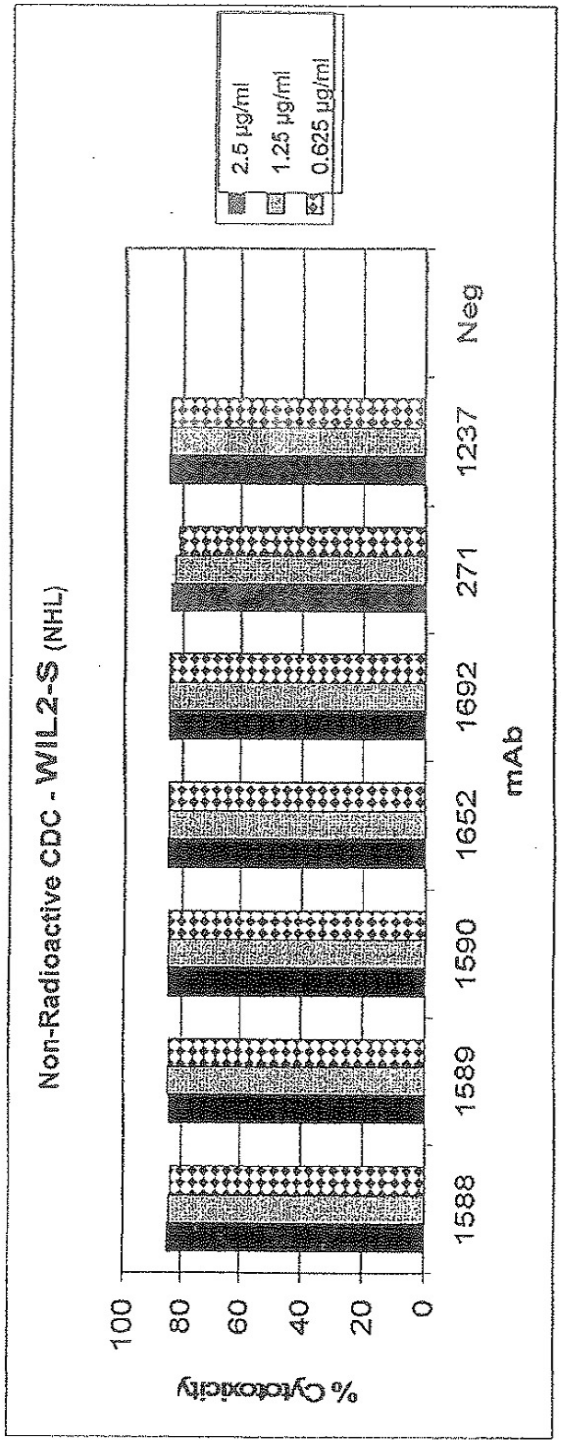


FIG. 17B

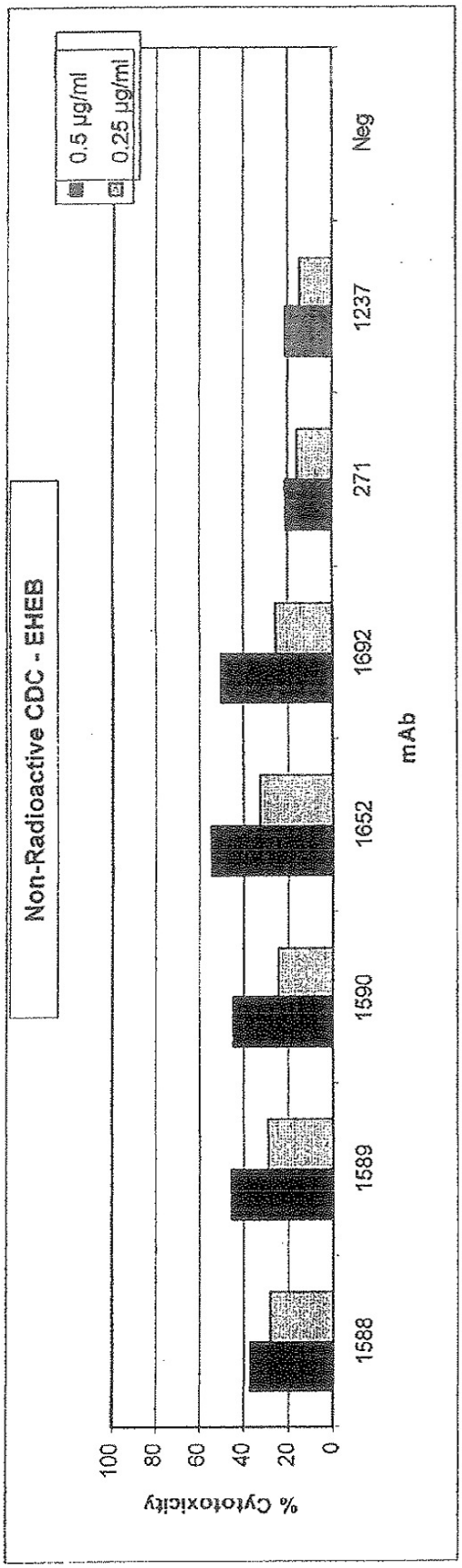


FIG. 18A

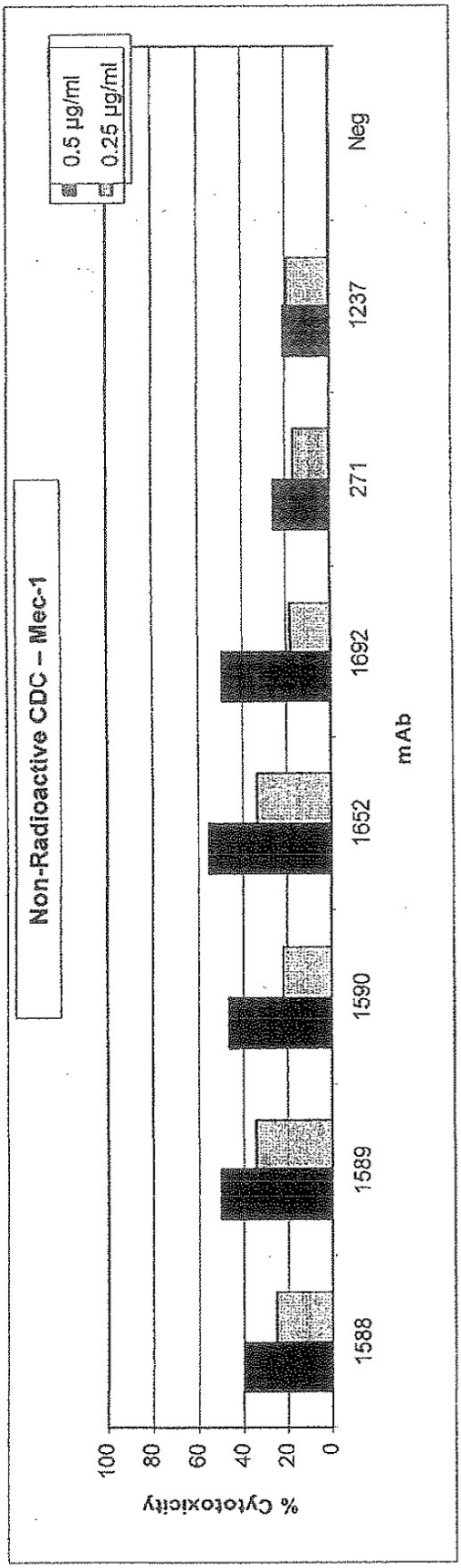


FIG. 18B

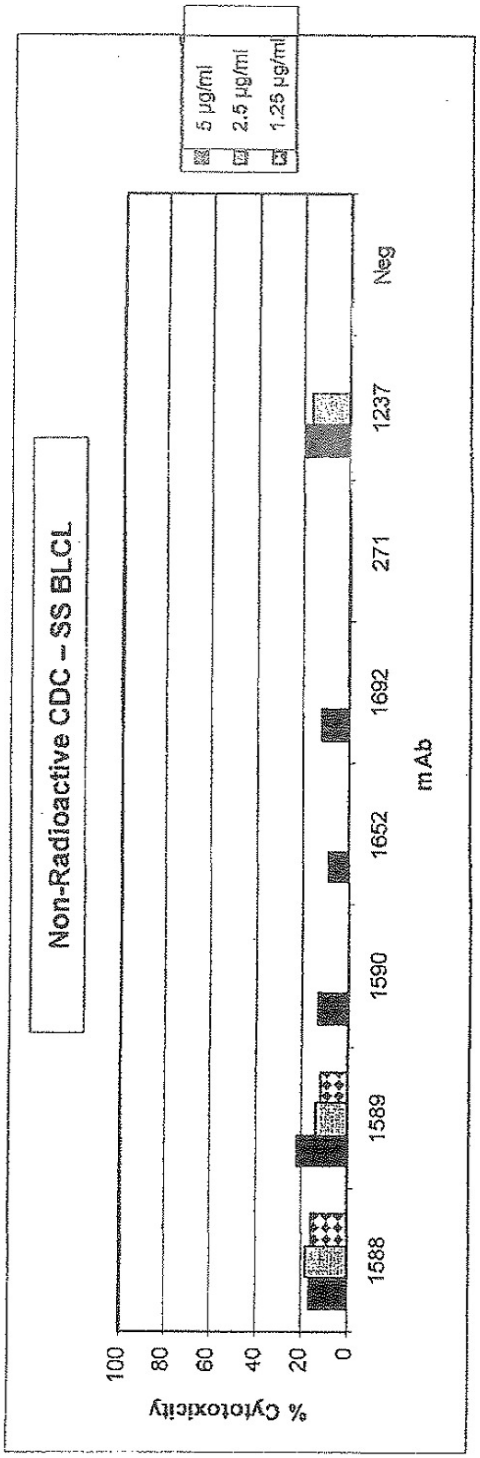


FIG. 19A

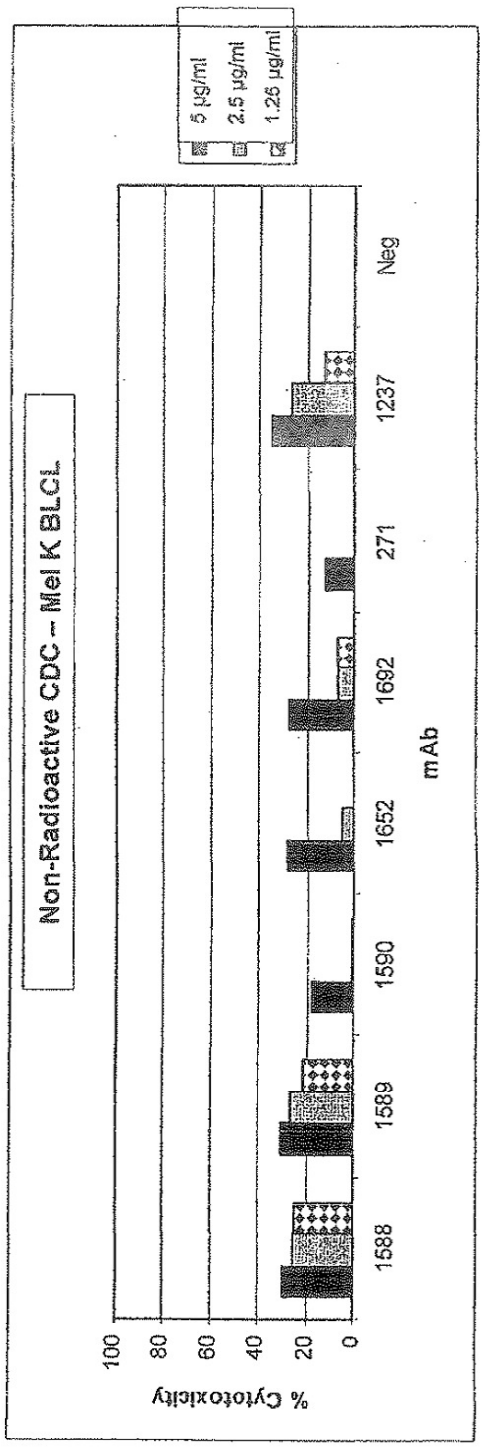


FIG. 19B

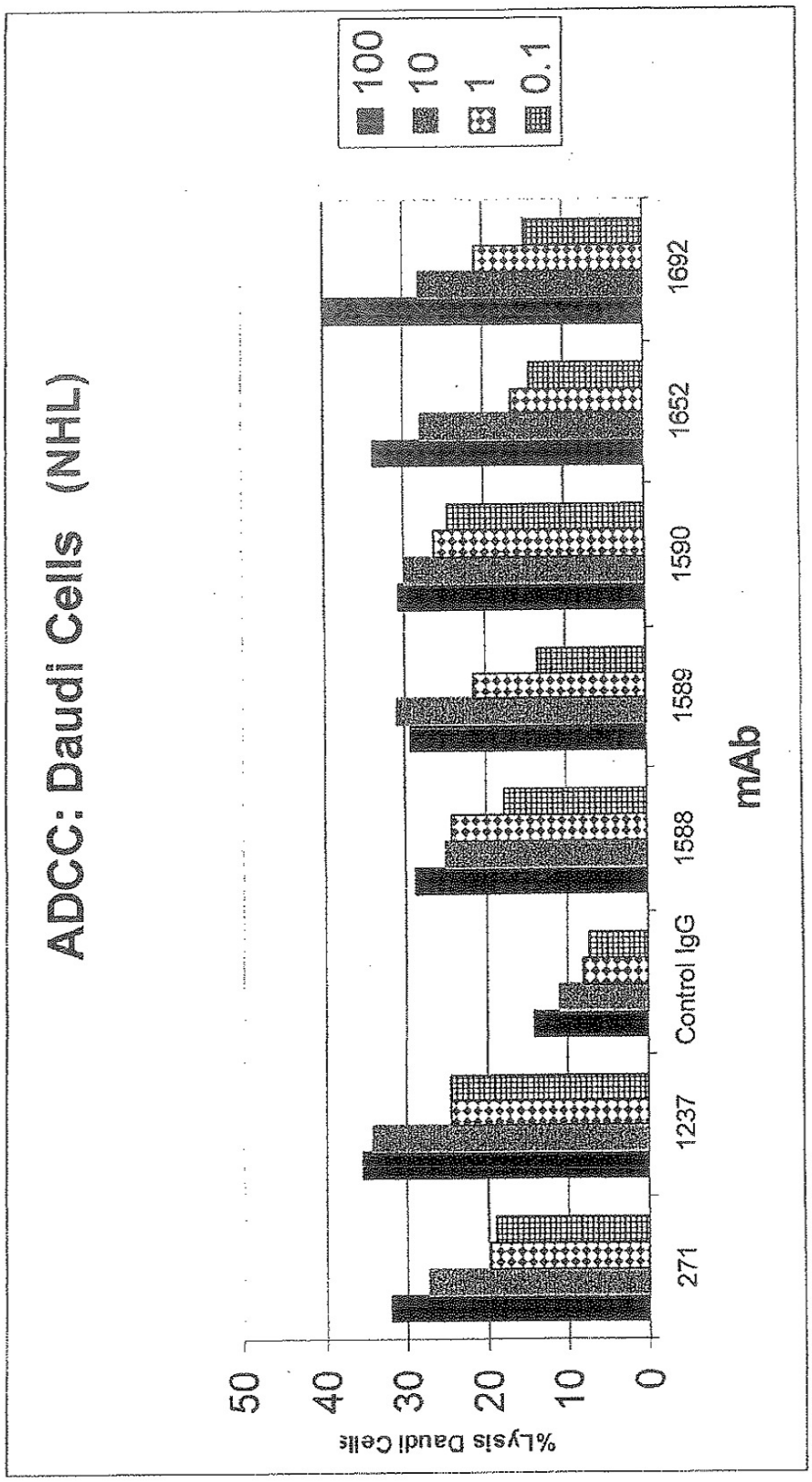

ADCC: Daudi Cells (NHL)
100
10
1
0.1
%Lysis Daudi Cells
0
10
20
30
40
50
271
1237
Control IgG
1588
1589
1590
1652
1692
mAb

FIG. 20

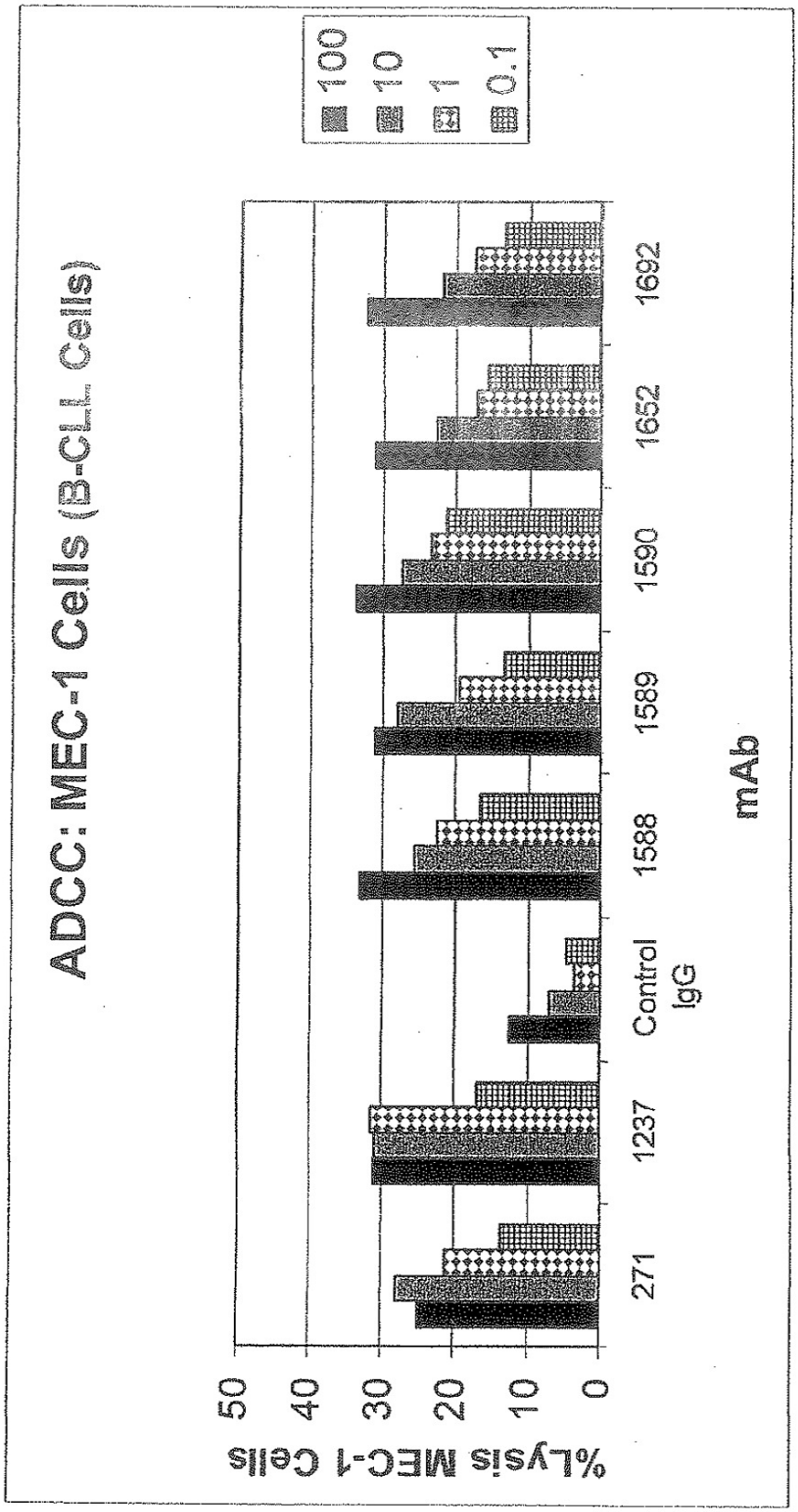

ADCC: MEC-1 Cells (B-CLL Cells)
100
10
1
0.1
%Lysis MEC-1 cells
0
10
20
30
40
50
271
1237
Control IgG
1588
1589
1590
1652
1692
mAb

FIG. 21

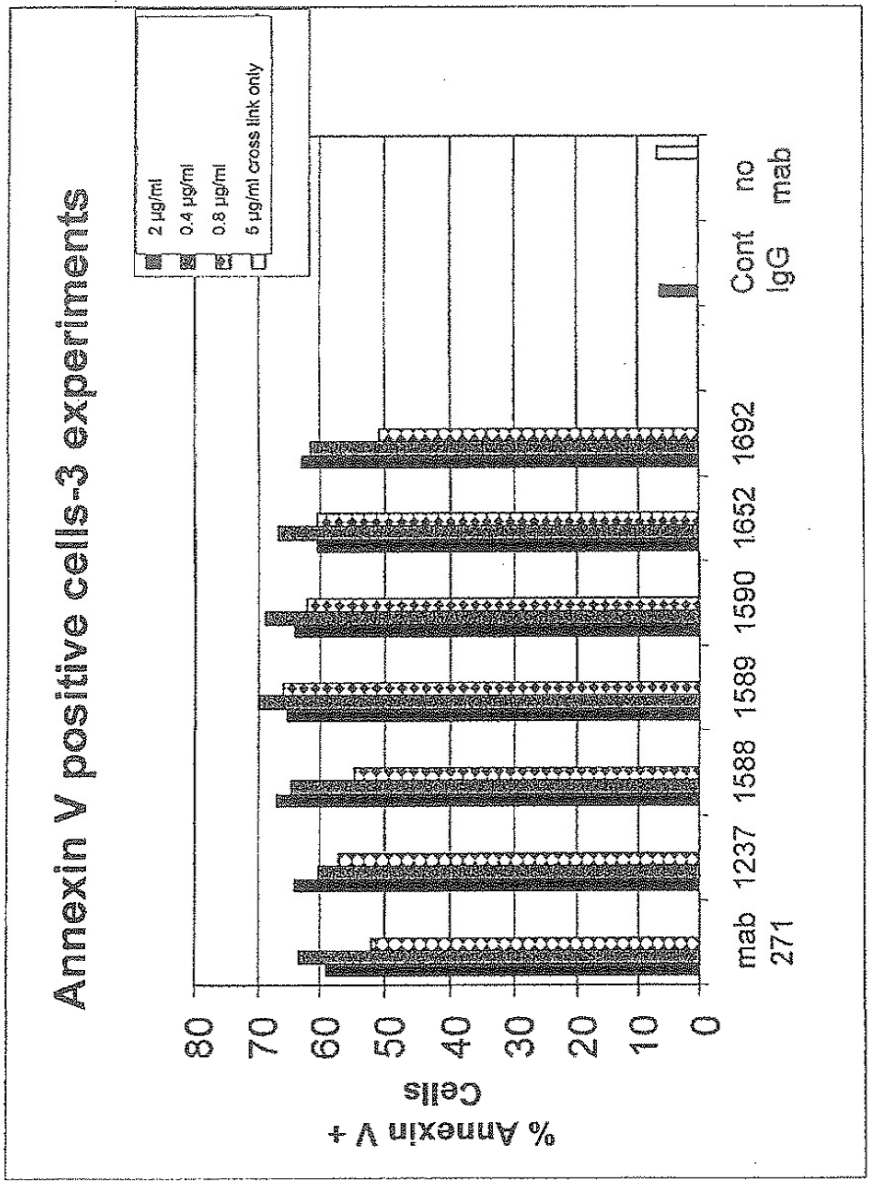

Annexin V positive cells-3 experiments
2 µg/ml
0.4 µg/ml
0.8 µg/ml
5 µg/ml cross link only
% Annexin V + Cells
0
10
20
30
40
50
60
70
80
mab 271
1237
1588
1589
1590
1652
1692
Cont IgG
no mab

FIG. 22

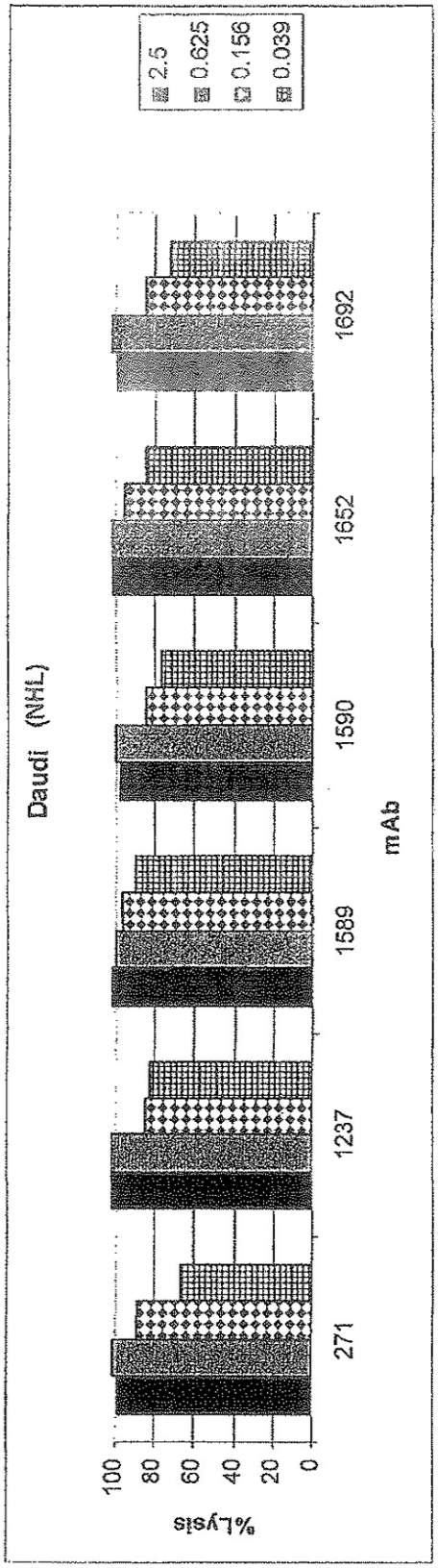

2.5
0.625
0.156
0.039
Daudi (NHL)
%Lysis
100
80
60
40
20
0
271
1237
1589
1590
1652
1692
mAb

FIG. 23

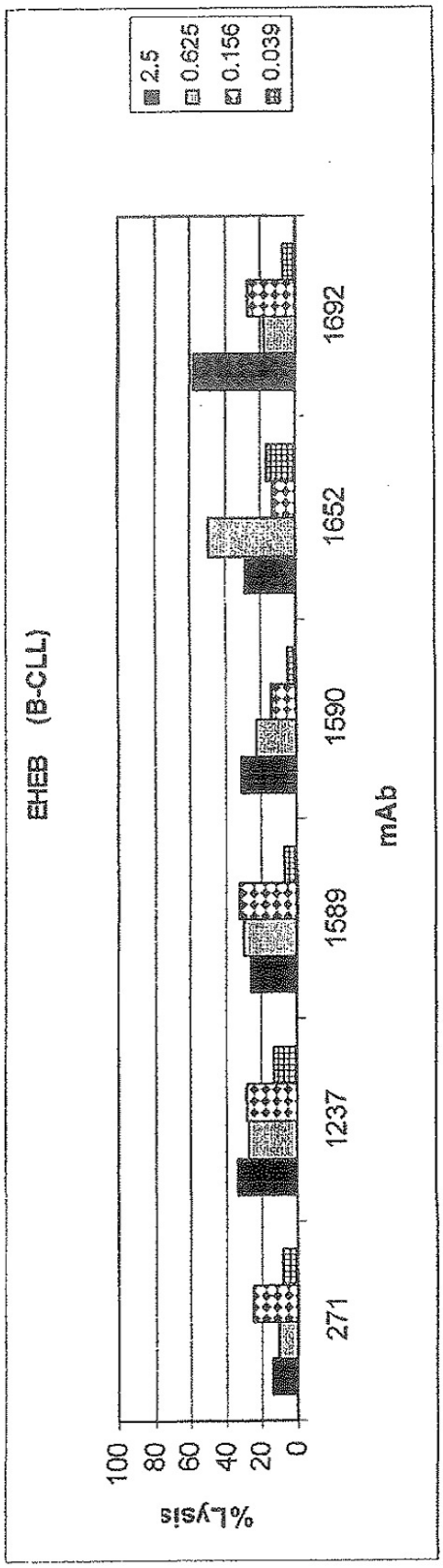


FIG. 24A

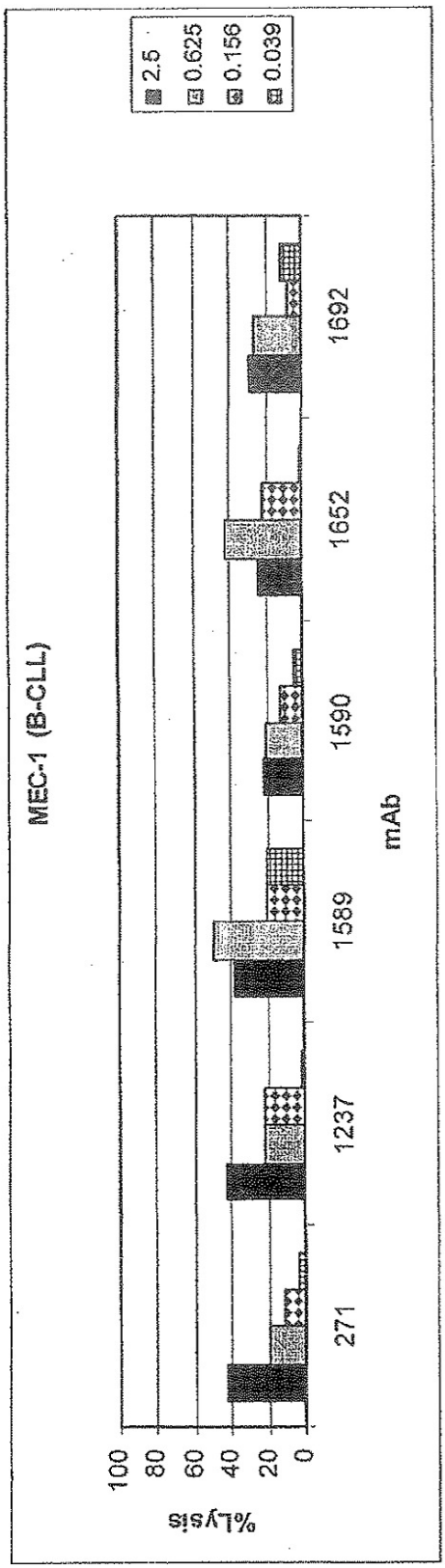


FIG. 24B

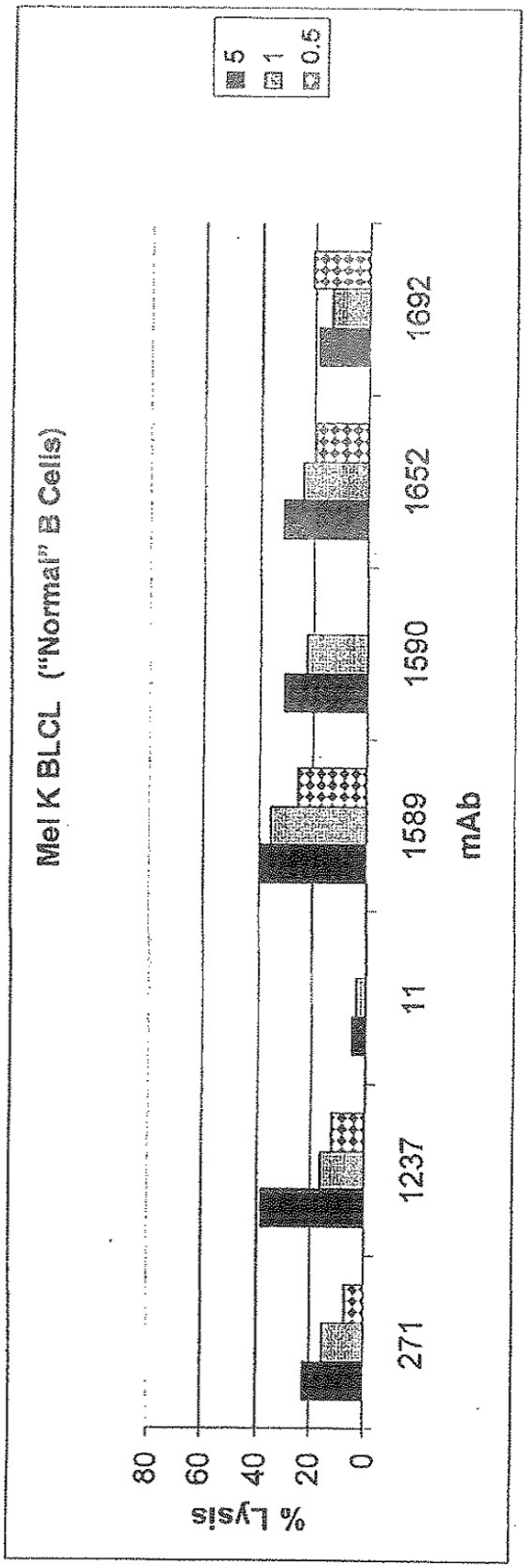

Mel K BLCL ("Normal" B Cells)
5
1
0.5
% Lysis
80
60
40
20
0
271
1237
11
1589
1590
1652
1692
mAb

FIG. 25

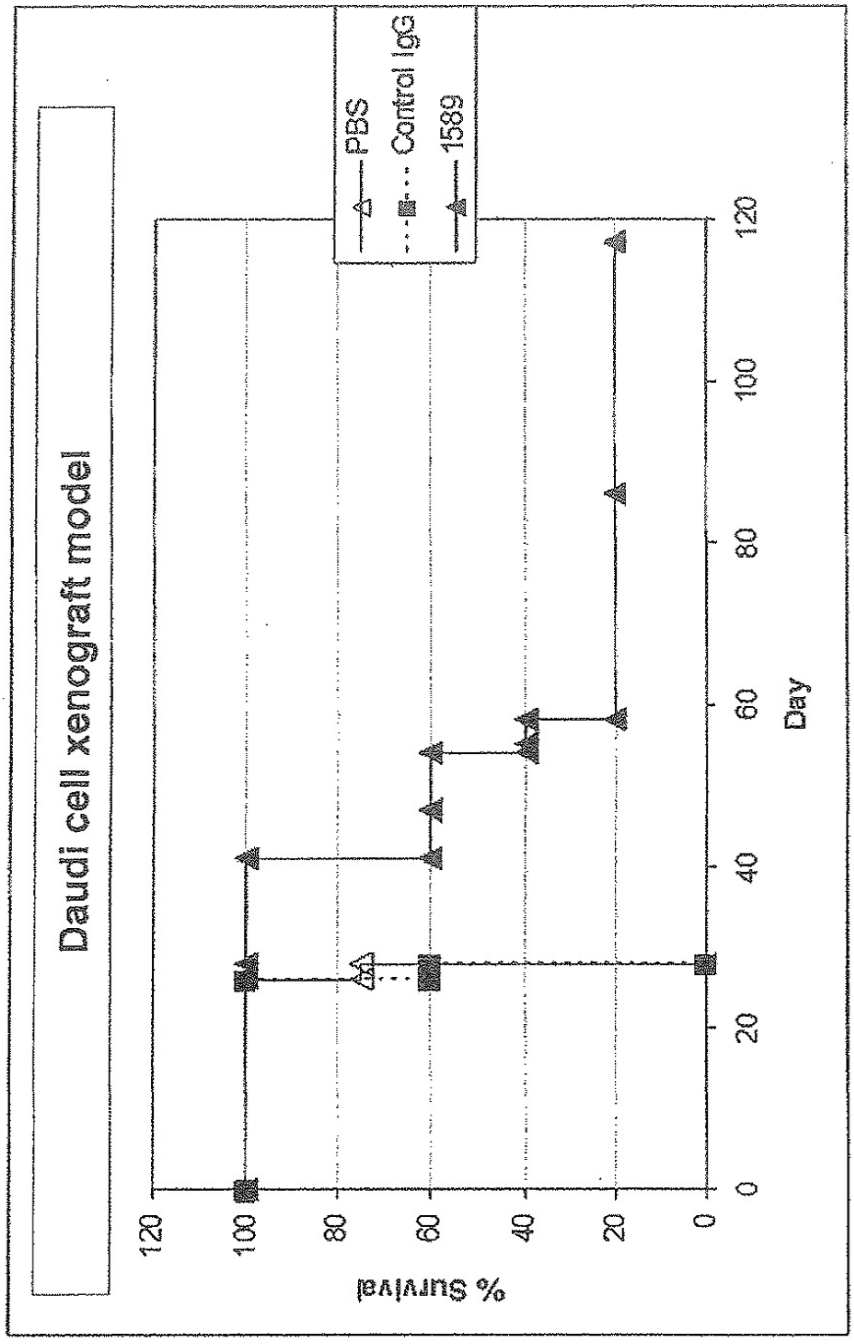

Daudi cell xenograft model
PBS
Control IgG
1589
% Survival
Day
0
20
40
60
80
100
120

FIG. 26