

FIG. 1

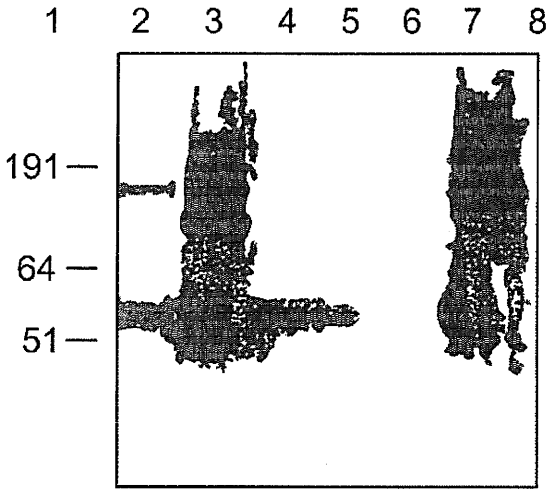


FIG. 2

FIG. 3

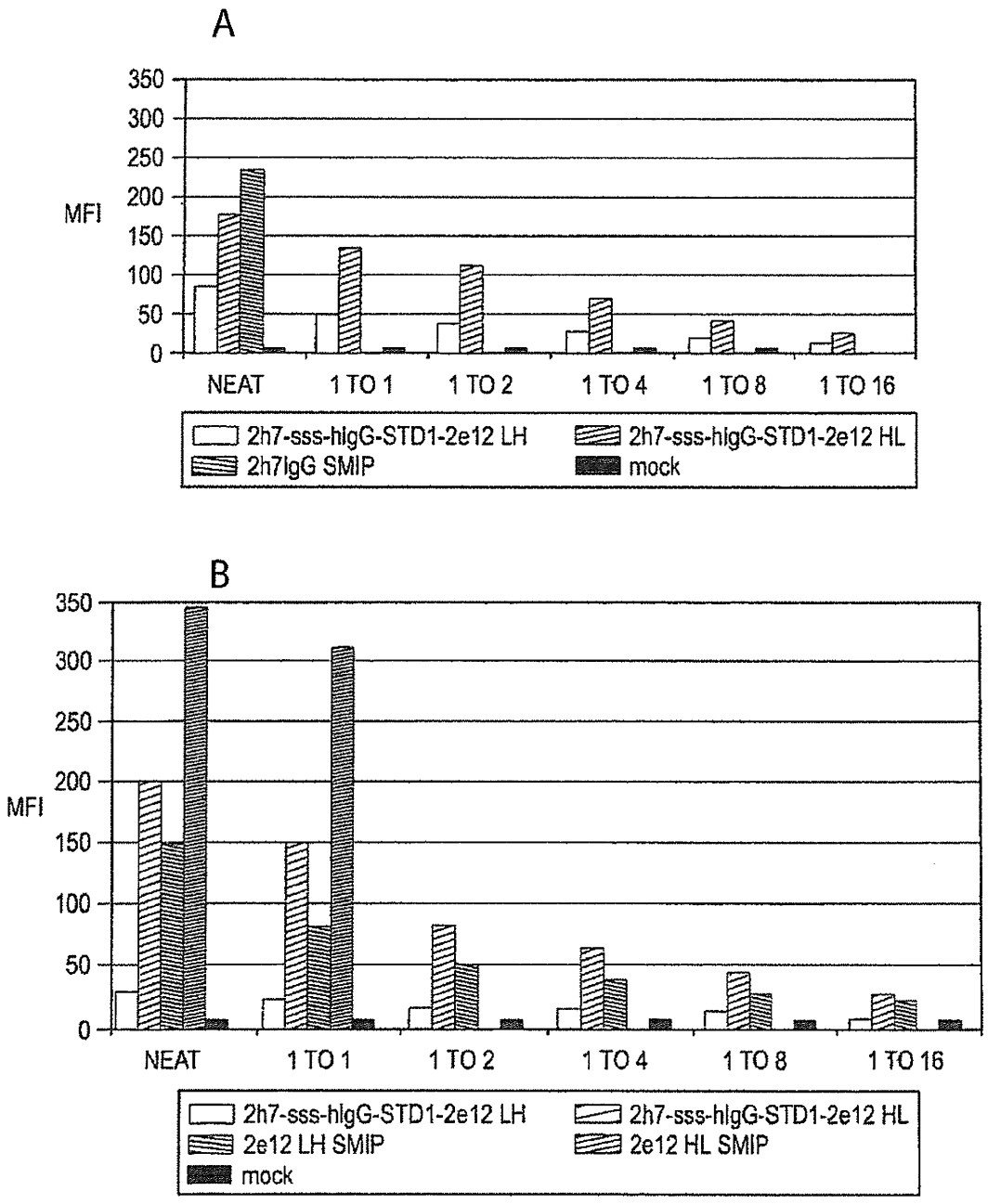

A
MFI
350
300
250
200
150
100
50
0
NEAT
1 TO 1
1 TO 2
1 TO 4
1 TO 8
1 TO 16
2h7-sss-hIgG-STD1-2e12 LH
2h7-sss-hIgG-STD1-2e12 HL
2h7IgG SMIP
mock
B
MFI
350
300
250
200
150
100
50
0
NEAT
1 TO 1
1 TO 2
1 TO 4
1 TO 8
1 TO 16
2h7-sss-hIgG-STD1-2e12 LH
2h7-sss-hIgG-STD1-2e12 HL
2e12 LH SMIP
2e12 HL SMIP
mock

FIG. 4

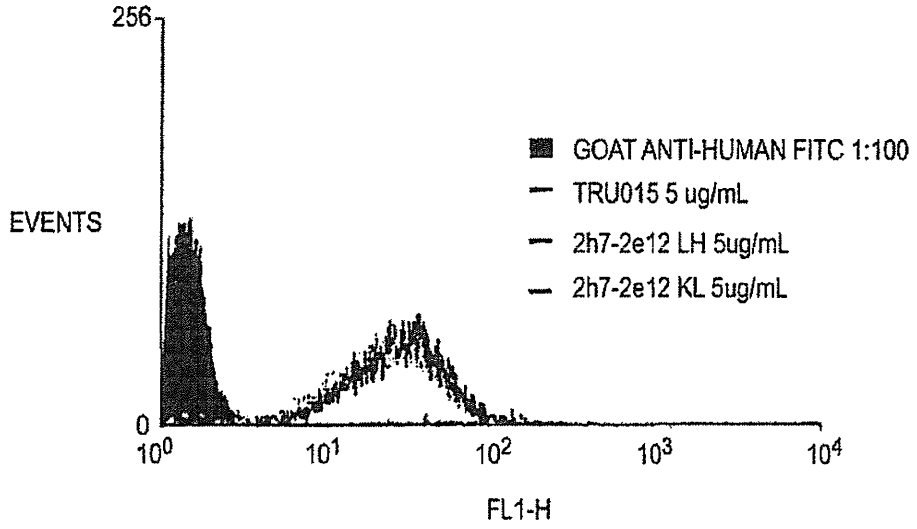

256
EVENTS
0
10^0
10^1
10^2
10^3
10^4
FL1-H
GOAT ANTI-HUMAN FITC 1:100
TRU015 5 ug/mL
2h7-2e12 LH 5ug/mL
2h7-2e12 KL 5ug/mL

FIG. 5

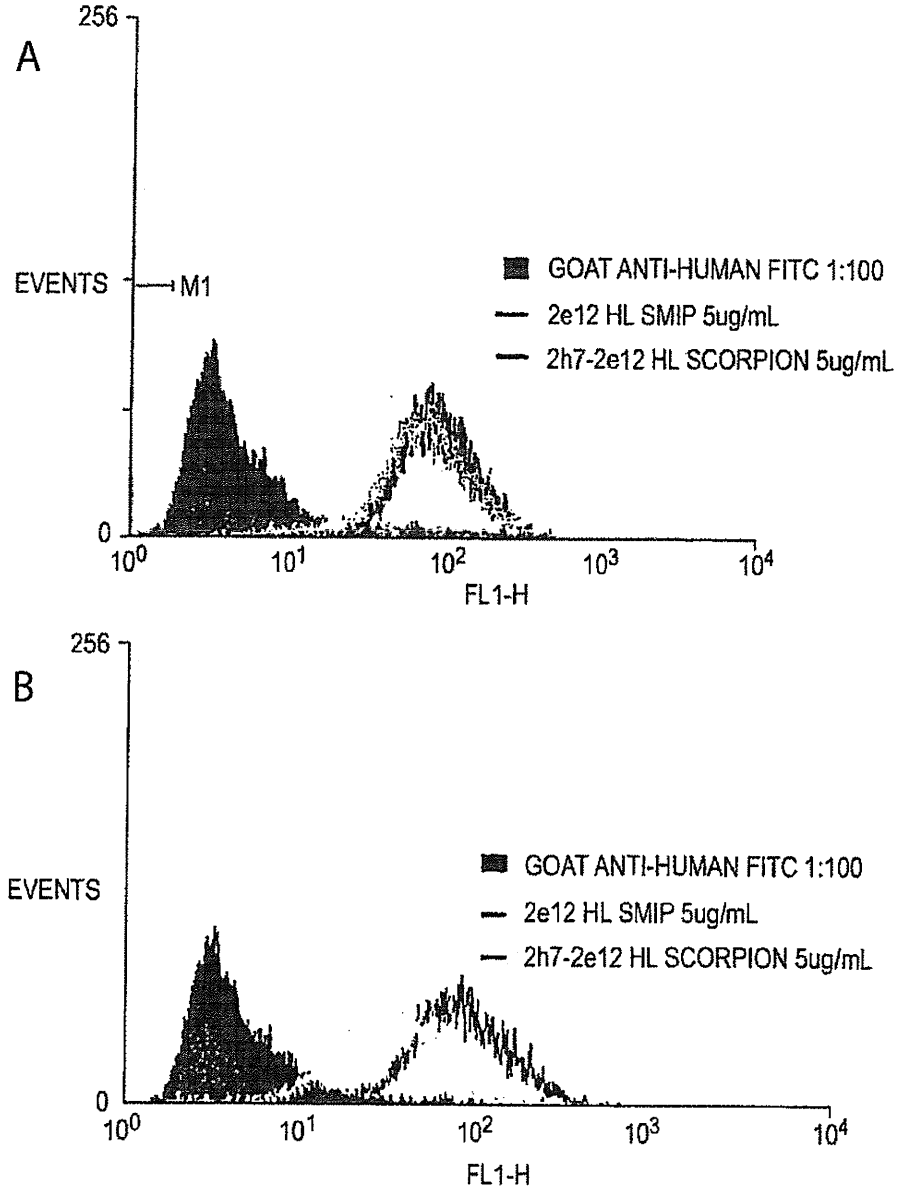

A
256
EVENTS
M1
0
10^0
10^1
10^2
10^3
10^4
FL1-H
GOAT ANTI-HUMAN FITC 1:100
2e12 HL SMIP 5ug/mL
2h7-2e12 HL SCORPION 5ug/mL
B
256
EVENTS
0
10^0
10^1
10^2
10^3
10^4
FL1-H
GOAT ANTI-HUMAN FITC 1:100
2e12 HL SMIP 5ug/mL
2h7-2e12 HL SCORPION 5ug/mL

FIG. 6A



BD1 Binding Domain 1	Linker 1	EFD Effector Function Domain	Linker 2 ID	Linker Length (aa) Linker Plus R. Site	CH3 COOH	Linker 2 Sequence (- restriction site)	Fusion Junction with BD2 (L2 RS--QVQ 2e12)	BD2 Binding Domain 2
2H7 V_L-V_H	Modified hIgGl hinge ccc->sss	hIgGl-CH2-CH3	STD	20	PGK	NYGGGGSGGGGSGGGGSG	NS QVQ	2e12 V_H-V_L
2H7 V_L-V_H	Modified hIgGl hinge ccc->sss	hIgGl-CH2-CH3	STD2	38	PGK	NYGGGGSGGGGSGGGGSG NYGGGGSGGGGSGGGGSG	NS QVQ	2e12 V_H-V_L
2H7 V_L-V_H	Modified hIgGl hinge ccc->sss	hIgGl-CH2-CH3	H1 L1=2e12 V_L-V_H	2 (RS)	PGK	--	NS QVQ	2e12 V_H-V_L
2H7 V_L-V_H	Modified hIgGl hinge ccc->sss	hIgGl-CH2-CH3	H2 L2=2e12 V_L-V_H	8	PGK	GGGGSG	NS QVQ	2e12 V_H-V_L
2H7 V_L-V_H	Modified hIgGl hinge ccc->sss	hIgGl-CH2-CH3	H3 L3=2e12 V_L-V_H	10	PGK	NYGGGGSG	NS QVQ	2e12 V_H-V_L
2H7 V_L-V_H	Modified hIgGl hinge ccc->sss	hIgGl-CH2-CH3	H4 L4=2e12 V_L-V_H	13	PGK	GGGGSGGGGSG	NS QVQ	2e12 V_H-V_L
2H7 V_L-V_H	Modified hIgGl hinge ccc->sss	hIgGl-CH2-CH3	H5 L5=2e12 V_L-V_H	15	PGK	NYGGGGSGGGGSG	NS QVQ	2e12 V_H-V_L
2H7 V_L-V_H	Modified hIgGl hinge ccc->sss	hIgGl-CH2-CH3	H6 L6=2e12 V_L-V_H	18	PGK	GGGGSGGGGSGGGGSG	NS QVQ	2e12 V_H-V_L
2H7 V_L-V_H	Modified hIgGl hinge ccc->sss	hIgGl-CH2-CH3	H7	8	PGK	GCPPCP	NS QVQ	2e12 V_H-V_L

FIG. 6B (1 of 5)

Constructs

Name	BD1	Hinge	EFD	Linker	BD2
2H7-sss-hIgG-STD1-2e12 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	STD1	2e12 (VL-VH)
2H7-sss-hIgG-STD1-2e12 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	STD1	2e12 (VH-VL)
2H7-sss-hIgG-STD2-2e12 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	STD2	2e12 (VL-VH)
2H7-sss-hIgG-STD2-2e12 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	STD2	2e12 (VH-VL)
2H7-csc-hIgG-STD1-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	STD1	2e12 (VL-VH)
2H7-csc-hIgG-STD2-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	STD2	2e12 (VL-VH)
2H7-csc-hIgG-STD1-2e12 HL	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	STD1	2e12 (VL-VH)
2H7-csc-hIgG-STD2-2e12 HL	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	STD2	2e12 (VH-VL)
2H7-sss-hIgG-H1-2e12 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H1	2e12 (VL-VH)
2H7-csc-hIgG-H1-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H1	2e12 (VL-VH)
2H7-sss-hIgG-H1-2e12 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H1	2e12 (VH-VL)
2H7-csc-hIgG-H1-2e12 HL	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H1	2e12 (VH-VL)
2H7-sss-hIgG-H2-2e12 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H2	2e12 (VH-VL)
2H7-csc-hIgG-H2-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H2	2e12 (VL-VH)
2H7-sss-hIgG-H2-2e12 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H2	2e12 (VH-VL)
2H7-csc-hIgG-H2-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H2	2e12 (VH-VL)
2H7-sss-hIgG-H3-2e12 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H3	2e12 (VL-VH)
2H7-csc-hIgG-H3-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H3	2e12 (VL-VH)
2H7-sss-hIgG-H3-2e12 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H3	2e12 (VH-VL)
2H7-csc-hIgG-H3-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H3	2e12 (VH-VL)
2H7-sss-hIgG-H4-2e12 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H4	2e12 (VL-VH)
2H7-csc-hIgG-H4-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H4	2e12 (VL-VH)
2H7-sss-hIgG-H4-2e12 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H4	2e12 (VH-VL)
2H7-csc-hIgG-H4-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H4	2e12 (VH-VL)

FIG. 6B (2 of 5)

Name	BD1	Hinge	EFD	Linker	BD2
2H7-sss-hIgG-H5-2e12 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H5	2e12 (VL-VH)
2H7-csc-hIgG-H5-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H5	2e12 (VL-VH)
2H7-sss-hIgG-H5-2e12 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H5	2e12 (VH-VL)
2H7-csc-hIgG-H5-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H5	2e12 (VH-VL)
2H7-sss-hIgG-H6-2e12 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H6	2e12 (VL-VH)
2H7-csc-hIgG-H6-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H6	2e12 (VL-VH)
2H7-sss-hIgG-H6-2e12 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H6	2e12 (VH-VL)
2H7-csc-hIgG-H6-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H6	2e12 (VH-VL)
2H7-sss-hIgG-H7-2e12 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H7	2e12 (VL-VH)
2H7-csc-hIgG-H7-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H7	2e12 (VL-VH)
2H7-sss-hIgG-H7-2e12 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H7	2e12 (VH-VL)
2H7-csc-hIgG-H7-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H7	2e12 (VH-VL)
2H7-sss-hIgG-STD1-G28-1 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	STD1	G28-1 (VL-VH)
2H7-sss-hIgG-STD1-G28-1 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	STD1	G28-1 (VL-VH)
2H7-sss-hIgG-STD2-G28-1 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	STD2	G28-1 (VL-VH)
2H7-sss-hIgG-STD2-G28-1 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	STD2	G28-1 (VH-VL)
2H7-csc-hIgG-STD1-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	STD1	G28-1 (VL-VH)
2H7-csc-hIgG-STD2-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	STD2	G28-1 (VH-VL)
2H7-csc-hIgG-STD1-G28-1 HL	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	STD1	G28-1 (VL-VH)
2H7-csc-hIgG-STD2-G28-1 HL	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	STD2	G28-1 (VH-VL)
2H7-sss-hIgG-H1-G28-1 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H1	G28-1 (VL-VH)
2H7-csc-hIgG-H1-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H1	G28-1 (VH-VL)
2H7-sss-hIgG-H1-G28-1 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H1	G28-1 (VL-VH)
2H7-csc-hIgG-H1-G28-1 HL	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H1	G28-1 (VH-VL)
2H7-sss-hIgG-H2-G28-1 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H2	G28-1 (VL-VH)

FIG. 6B (3 of 5)

Name	*BD1*	*Hinge*	*EFD*	*Linker*	*BD2*
2H7-csc-hIgG-H2-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H2	G28-1 (VH-VL)
2H7-sss-hIgG-H2-G28-1 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H2	G28-1 (VL-VH)
2H7-csc-hIgG-H2-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H2	G28-1 (VH-VL)
2H7-sss-hIgG-H3-G28-1 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H3	G28-1 (VL-VH)
2H7-csc-hIgG-H3-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H3	G28-1 (VH-VL)
2H7-sss-hIgG-H3-G28-1 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H3	G28-1 (VL-VH)
2H7-csc-hIgG-H3-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H3	G28-1 (VH-VL)
2H7-sss-hIgG-H4-G28-1 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H4	G28-1 (VL-VH)
2H7-csc-hIgG-H4-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H4	G28-1 (VH-VL)
2H7-sss-hIgG-H4-G28-1 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H4	G28-1 (VL-VH)
2H7-csc-hIgG-H4-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H4	G28-1 (VH-VL)
2H7-sss-hIgG-H5-G28-1 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H5	G28-1 (VH-VL)
2H7-sss-hIgG-H5-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H5	G28-1 (VH-VL)
2H7-sss-hIgG-H5-G28-1 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H5	G28-1 (VH-VL)
2H7-csc-hIgG-H5-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H5	G28-1 (VH-VL)
2H7-sss-hIgG-H6-G28-1 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H6	G28-1 (VL-VH)
2H7-csc-hIgG-H6-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H6	G28-1 (VL-VH)
2H7-sss-hIgG-H6-G28-1 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H6	G28-1 (VH-VL)
2H7-csc-hIgG-H6-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H6	G28-1 (VH-VL)
2H7-sss-hIgG-H7-G28-1 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H7	G28-1 (VL-VH)
2H7-csc-hIgG-H7-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H7	G28-1 (VL-VH)
2H7-sss-hIgG-H7-G28-1 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H7	G28-1 (VH-VL)
2H7-csc-hIgG-H7-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H7	G28-1 (VH-VL)
2H7-sss-hIgG-STD1-G19-4 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	STD1	G19-4 (VL-VH)
2H7-sss-hIgG-STD1-G19-4 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	STD1	G19-4 (VH-VL)

FIG. 6B (4 of 5)

Name	BD1	Hinge	EFD	Linker	BD2
2H7-sss-hIgG-STD2-G19-4 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	STD2	G19-4 (VL-VH)
2H7-sss-hIgG-STD2-G19-4 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	STD2	G19-4 (VH-VL)
2H7-csc-hIgG-STD1-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	STD1	G19-4 (VL-VH)
2H7-csc-hIgG-STD2-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	STD2	G19-4 (VH-VL)
2H7-csc-hIgG-STD1-G19-4 HL	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	STD1	G19-4 (VL-VH)
2H7-csc-hIgG-STD2-G19-4 HL	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	STD2	G19-4 (VH-VL)
2H7-sss-hIgG-H1-G19-4 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H1	G19-4 (VL-VH)
2H7-csc-hIgG-H1-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H1	G19-4 (VH-VL)
2H7-sss-hIgG-H1-G19-4 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H1	G19-4 (VL-VH)
2H7-csc-hIgG-H1-G19-4 HL	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H1	G19-4 (VH-VL)
2H7-sss-hIgG-H2-G19-4 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H2	G19-4 (VL-VH)
2H7-csc-hIgG-H2-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H2	G19-4 (VL-VH)
2H7-sss-hIgG-H2-G19-4 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H2	G19-4 (VL-VH)
2H7-csc-hIgG-H2-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H2	G19-4 (VL-VH)
2H7-sss-hIgG-H3-G19-4 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H3	G19-4 (VL-VH)
2H7-csc-hIgG-H3-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H3	G19-4 (VH-VL)
2H7-sss-hIgG-H3-G19-4 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H3	G19-4 (VL-VH)
2H7-csc-hIgG-H3-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H3	G19-4 (VH-VL)
2H7-sss-hIgG-H4-G19-4 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H4	G19-4 (VL-VH)
2H7-csc-hIgG-H4-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H4	G19-4 (VH-VL)
2H7-sss-hIgG-H4-G19-4 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H4	G19-4 (VL-VH)
2H7-csc-hIgG-H4-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H4	G19-4 (VH-VL)
2H7-sss-hIgG-H5-G19-4 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H5	G19-4 (VL-VH)
2H7-csc-hIgG-H5-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H5	G19-4 (VH-VL)
2H7-sss-hIgG-H5-G19-4 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H5	G19-4 (VL-VH)

FIG. 6B (5 of 5)

Name	BD1	Hinge	EFD	Linker	BD2
2H7-csc-hIgG-H5-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H5	G19-4 (VH-VL)
2H7-sss-hIgG-H6-G19-4 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H6	G19-4 (VL-VH)
2H7-csc-hIgG-H6-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H6	G19-4 (VL-VH)
2H7-sss-hIgG-H6-G19-4 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H6	G19-4 (VH-VL)
2H7-csc-hIgG-H6-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H6	G19-4 (VH-VL)
2H7-sss-hIgG-H7-G19-4 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H7	G19-4 (VL-VH)
2H7-csc-hIgG-H7-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H7	G19-4 (VL-VH)
2H7-sss-hIgG-H7-G19-4 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H7	G19-4 (VH-VL)
2H7-csc-hIgG-H7-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H7	G19-4 (VH-VL)

FIG. 7

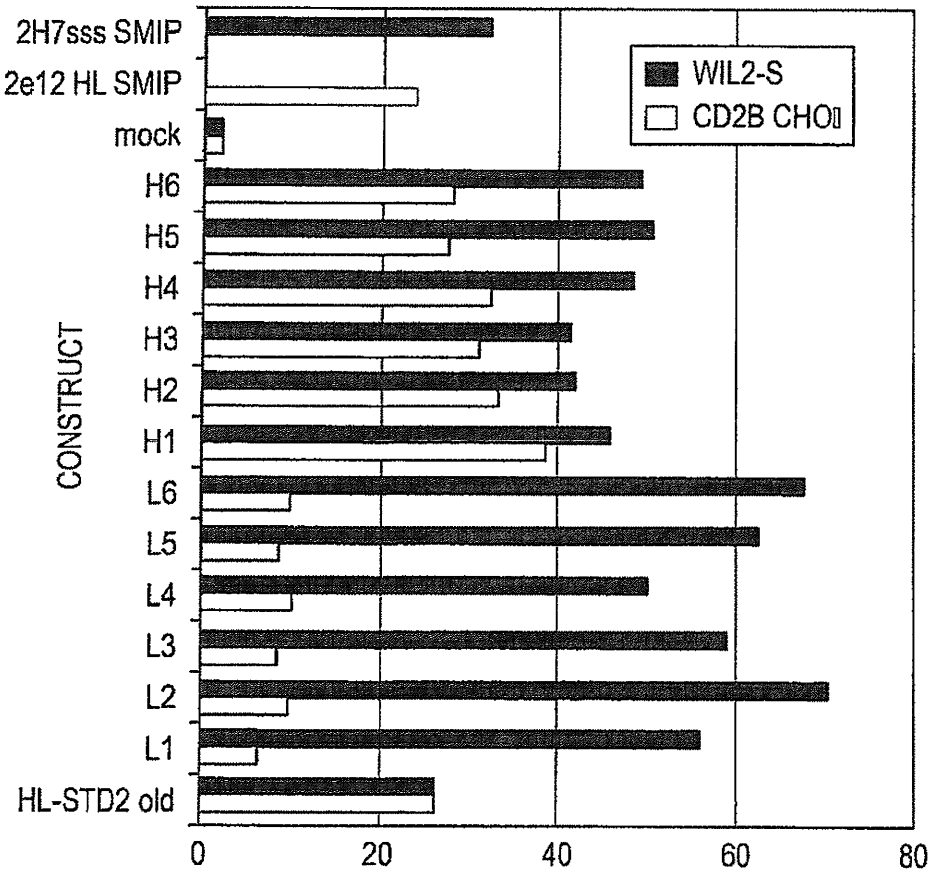

2H7sss SMIP
2e12 HL SMIP
mock
H6
H5
H4
H3
H2
H1
L6
L5
L4
L3
L2
L1
HL-STD2 old
CONSTRUCT
WIL2-S
CD2B CHO
0
20
40
60
80

FIG. 8

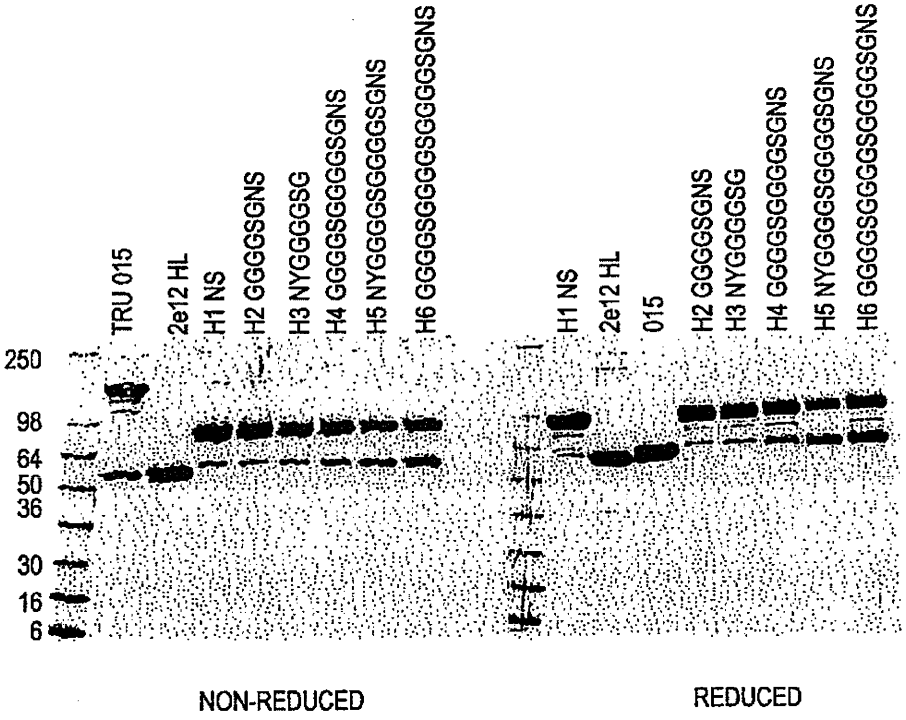

TRU 015
2e12 HL
H1 NS
H2 GGGGSGNS
H3 NYGGGGSG
H4 GGGGSGGGGSGNS
H5 NYGGGGSGGGGSGNS
H6 GGGGSGGGGSGGGGSGNS
H1 NS
2e12 HL
015
H2 GGGGSGNS
H3 NYGGGGSG
H4 GGGGSGGGGSGNS
H5 NYGGGGSGGGGSGNS
H6 GGGGSGGGGSGGGGSGNS
250
98
64
50
36
30
16
6
NON-REDUCED
REDUCED

FIG. 9

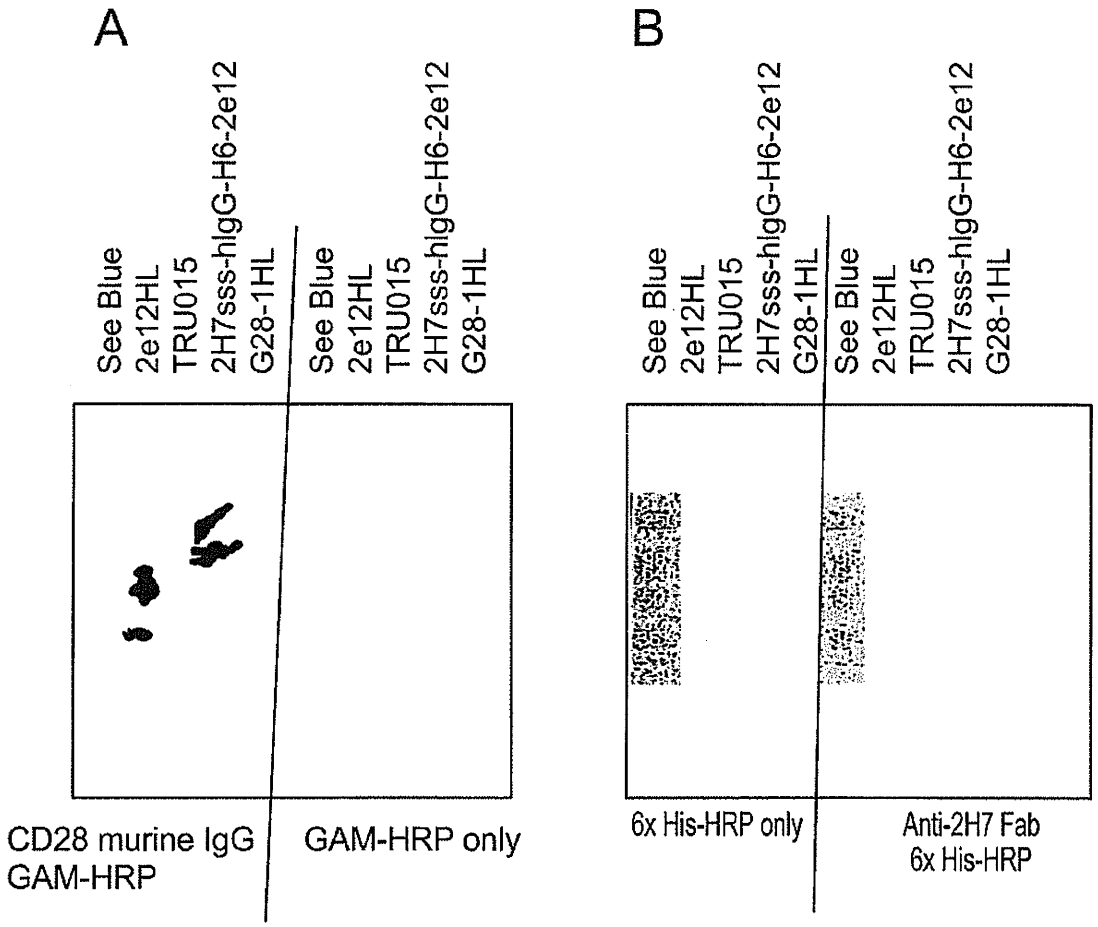

A
See Blue
2e12HL
TRU015
2H7sss-hlgG-H6-2e12
G28-1HL
See Blue
2e12HL
TRU015
2H7sss-hlgG-H6-2e12
G28-1HL
CD28 murine IgG
GAM-HRP
GAM-HRP only
B
See Blue
2e12HL
TRU015
2H7sss-hlgG-H6-2e12
G28-1HL
See Blue
2e12HL
TRU015
2H7sss-hlgG-H6-2e12
G28-1HL
6x His-HRP only
Anti-2H7 Fab
6x His-HRP

FIG. 10

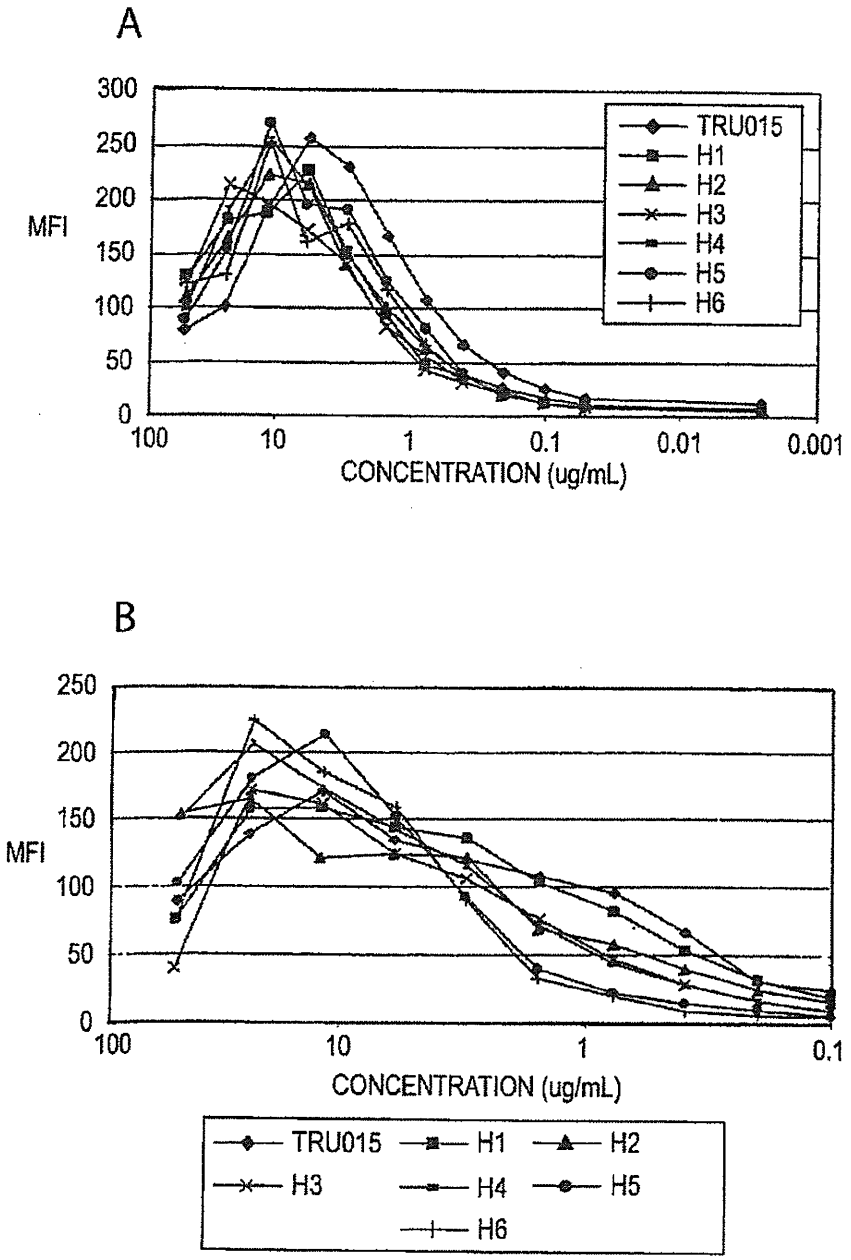

A
300
250
200
150
100
50
0
MFI
100
10
1
0.1
0.01
0.001
CONCENTRATION (ug/mL)
TRU015
H1
H2
H3
H4
H5
H6
B
250
200
150
100
50
0
MFI
100
10
1
0.1
CONCENTRATION (ug/mL)
TRU015
H1
H2
H3
H4
H5
H6

FIG. 11

Linker ID	Retention Time for POI	% POI	% Other Forms	SDS-PAGE Analysis of Cleavage at EFD/BD2 junction
H1	7.620	70.9	29.0	(Yes)
H2	7.589	67.5	32.5	(Yes)
H3	7.605	68.7	31.4	Yes
H4	7.622 (shoulder)	68.0	32.0	Yes
H5	7.933/7.680 (doublet with shoulder)	32.42/33.94	33.63	YES
H6	7.901/7.69 (doublet with shoulder)	34.5/29.3	36.1	YES
H7	7.788	84.2	15.8 (HMW)	NO

FIG. 12

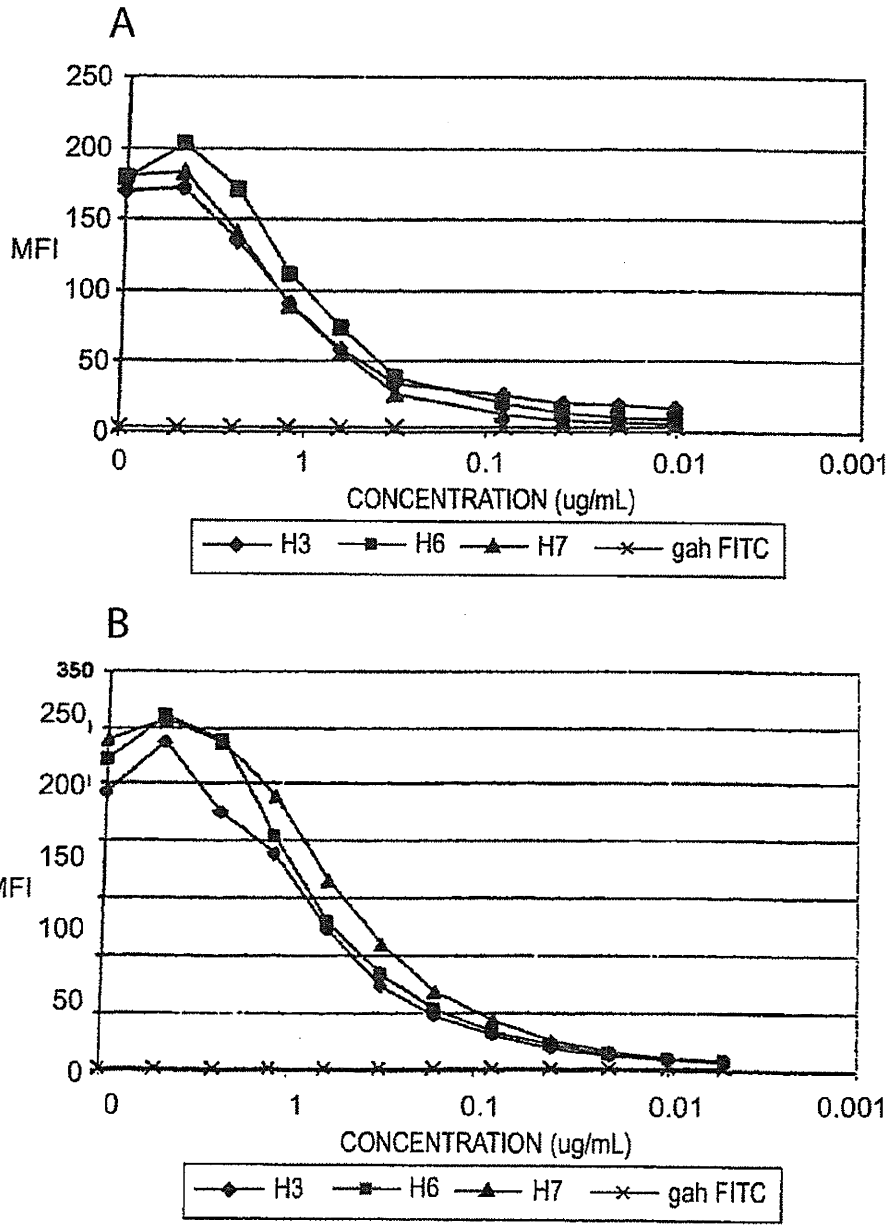

A
250
200
150
MFI
100
50
0
0
1
0.1
0.01
0.001
CONCENTRATION (ug/mL)
H3
H6
H7
gah FITC
B
350
250
200
150
MFI
100
50
0
0
1
0.1
0.01
0.001
CONCENTRATION (ug/mL)
H3
H6
H7
gah FITC

FIG. 13

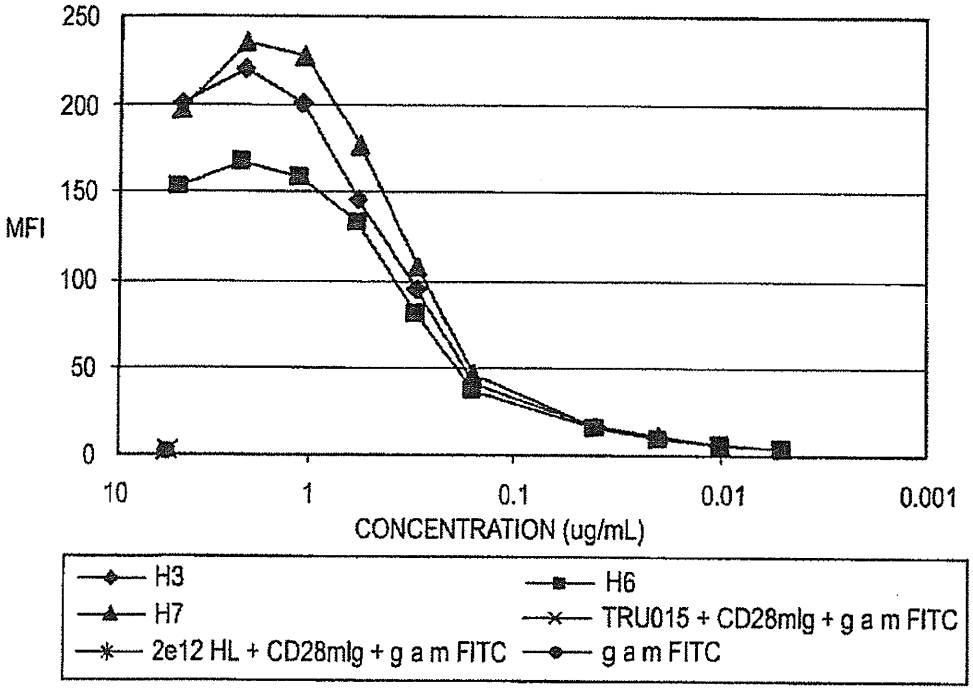

250
200
150
100
50
0
MFI
10
1
0.1
0.01
0.001
CONCENTRATION (ug/mL)
H3
H6
H7
TRU015 + CD28mIg + g a m FITC
2e12 HL + CD28mIg + g a m FITC
g a m FITC

FIG. 14

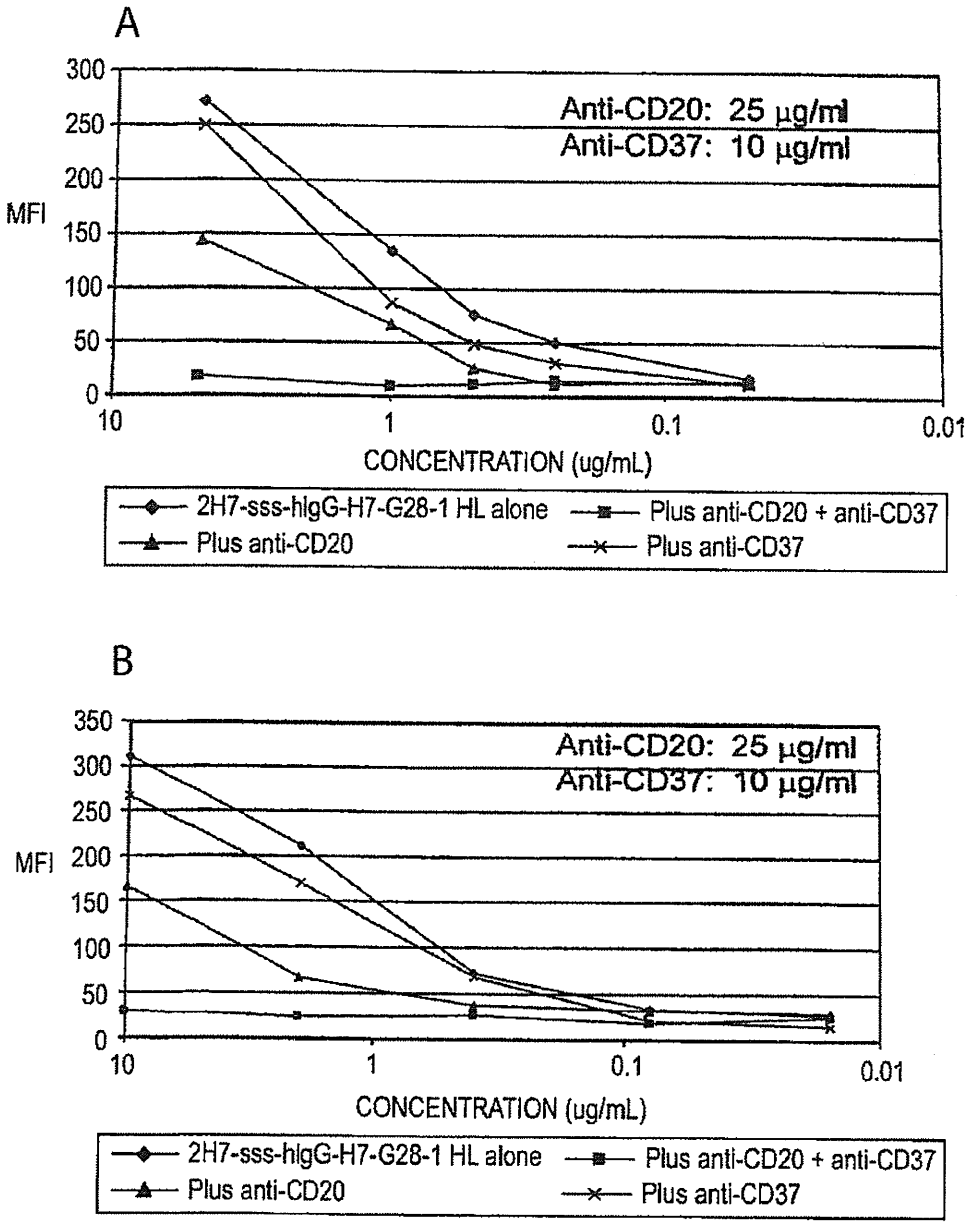

A
300
250
200
150
100
50
0
MFI
Anti-CD20: 25 µg/ml
Anti-CD37: 10 µg/ml
10
1
0.1
0.01
CONCENTRATION (ug/mL)
2H7-sss-hIgG-H7-G28-1 HL alone
Plus anti-CD20 + anti-CD37
Plus anti-CD20
Plus anti-CD37
B
350
300
250
200
150
100
50
0
MFI
Anti-CD20: 25 µg/ml
Anti-CD37: 10 µg/ml
10
1
0.1
0.01
CONCENTRATION (ug/mL)
2H7-sss-hIgG-H7-G28-1 HL alone
Plus anti-CD20 + anti-CD37
Plus anti-CD20
Plus anti-CD37

FIG. 15

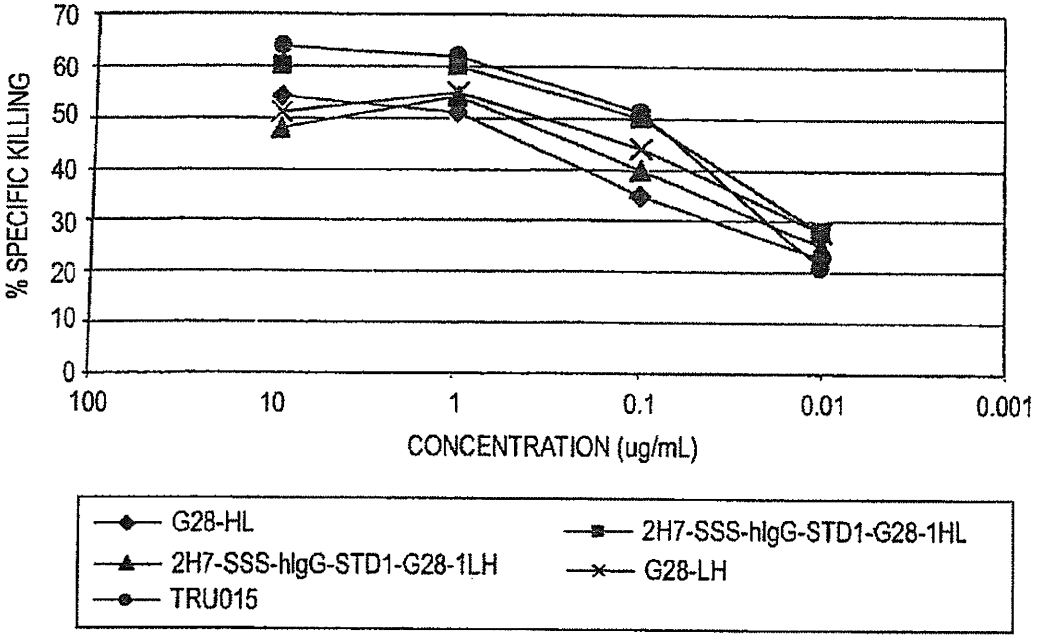

% SPECIFIC KILLING
70
60
50
40
30
20
10
0
100
10
1
0.1
0.01
0.001
CONCENTRATION (ug/mL)
G28-HL
2H7-SSS-hIgG-STD1-G28-1HL
2H7-SSS-hIgG-STD1-G28-1LH
G28-LH
TRU015

FIG. 16

24 Hours	CD3+	CD19+	CD40+
Media	77%	8%	7%
TRU015	76%	8.5%	7.9%
G28-1 SMIP	78%	8. 9%	7.8%
TRU015+G28-1 SMIP	76.3%	8.1%	7.5%
2H7-sshIgG1-G28-1HL (H7)	78.4%	8.9%	7.9%

72 Hours	CD3+	CD19+	CD40+
Media	78.5%	7.9%	7.7%
TRU015	85.2%	2.3%	2.7%
G28-1 SMIP	86.7%	2.2%	2.3%
TRU015+G28-1 SMIP	87.1%	1.1%	1.1%
2H7-sshIgG1-G28-1 HL (H7)	96.7%	.08%	.77%

FIG. 17

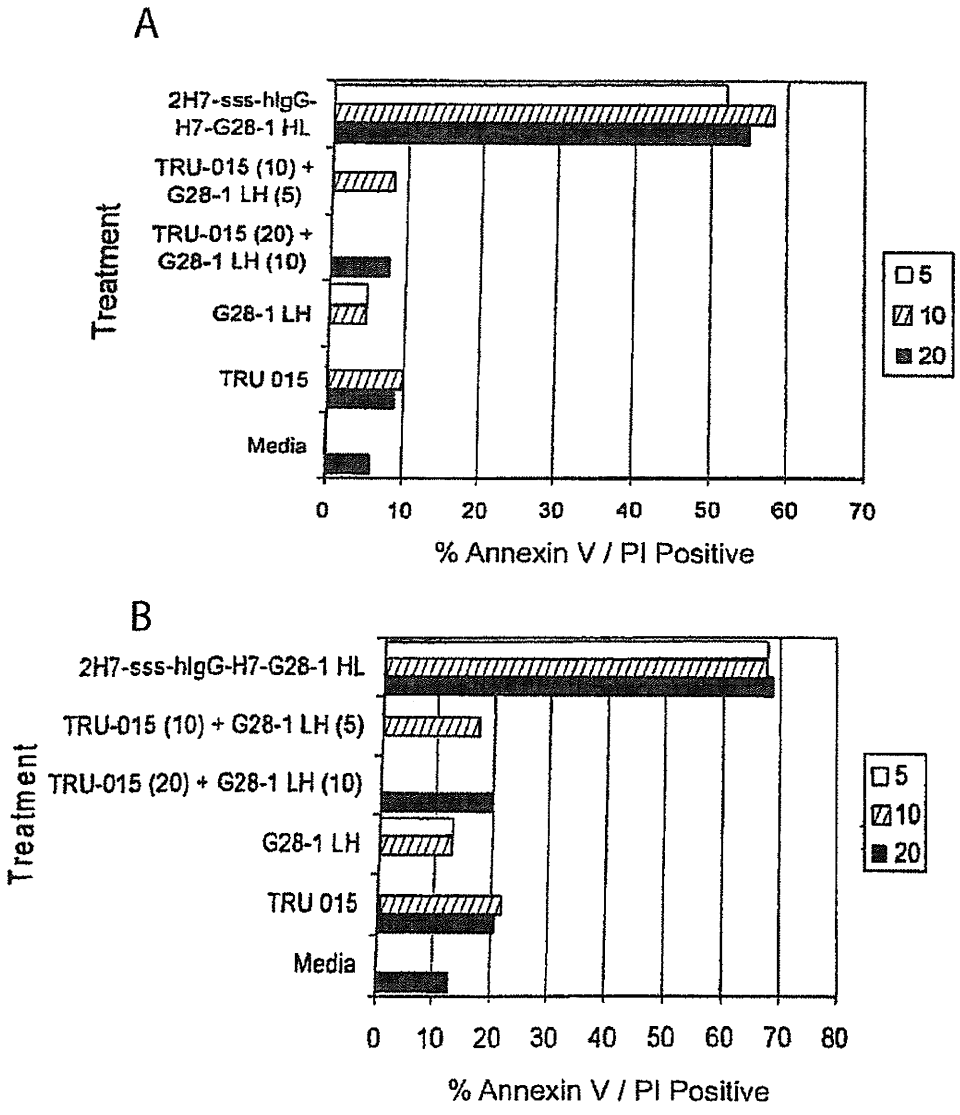

A
2H7-sss-hIgG-H7-G28-1 HL
TRU-015 (10) + G28-1 LH (5)
TRU-015 (20) + G28-1 LH (10)
G28-1 LH
TRU 015
Media
Treatment
0 10 20 30 40 50 60 70
% Annexin V / PI Positive
5
10
20
B
2H7-sss-hIgG-H7-G28-1 HL
TRU-015 (10) + G28-1 LH (5)
TRU-015 (20) + G28-1 LH (10)
G28-1 LH
TRU 015
Media
Treatment
0 10 20 30 40 50 60 70 80
% Annexin V / PI Positive
5
10
20

FIG. 18

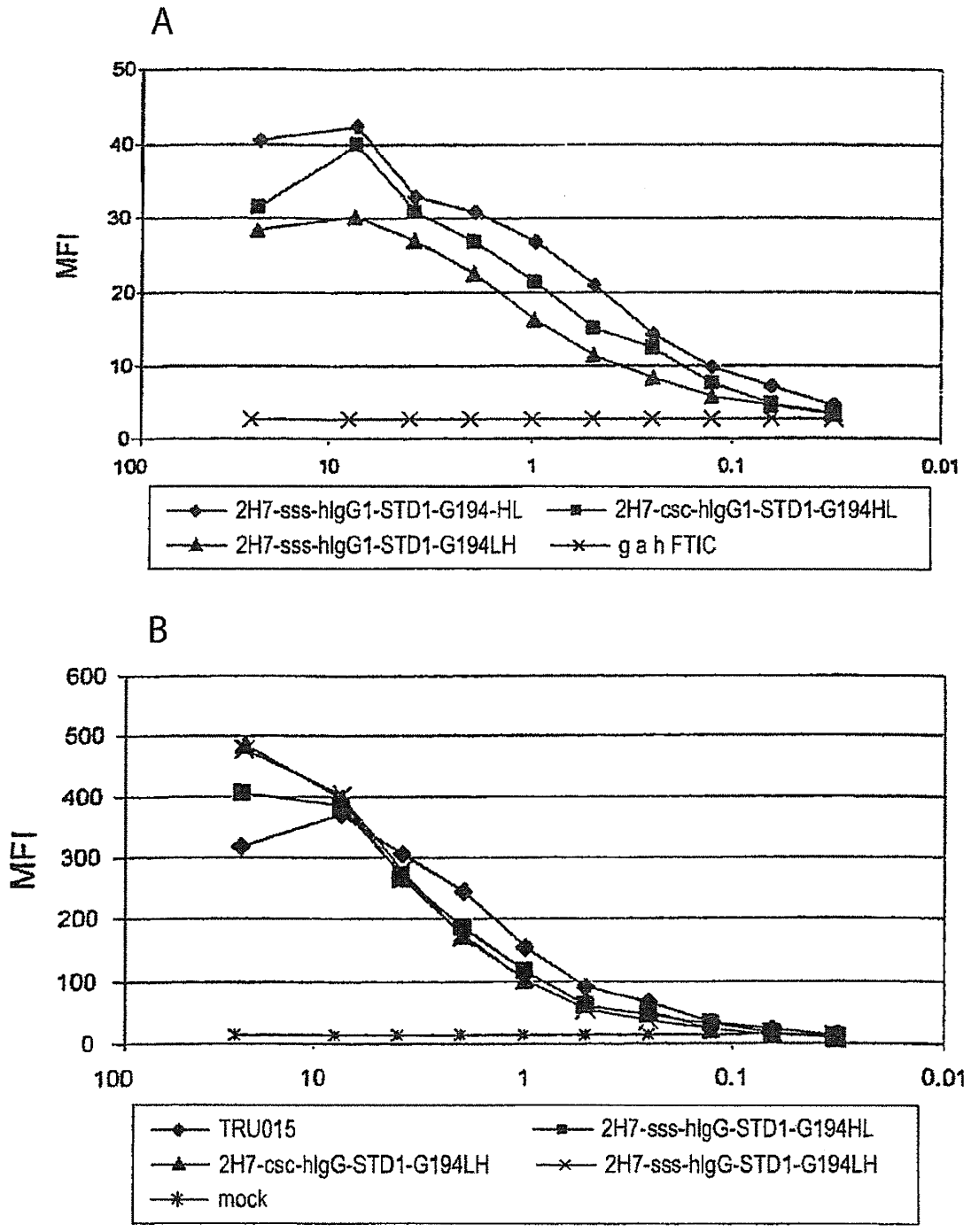
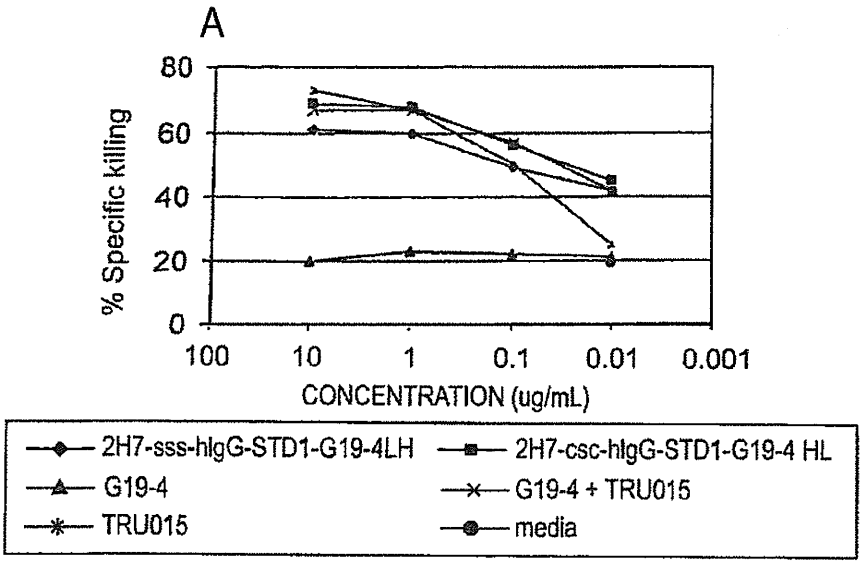

A
MFI
50
40
30
20
10
0
100
10
1
0.1
0.01
2H7-sss-hIgG1-STD1-G194-HL
2H7-csc-hIgG1-STD1-G194HL
2H7-sss-hIgG1-STD1-G194LH
g a h FTIC
B
MFI
600
500
400
300
200
100
0
100
10
1
0.1
0.01
TRU015
2H7-sss-hIgG-STD1-G194HL
2H7-csc-hIgG-STD1-G194LH
2H7-sss-hIgG-STD1-G194LH
mock

FIG. 19


A
80
60
40
20
0
% Specific killing
100
10
1
0.1
0.01
0.001
CONCENTRATION (ug/mL)
2H7-sss-hIgG-STD1-G19-4LH
2H7-csc-hIgG-STD1-G19-4 HL
G19-4
G19-4 + TRU015
TRU015
media

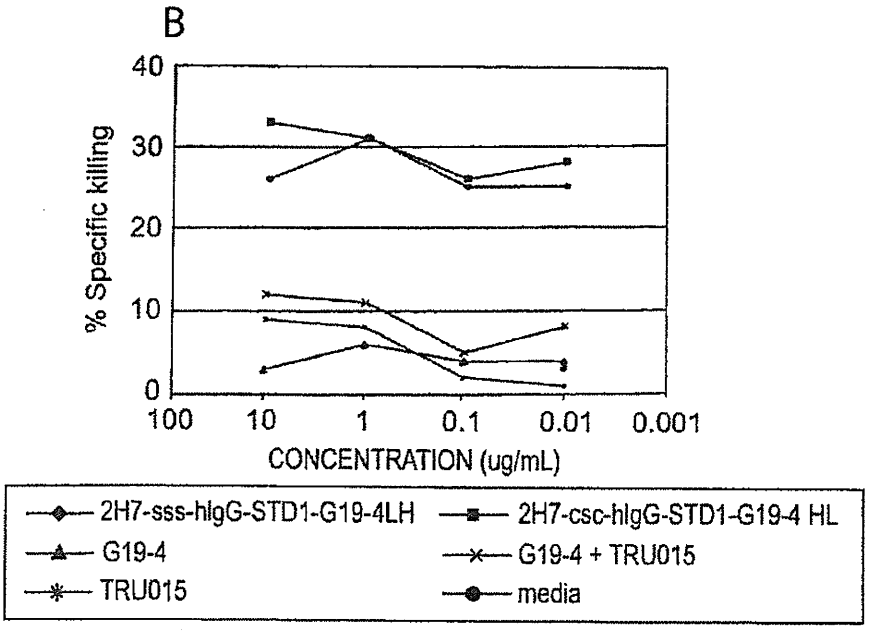

B
40
30
20
10
0
% Specific killing
100
10
1
0.1
0.01
0.001
CONCENTRATION (ug/mL)
2H7-sss-hIgG-STD1-G19-4LH
2H7-csc-hIgG-STD1-G19-4 HL
G19-4
G19-4 + TRU015
TRU015
media

FIG. 20

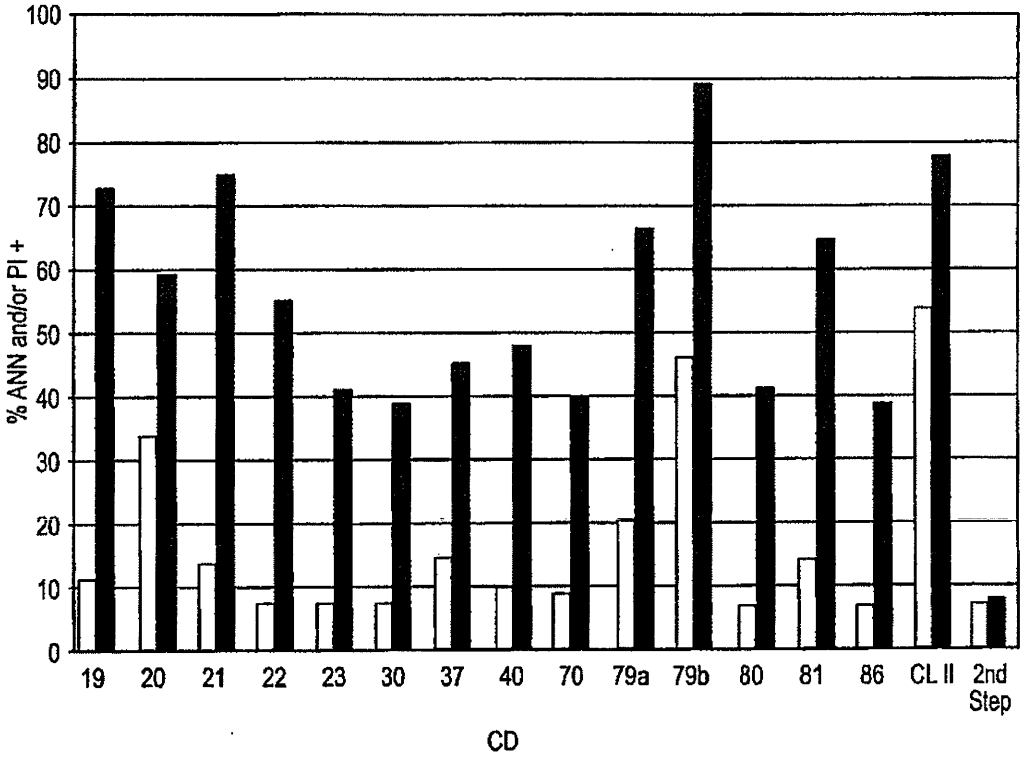

100
90
80
70
60
50
40
30
20
10
0
% ANN and/or PI +
19
20
21
22
23
30
37
40
70
79a
79b
80
81
86
CL II
2nd Step
CD

FIG. 21

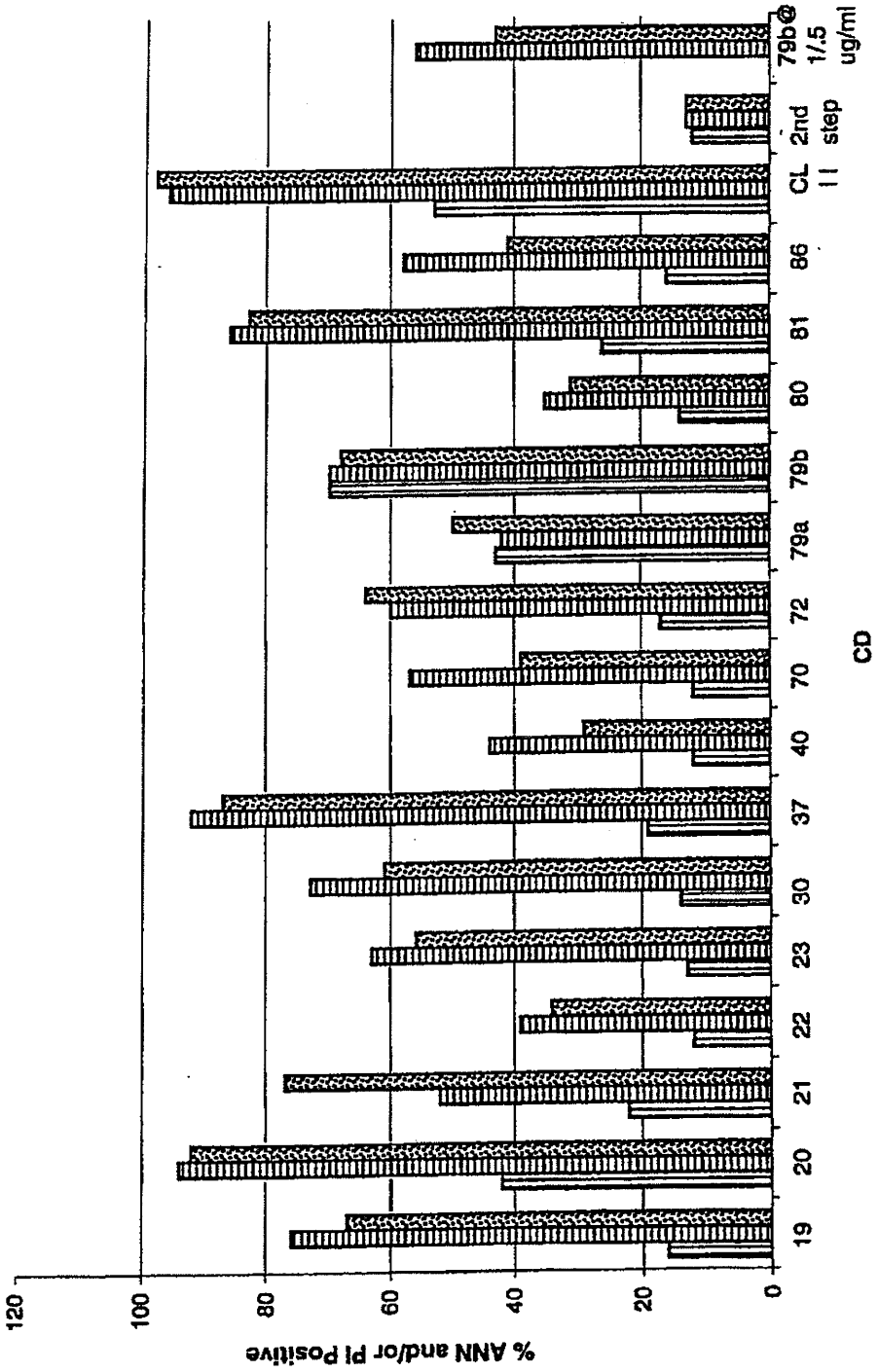

% ANN and/or PI Positive
120
100
80
60
40
20
0
19
20
21
22
23
30
37
40
70
72
79a
79b
80
81
86
CL
2nd step 1/.5 ug/ml
79b@
CD

FIG. 22

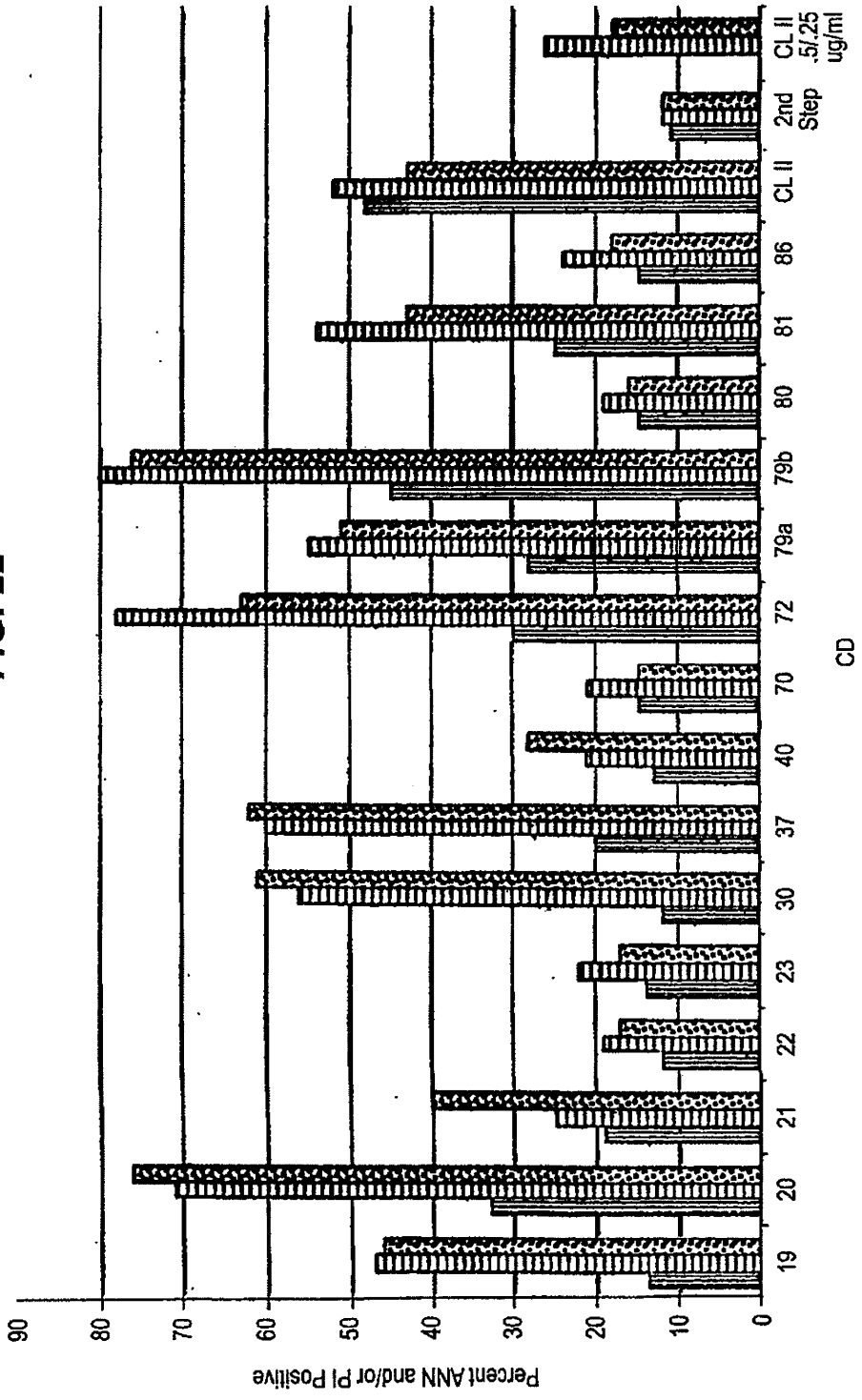

Percent ANN and/or PI Positive
0
10
20
30
40
50
60
70
80
90
19
20
21
22
23
30
37
40
70
72
79a
79b
80
81
86
CL II
2nd Step
CL II .5/.25 ug/ml
CD

FIG. 23

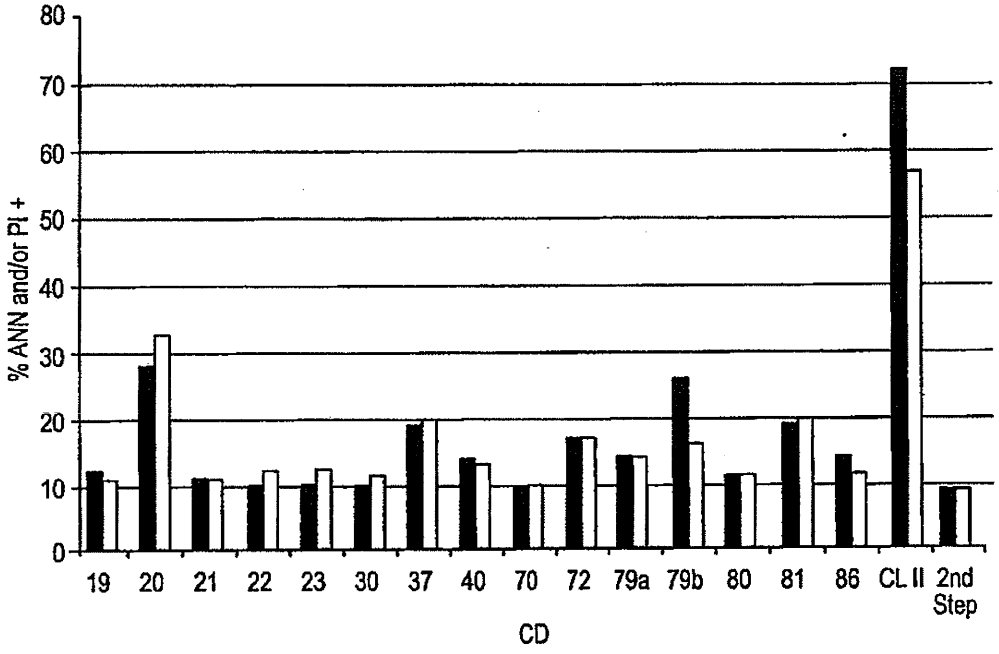

80
70
60
50
40
30
20
10
0
% ANN and/or PI +
19
20
21
22
23
30
37
40
70
72
79a
79b
80
81
86
CL II
2nd Step
CD

FIG. 24

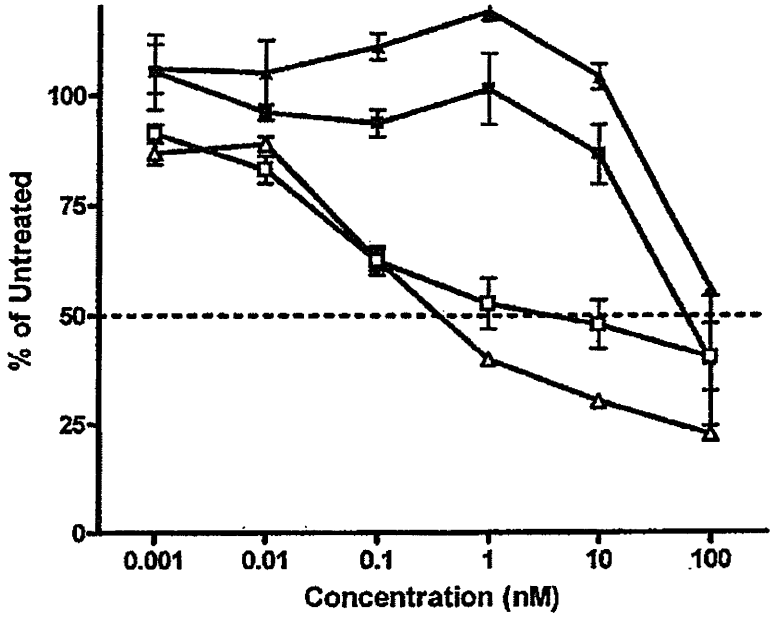

% of Untreated
100
75
50
25
0
0.001
0.01
0.1
1
10
100
Concentration (nM)

FIG. 25

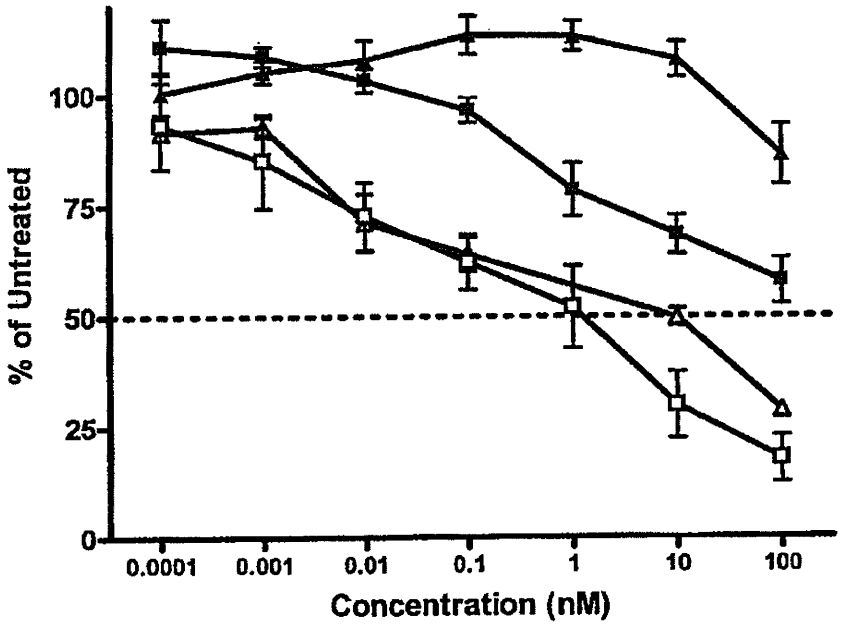

% of Untreated
100
75
50
25
0
0.0001
0.001
0.01
0.1
1
10
100
Concentration (nM)

FIG. 26

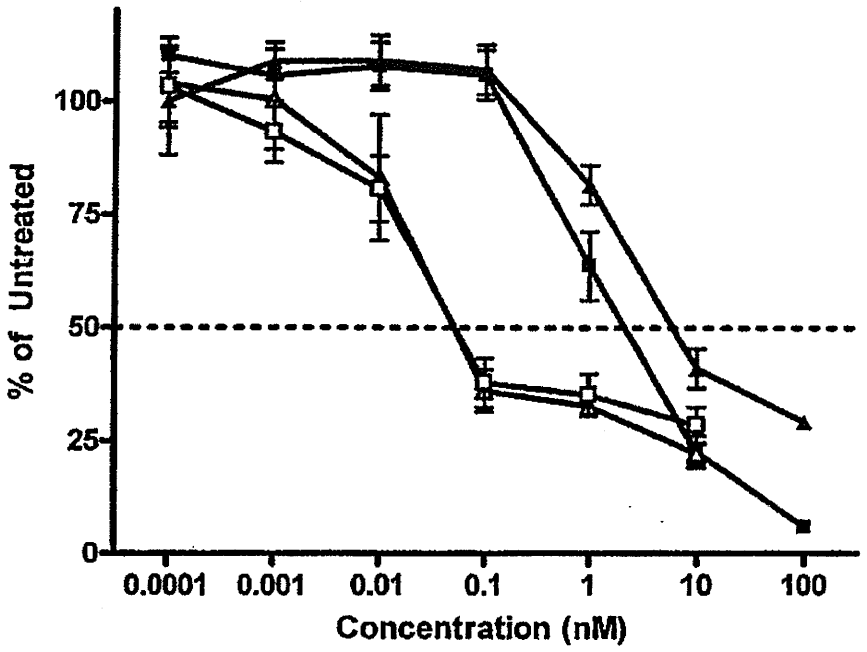

% of Untreated
100
75
50
25
0
0.0001
0.001
0.01
0.1
1
10
100
Concentration (nM)

FIG. 27

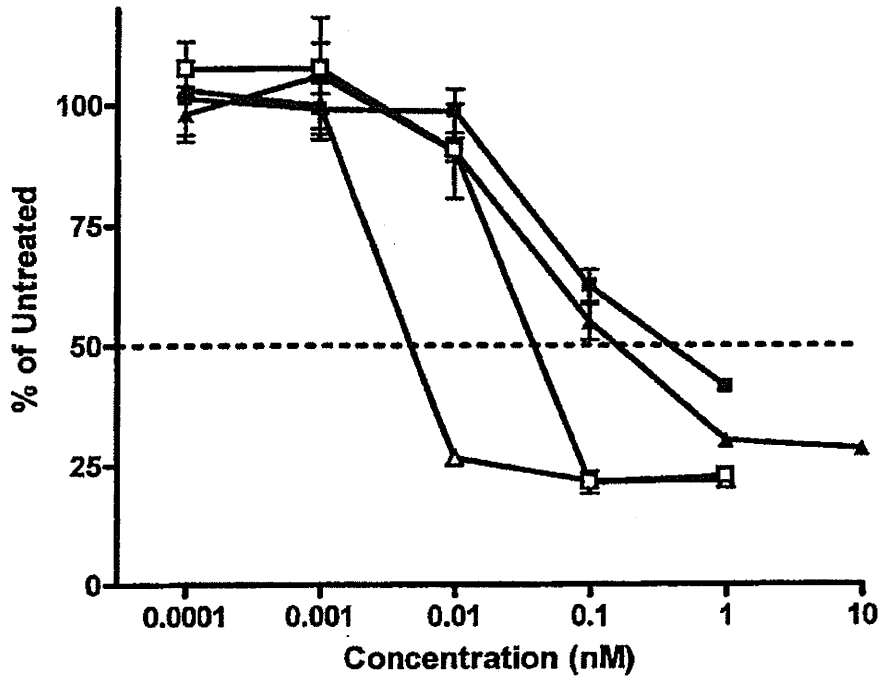

% of Untreated
100
75
50
25
0
0.0001
0.001
0.01
0.1
1
10
Concentration (nM)

FIG. 28

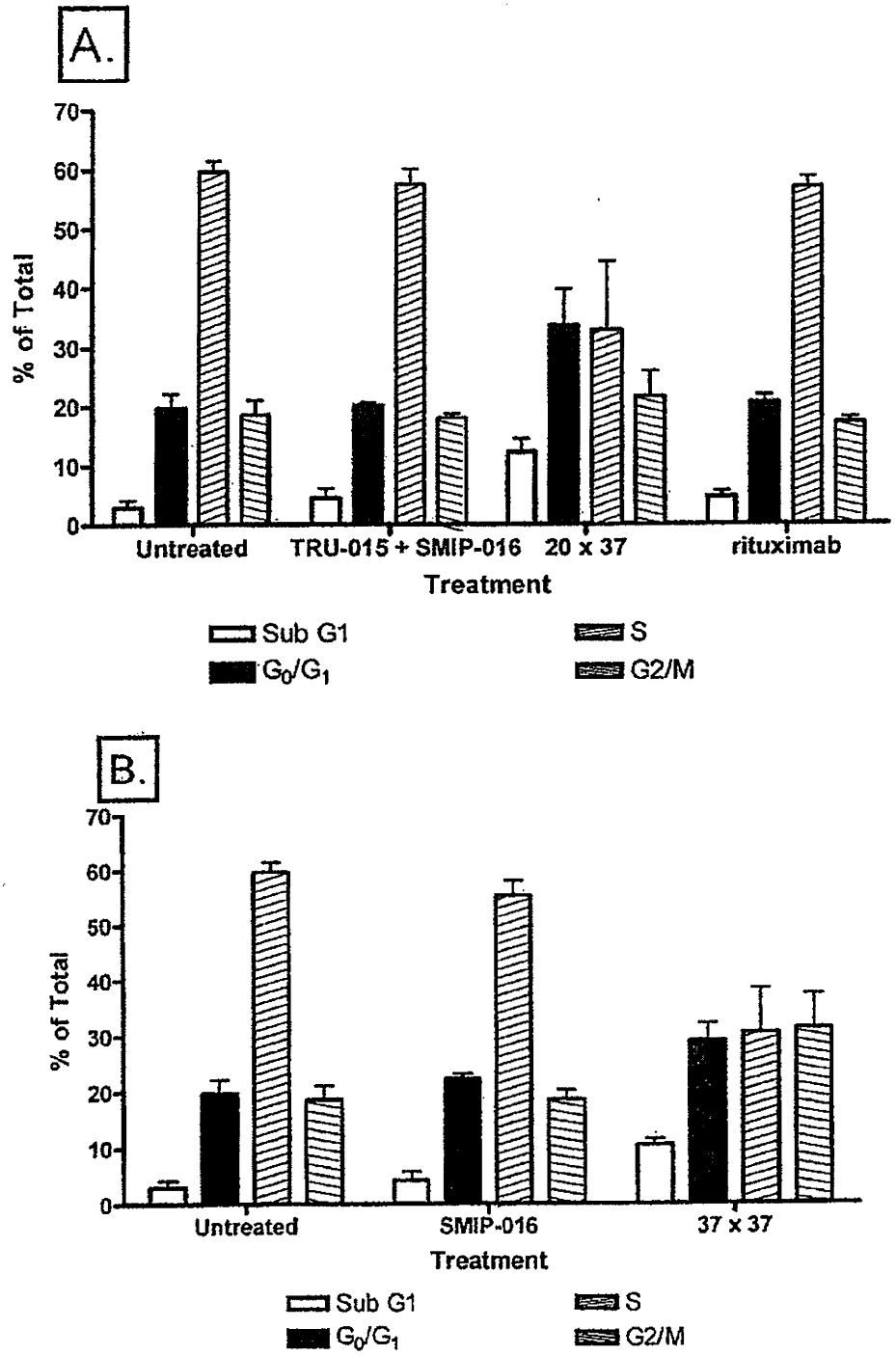

A.
70
60
50
40
30
20
10
0
% of Total
Untreated
TRU-015 + SMIP-016
20 x 37
rituximab
Treatment
Sub G1
S
G_0/G_1
G2/M
B.
70
60
50
40
30
20
10
0
% of Total
Untreated
SMIP-016
37 x 37
Treatment
Sub G1
S
G_0/G_1
G2/M

FIG. 29

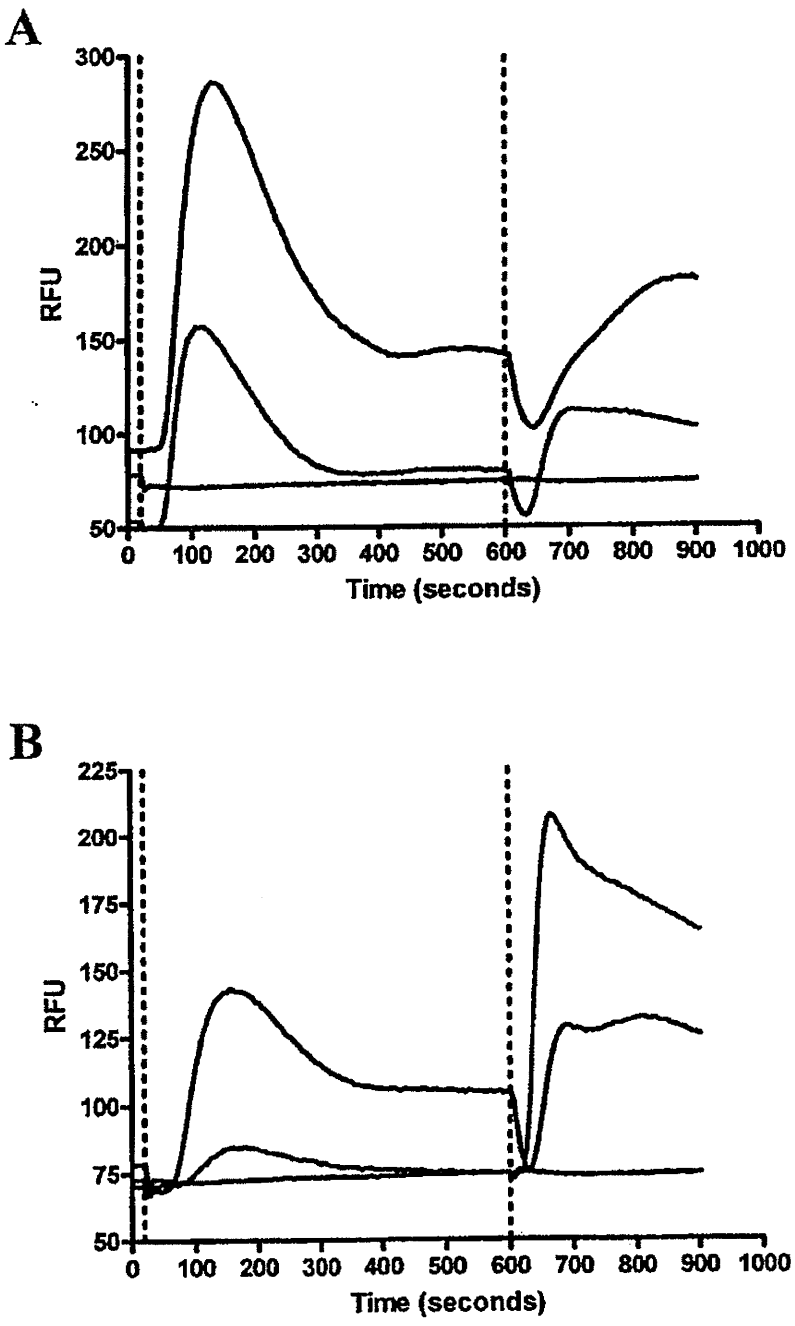

A
300
250
200
150
100
50
RFU
0
100
200
300
400
500
600
700
800
900
1000
Time (seconds)
B
225
200
175
150
125
100
75
50
RFU
0
100
200
300
400
500
600
700
800
900
1000
Time (seconds)

FIG. 30

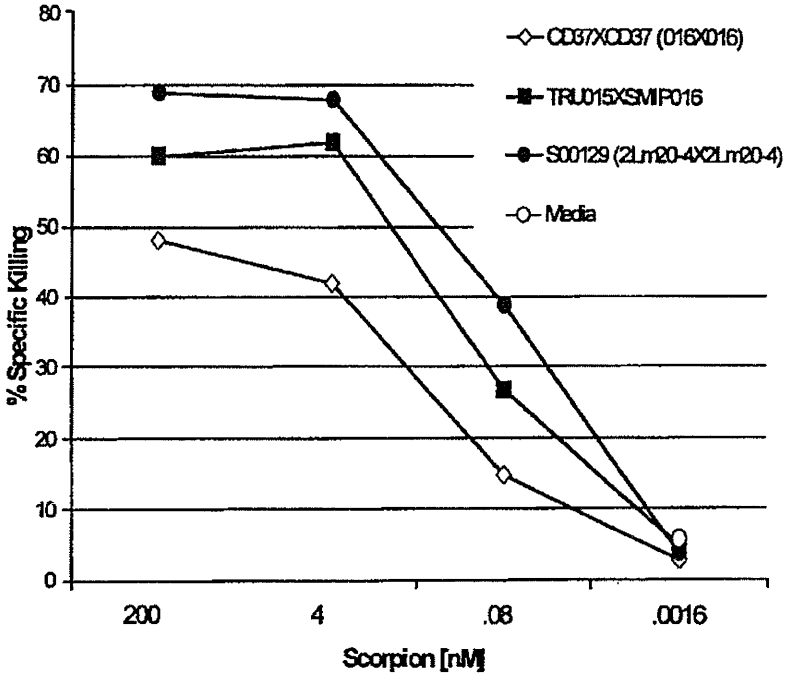

80
70
60
50
40
30
20
10
0
% Specific Killing
200
4
.08
.0016
Scorpion [nM]
CD37XCD37 (016X016)
TRU015XSMIP016
S00129 (2Lm20-4X2Lm20-4)
Media

FIG. 31

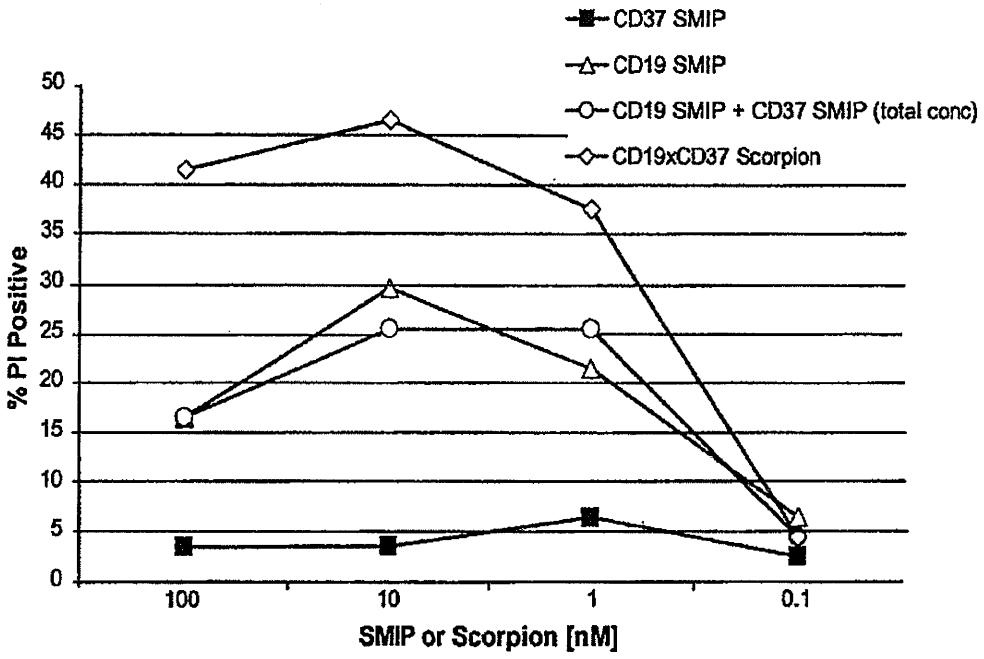

CD37 SMIP
CD19 SMIP
CD19 SMIP + CD37 SMIP (total conc)
CD19xCD37 Scorpion
% PI Positive
50
45
40
35
30
25
20
15
10
5
0
100
10
1
0.1
SMIP or Scorpion [nM]

FIG. 32A

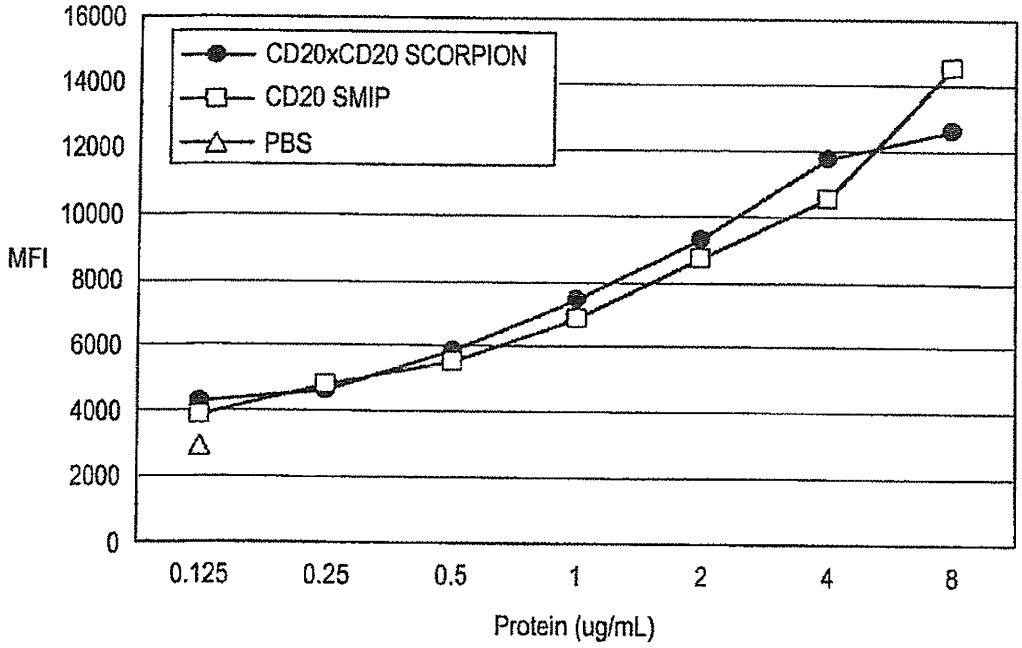

16000
14000
12000
10000
8000
6000
4000
2000
0
MFI
CD20xCD20 SCORPION
CD20 SMIP
PBS
0.125
0.25
0.5
1
2
4
8
Protein (ug/mL)

FIG. 32B

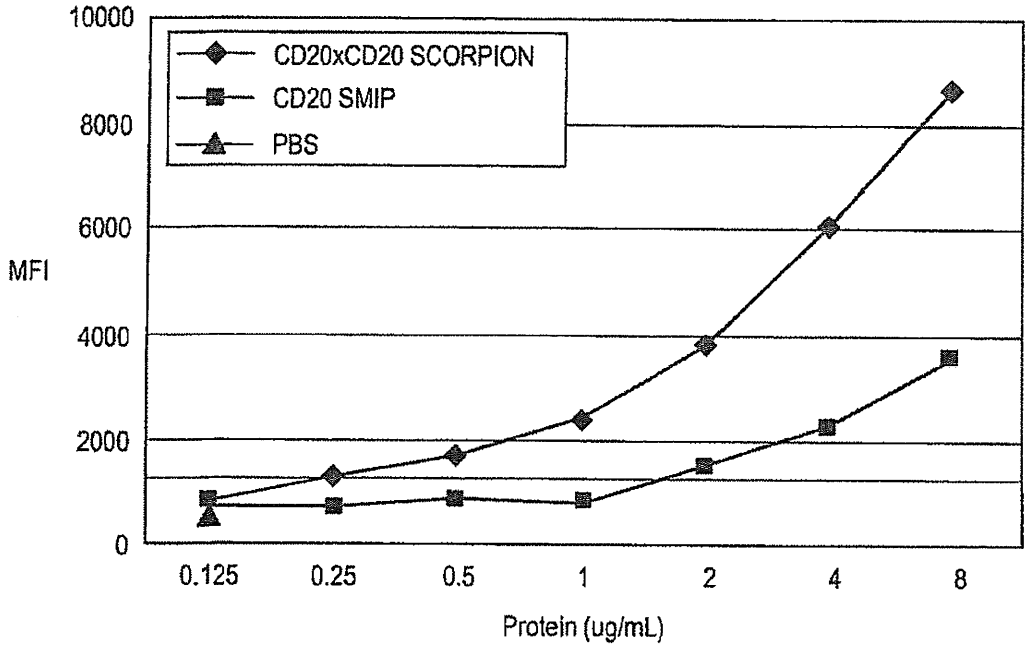

10000
8000
6000
4000
2000
0
MFI
CD20xCD20 SCORPION
CD20 SMIP
PBS
0.125
0.25
0.5
1
2
4
8
Protein (ug/mL)

FIG. 33

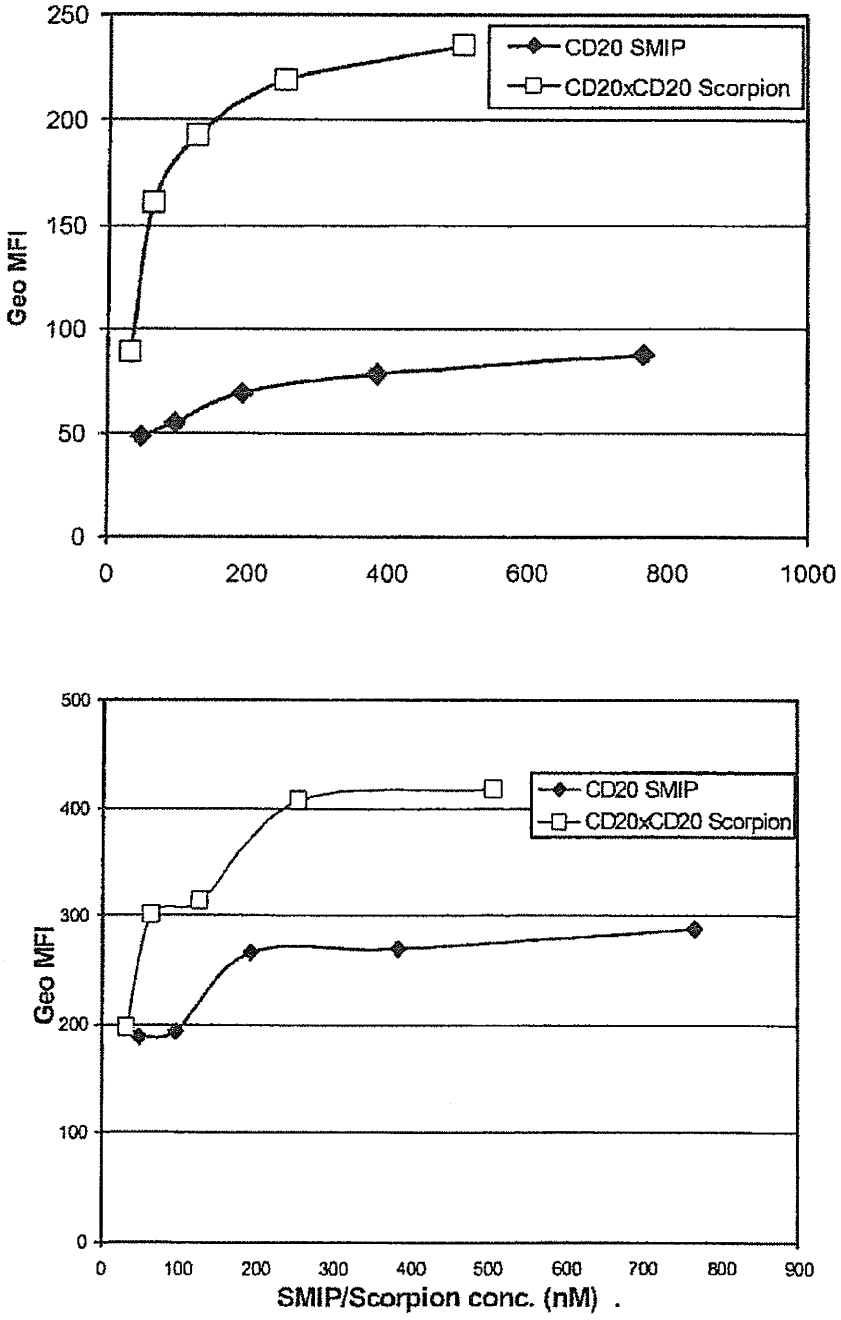

250
200
150
100
50
0
Geo MFI
0
200
400
600
800
1000
CD20 SMIP
CD20xCD20 Scorpion
500
400
300
200
100
0
Geo MFI
0
100
200
300
400
500
600
700
800
900
SMIP/Scorpion conc. (nM)
CD20 SMIP
CD20xCD20 Scorpion

FIG. 34

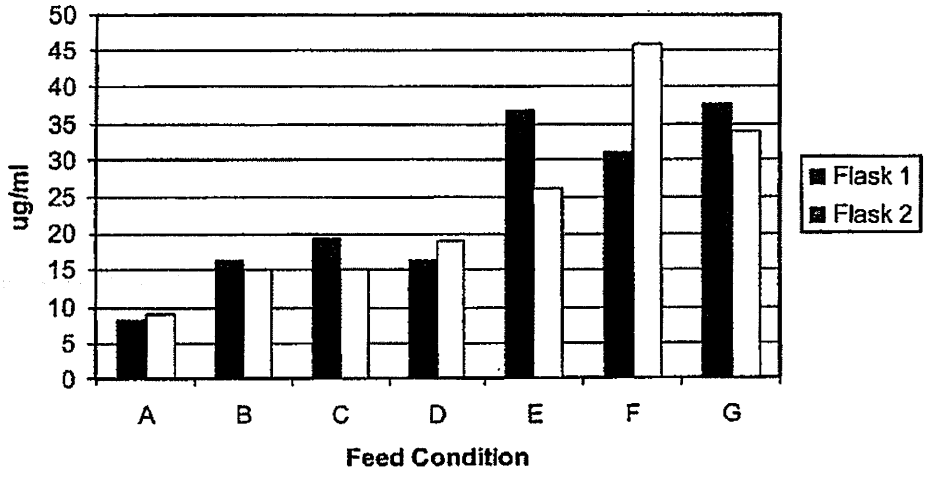

50
45
40
35
30
25
20
15
10
5
0
ug/ml
A
B
C
D
E
F
G
Feed Condition
Flask 1
Flask 2

FIG. 35

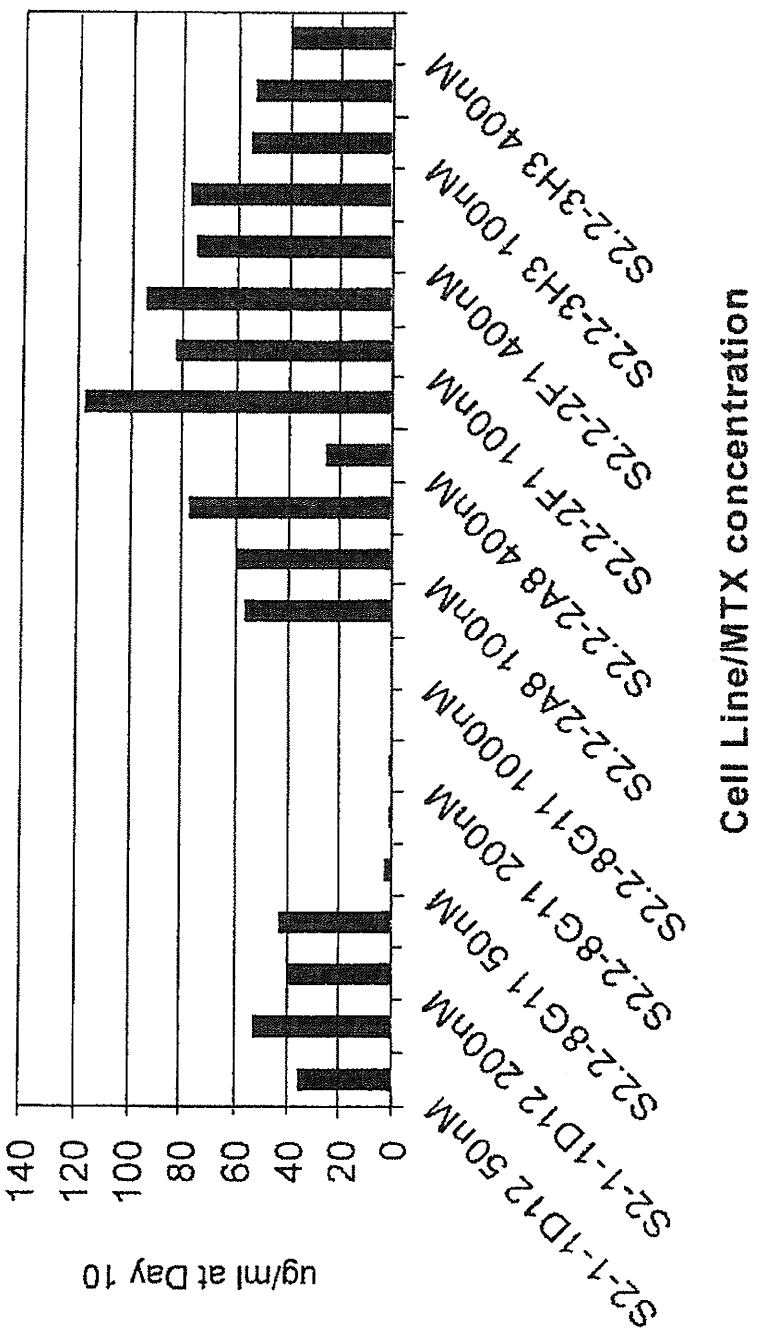

ug/ml at Day 10
140
120
100
80
60
40
20
0
S2-1-1D12 50nM
S2-1-1D12 200nM
S2.2-8G11 50nM
S2.2-8G11 200nM
S2.2-8G11 1000nM
S2.2-2A8 100nM
S2.2-2A8 400nM
S2.2-2F1 100nM
S2.2-2F1 400nM
S2.2-3H3 100nM
S2.2-3H3 400nM
Cell Line/MTX concentration

FIG. 36

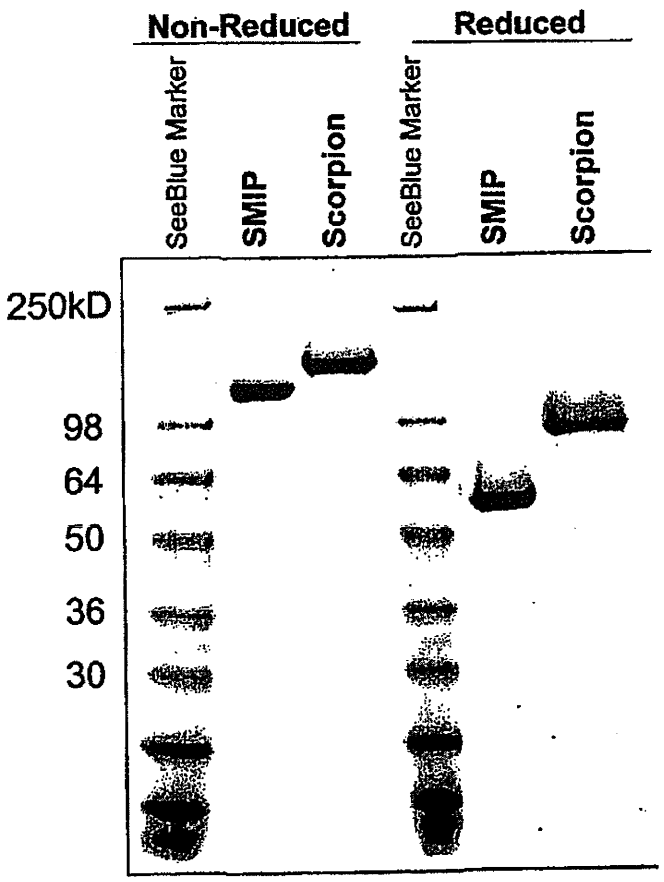

Non-Reduced
Reduced
SeeBlue Marker
SMIP
Scorpion
SeeBlue Marker
SMIP
Scorpion
250kD
98
64
50
36
30

FIG. 37

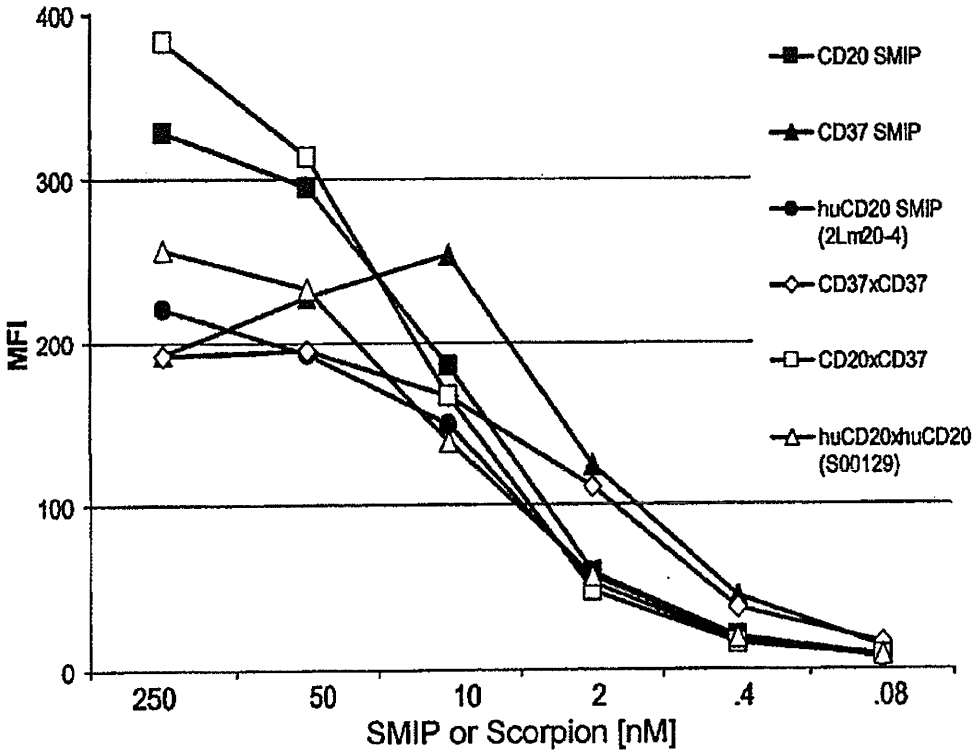

400
300
200
100
0
MFI
250
50
10
2
.4
.08
SMIP or Scorpion [nM]
CD20 SMIP
CD37 SMIP
huCD20 SMIP (2Lm20-4)
CD37xCD37
CD20xCD37
huCD20xhuCD20 (S00129)

FIG. 38

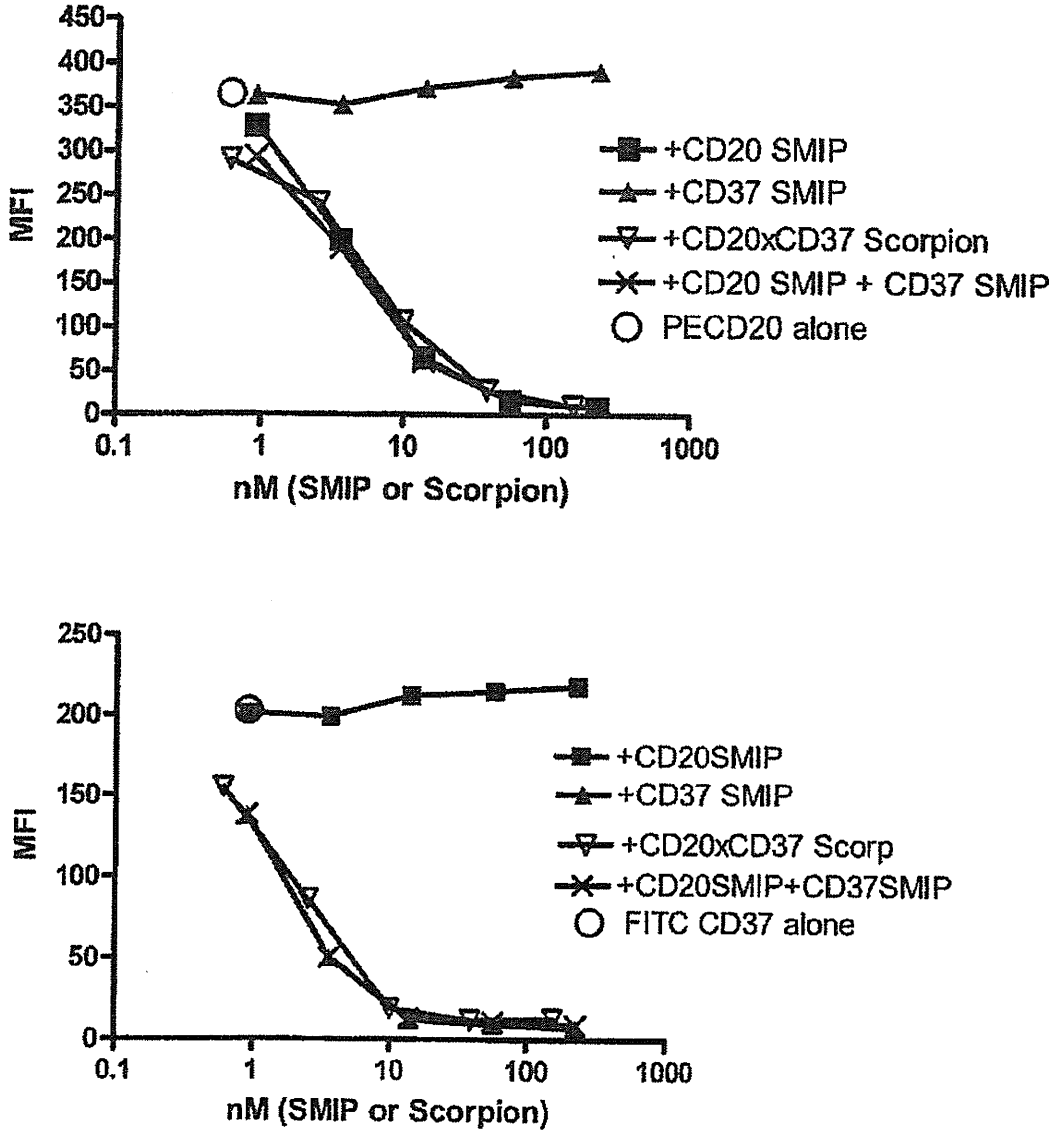

MFI
450
400
350
300
250
200
150
100
50
0
0.1
1
10
100
1000
nM (SMIP or Scorpion)
+CD20 SMIP
+CD37 SMIP
+CD20xCD37 Scorpion
+CD20 SMIP + CD37 SMIP
PECD20 alone
MFI
250
200
150
100
50
0
0.1
1
10
100
1000
nM (SMIP or Scorpion)
+CD20SMIP
+CD37 SMIP
+CD20xCD37 Scorp
+CD20SMIP+CD37SMIP
FITC CD37 alone

FIG. 39

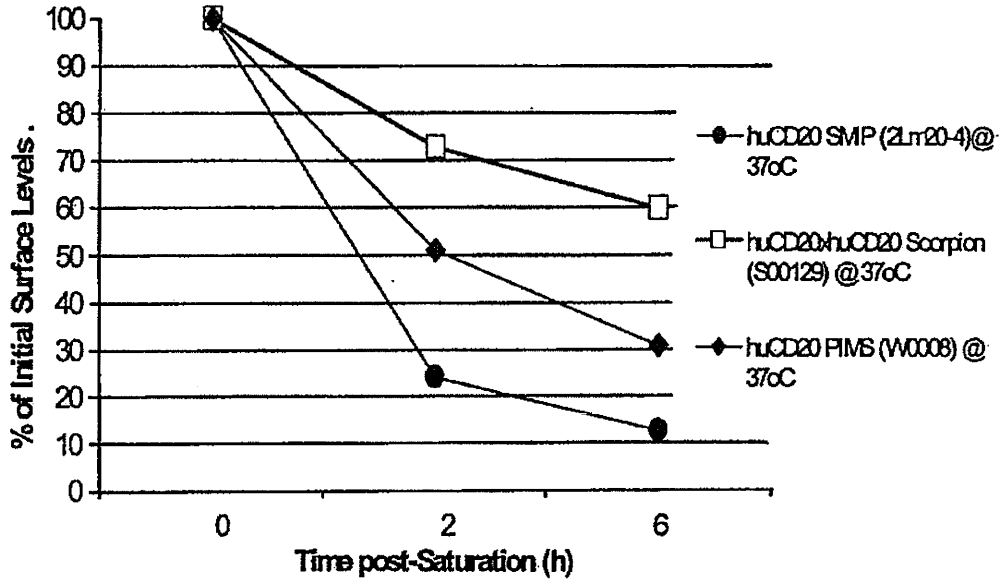

100
90
80
70
60
50
40
30
20
10
0
% of Initial Surface Levels .
0
2
6
Time post-Saturation (h)
huCD20 SMIP (2Lm20-4)@ 37oC
huCD20/huCD20 Scorpion (S00129) @37oC
huCD20 PIMS (W0008) @ 37oC

FIG. 40

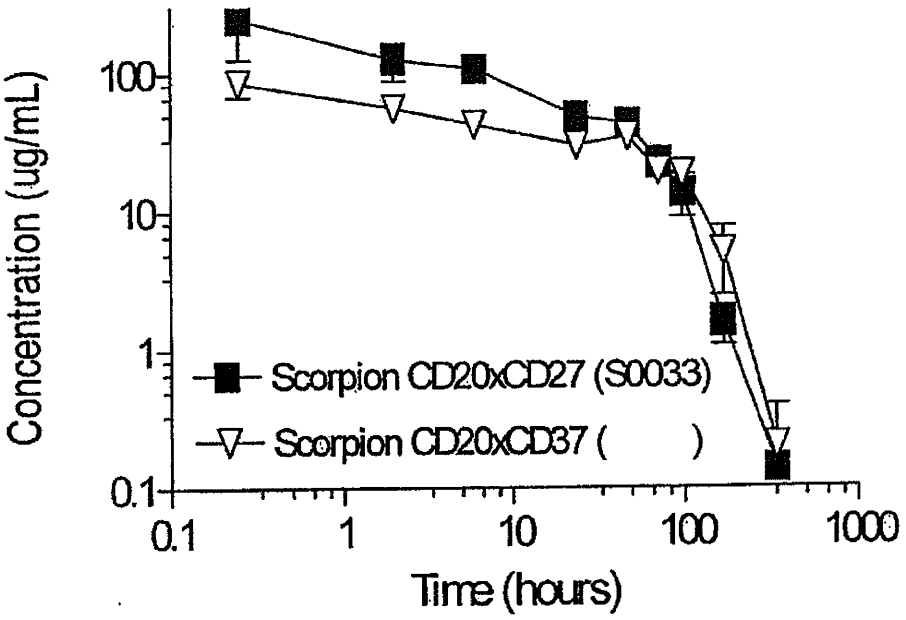

Concentration (ug/mL)
100
10
1
0.1
0.1
1
10
100
1000
Time (hours)
Scorpion CD20xCD27 (S0033)
Scorpion CD20xCD37 ( )

FIG. 41

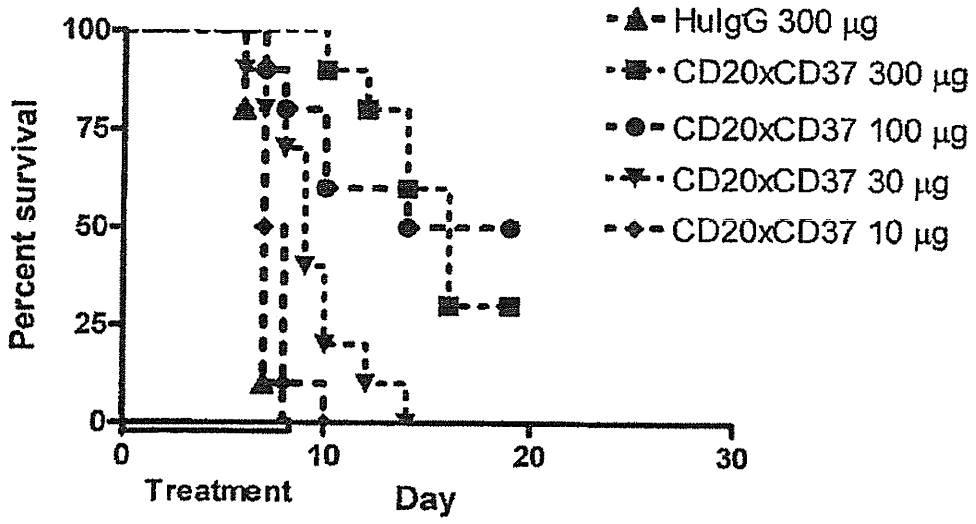

Percent survival
100
75
50
25
0
0
10
20
30
Treatment
Day
HuIgG 300 µg
CD20xCD37 300 µg
CD20xCD37 100 µg
CD20xCD37 30 µg
CD20xCD37 10 µg

FIG. 42

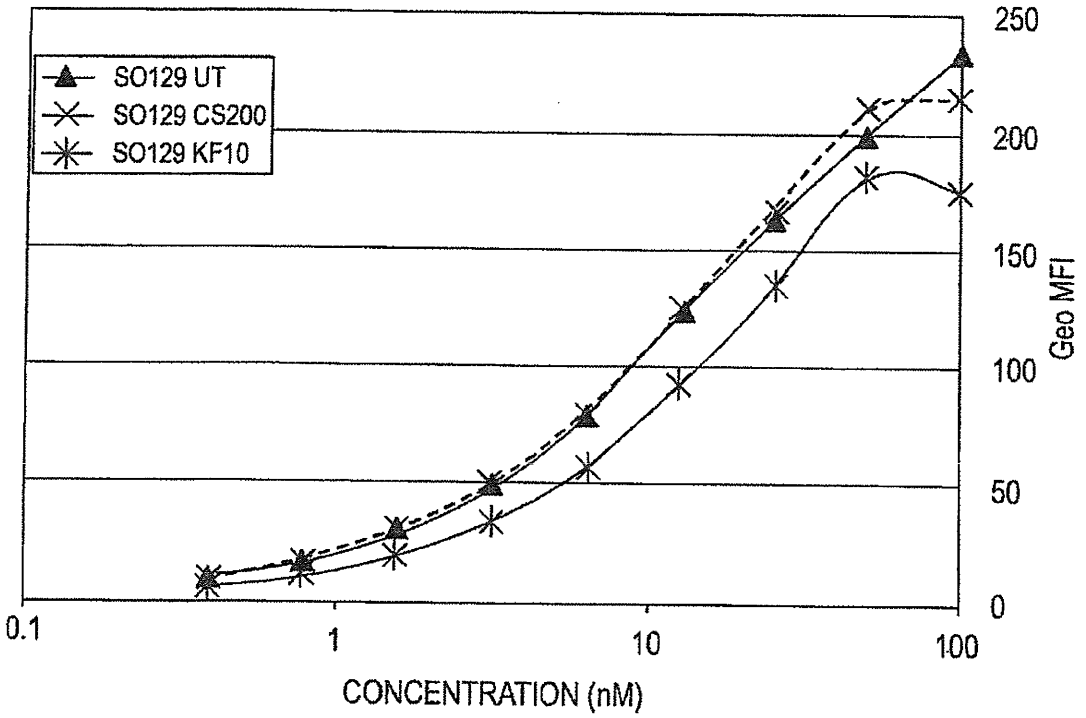

SO129 UT
SO129 CS200
SO129 KF10
250
200
150
100
50
0
Geo MFI
0.1
1
10
100
CONCENTRATION (nM)

FIG. 43

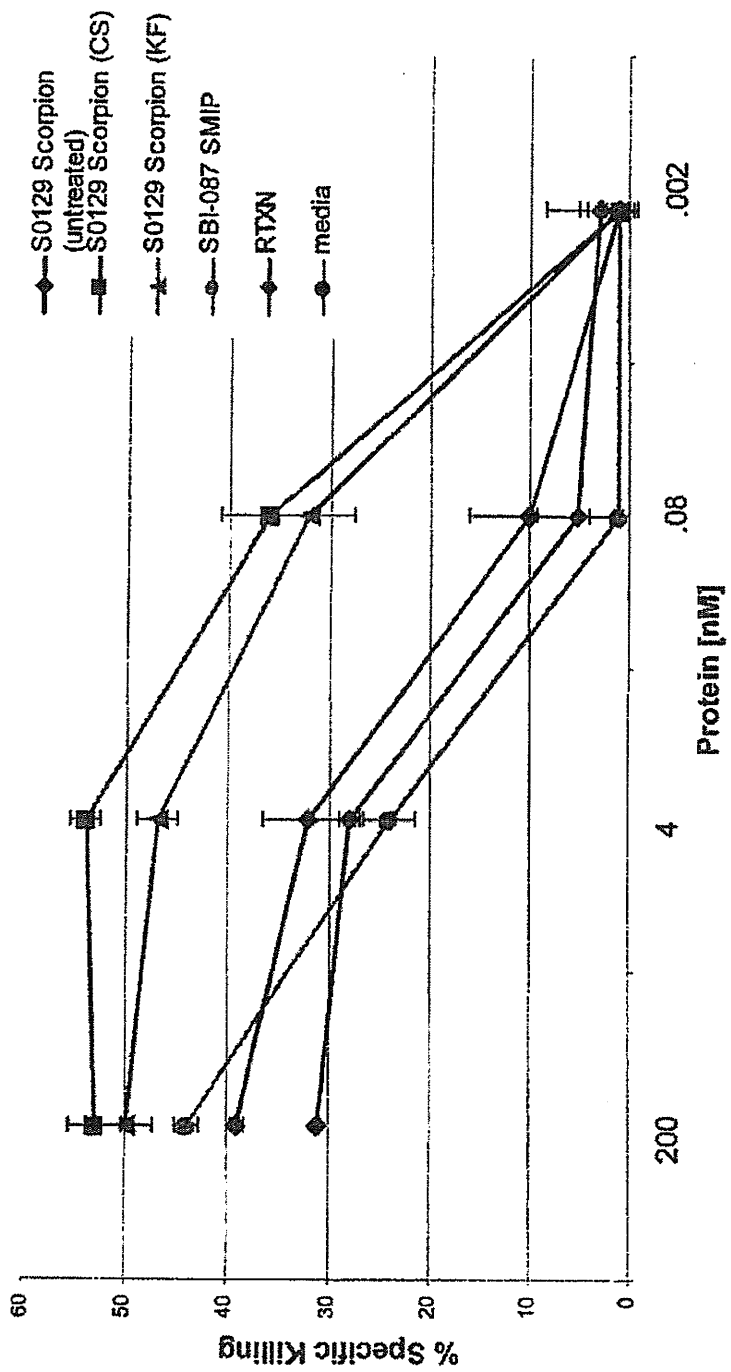

S0129 Scorpion (untreated)
S0129 Scorpion (CS)
S0129 Scorpion (KF)
SBI-087 SMIP
RTXN
media
% Specific Killing
60
50
40
30
20
10
0
200
4
.08
.002
Protein [nM]

FIG. 44

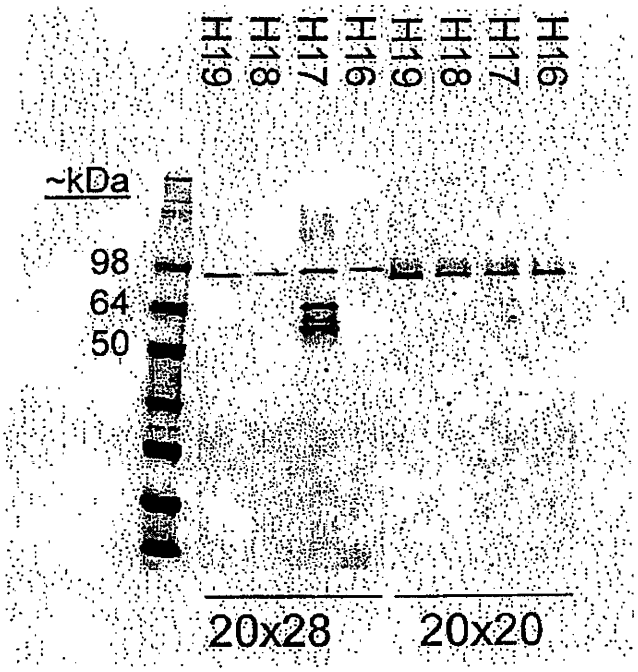


FIG. 45

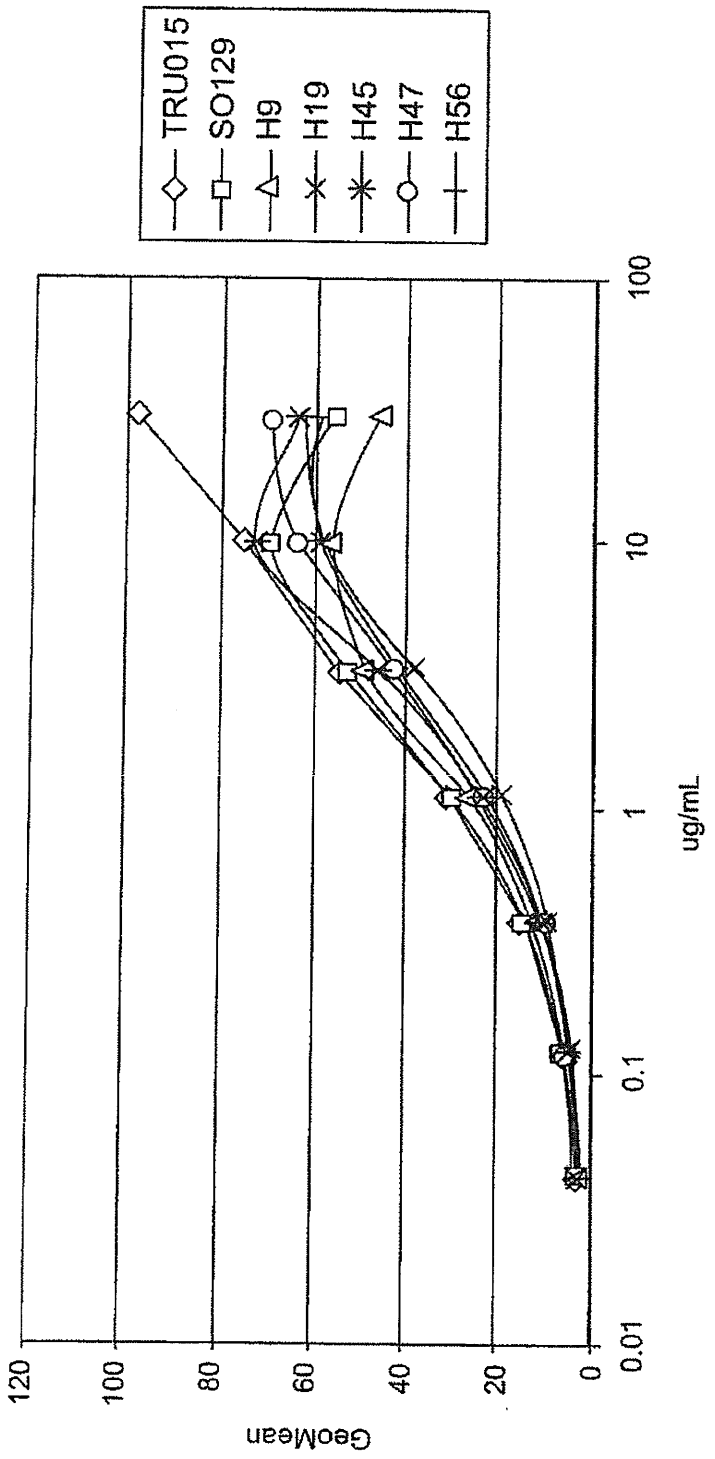

TRU015
SO129
H9
H19
H45
H47
H56
GeoMean
120
100
80
60
40
20
0
0.01
0.1
1
10
100
ug/mL

FIG. 46

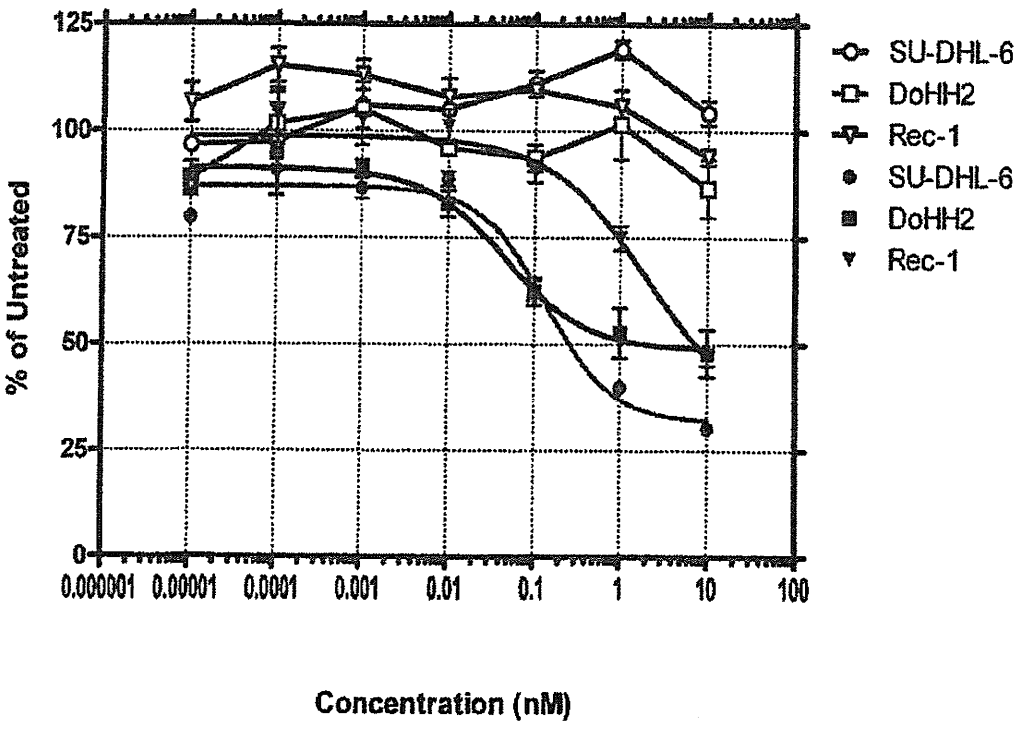

125
100
75
50
25
0
% of Untreated
0.000001
0.00001
0.0001
0.001
0.01
0.1
1
10
100
Concentration (nM)
SU-DHL-6
DoHH2
Rec-1
SU-DHL-6
DoHH2
Rec-1

FIG. 47

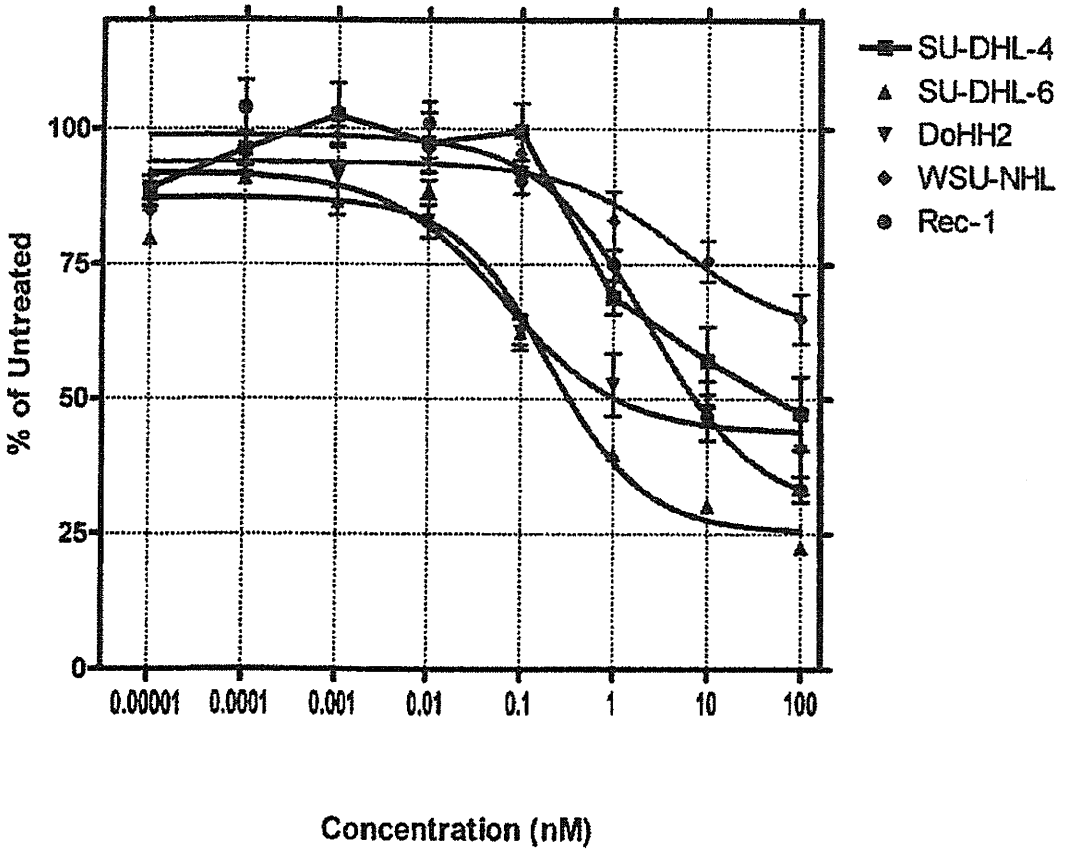

% of Untreated
100
75
50
25
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
Concentration (nM)
SU-DHL-4
SU-DHL-6
DoHH2
WSU-NHL
Rec-1

FIG. 48

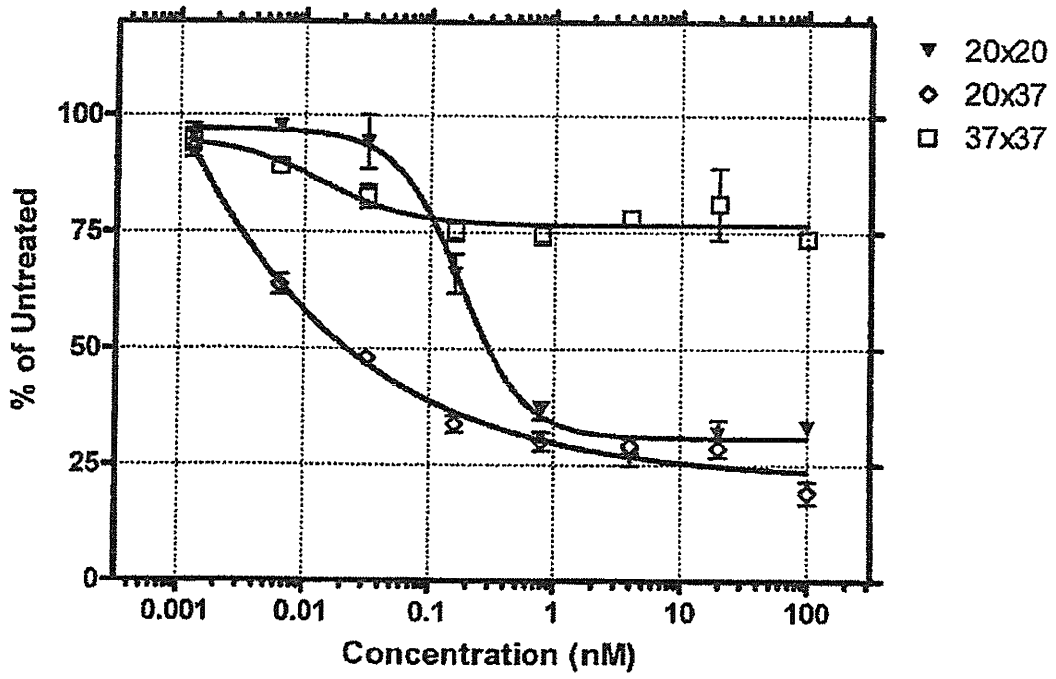

% of Untreated
100
75
50
25
0
0.001
0.01
0.1
1
10
100
Concentration (nM)
20x20
20x37
37x37

FIG. 49

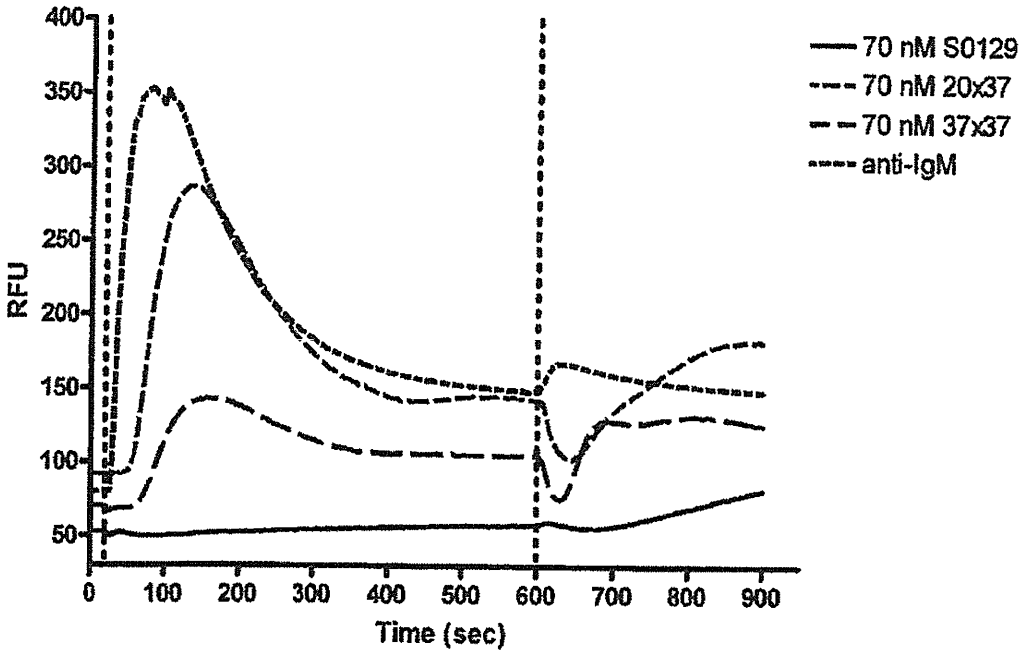

400
350
300
250
200
150
100
50
RFU
0
100
200
300
400
500
600
700
800
900
Time (sec)
70 nM S0129
70 nM 20x37
70 nM 37x37
anti-IgM

FIG. 50

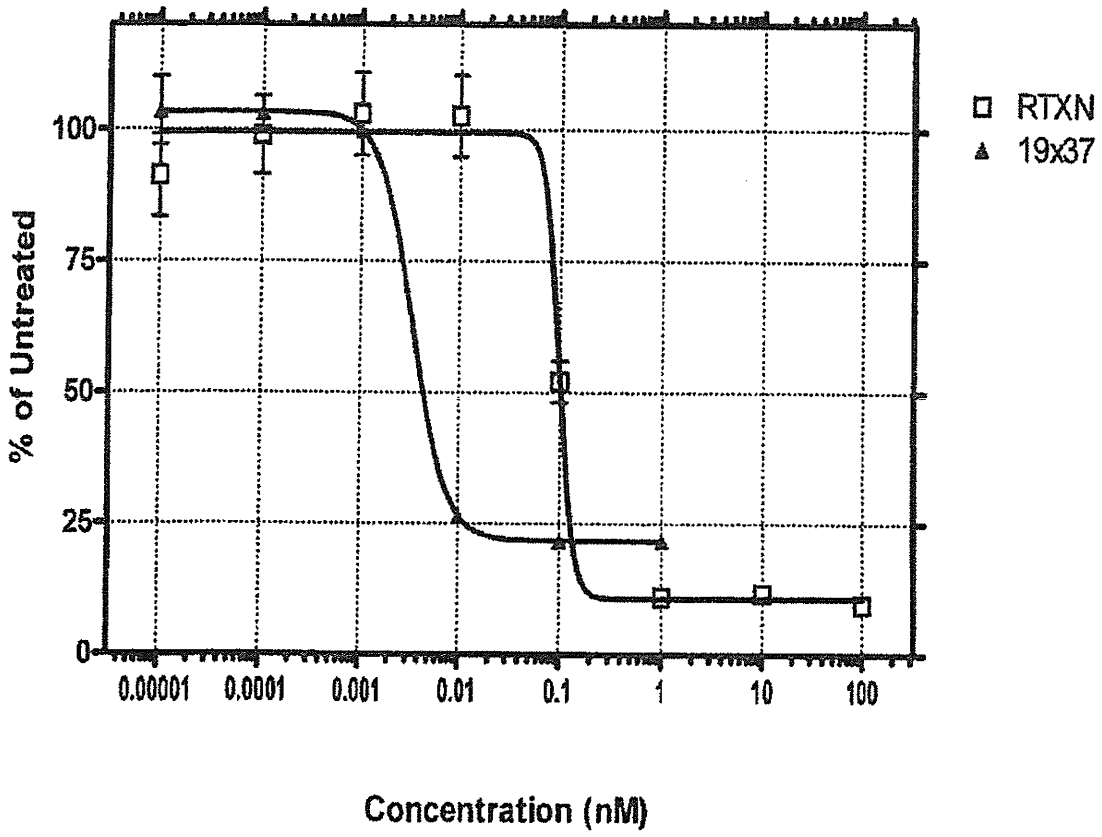

% of Untreated
100
75
50
25
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
Concentration (nM)
RTXN
19x37

FIG. 51

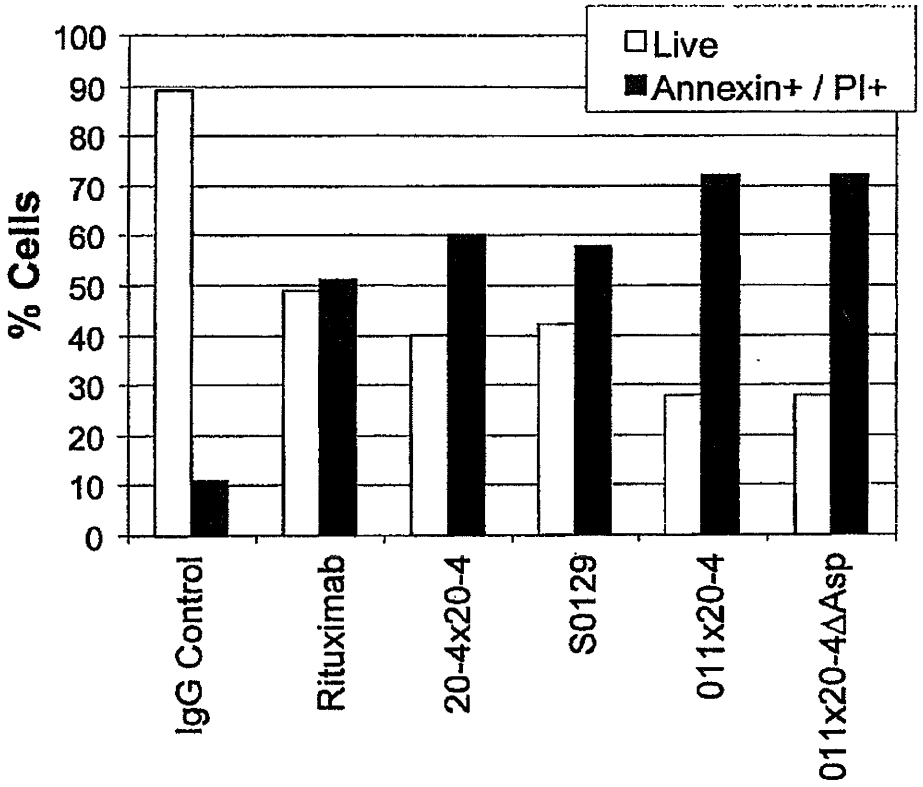

Live
Annexin+ / PI+
% Cells
100
90
80
70
60
50
40
30
20
10
0
IgG Control
Rituximab
20-4x20-4
S0129
011x20-4
011x20-4ΔAsp

FIG. 52

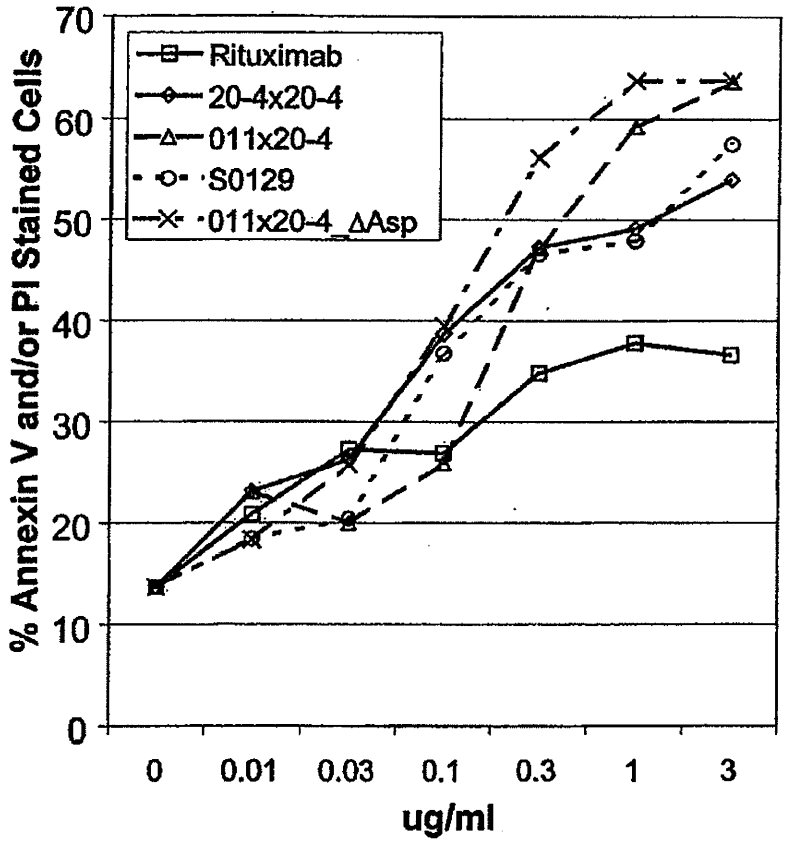

% Annexin V and/or PI Stained Cells
70
60
50
40
30
20
10
0
Rituximab
20-4x20-4
011x20-4
S0129
011x20-4_ΔAsp
0
0.01
0.03
0.1
0.3
1
3
ug/ml

FIG. 53

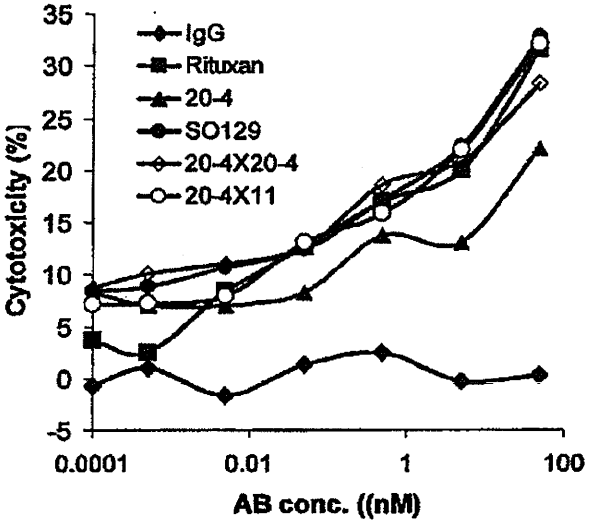

IgG
Rituxan
20-4
SO129
20-4X20-4
20-4X11
Cytotoxicity (%)
35
30
25
20
15
10
5
0
-5
0.0001
0.01
1
100
AB conc. ((nM)

FIG. 54

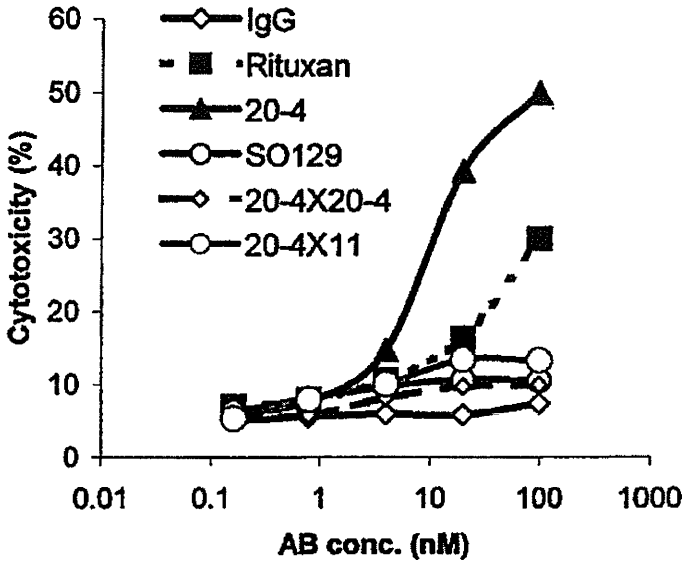

IgG
Rituxan
20-4
SO129
20-4X20-4
20-4X11
Cytotoxicity (%)
60
50
40
30
20
10
0
0.01
0.1
1
10
100
1000
AB conc. (nM)

FIG. 55

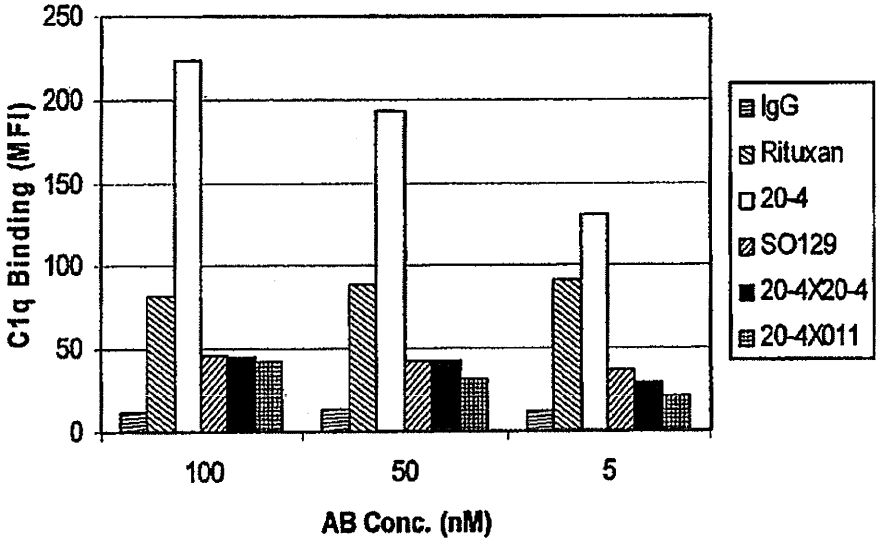

250
200
150
100
50
0
C1q Binding (MFI)
100
50
5
AB Conc. (nM)
IgG
Rituxan
20-4
SO129
20-4X20-4
20-4X011

FIG. 56

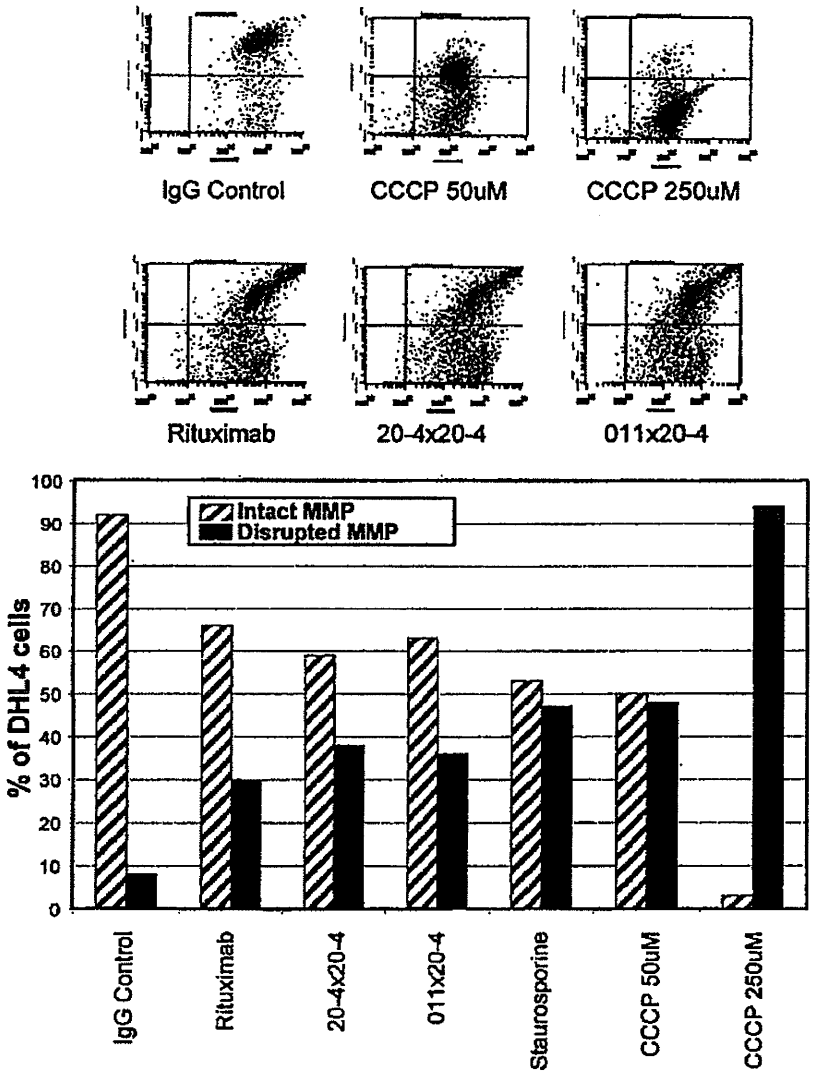

IgG Control
CCCP 50uM
CCCP 250uM
Rituximab
20-4x20-4
011x20-4
Intact MMP
Disrupted MMP
% of DHL4 cells
100
90
80
70
60
50
40
30
20
10
0
IgG Control
Rituximab
20-4X20-4
011X20-4
Staurosporine
CCCP 50uM
CCCP 250uM

FIG. 57

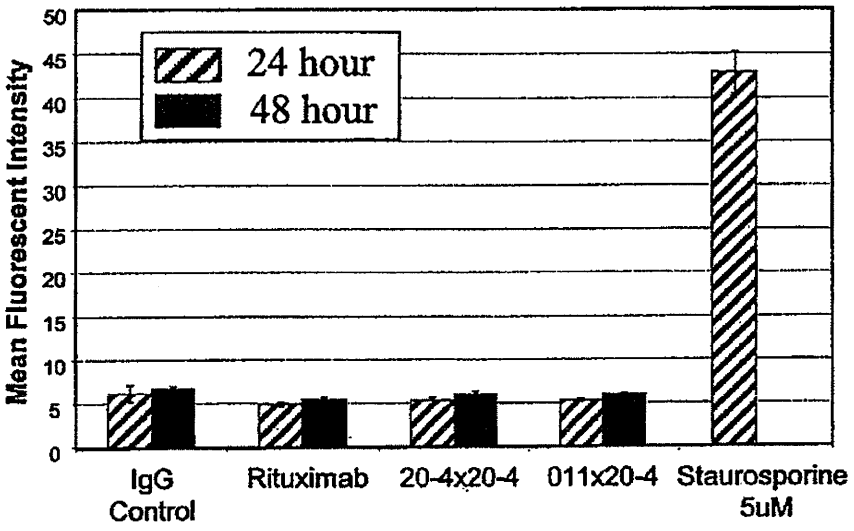

Mean Fluorescent Intensity
50
45
40
35
30
25
20
15
10
5
0
24 hour
48 hour
IgG Control
Rituximab
20-4x20-4
011x20-4
Staurosporine 5uM

FIG. 58

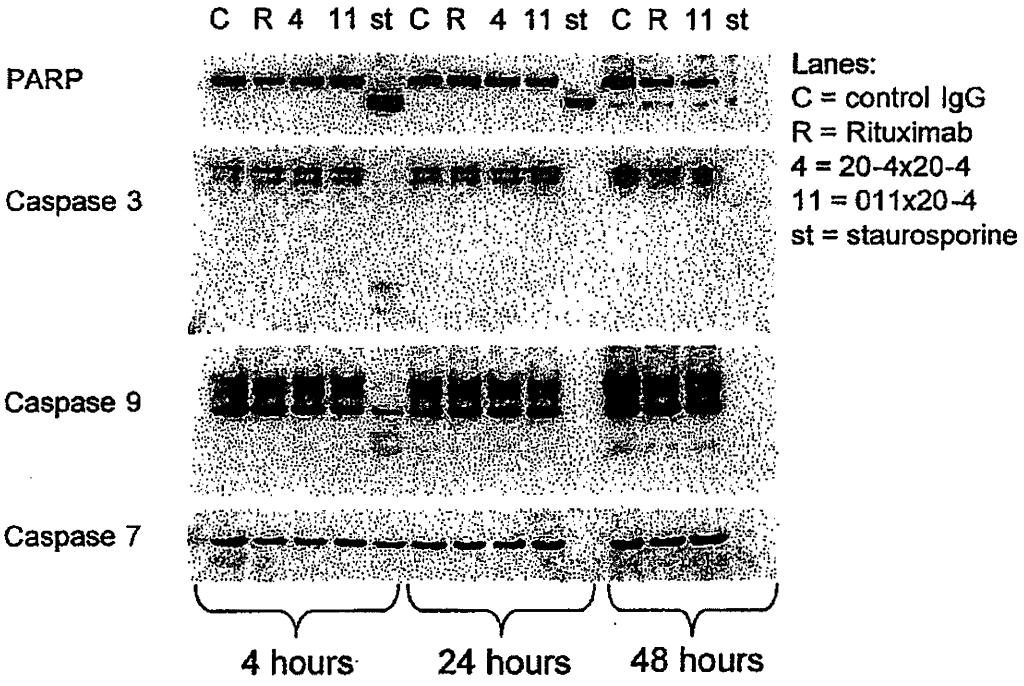

C R 4 11 st C R 4 11 st C R 11 st
PARP
Caspase 3
Caspase 9
Caspase 7
Lanes:
C = control IgG
R = Rituximab
4 = 20-4x20-4
11 = 011x20-4
st = staurosporine
4 hours
24 hours
48 hours

FIG. 59

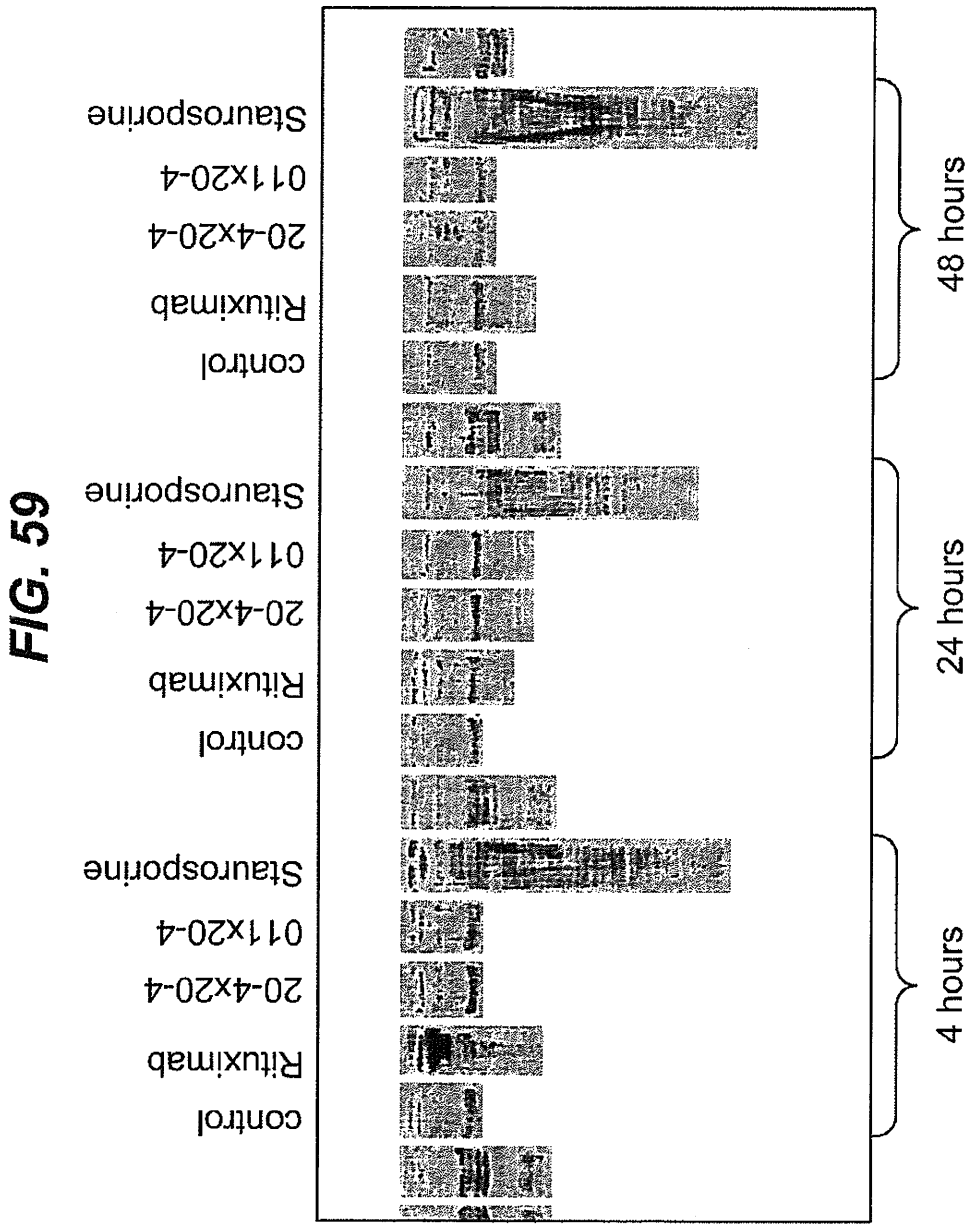
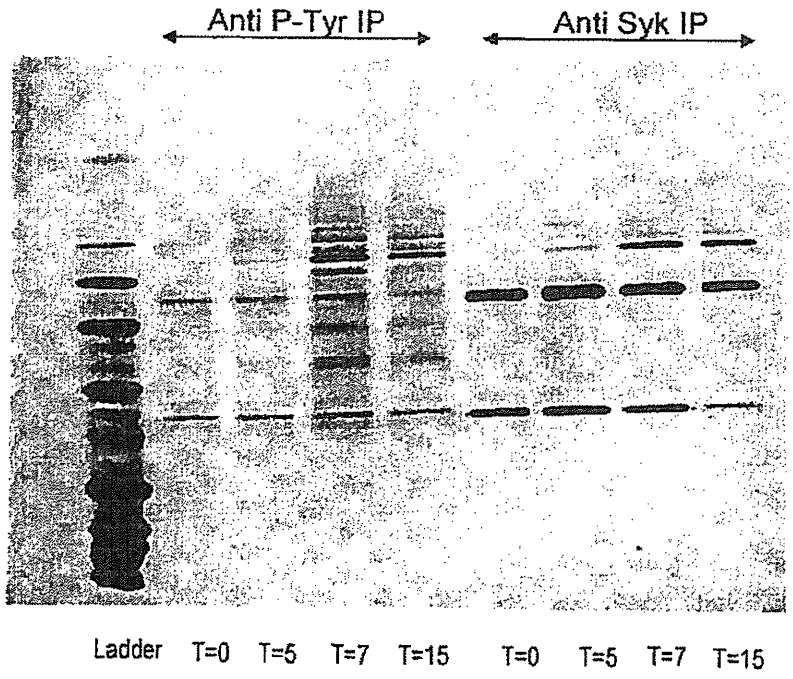

control
Rituximab
20-4x20-4
011x20-4
Staurosporine
control
Rituximab
20-4x20-4
011x20-4
Staurosporine
control
Rituximab
20-4x20-4
011x20-4
Staurosporine
4 hours
24 hours
48 hours

FIG. 60



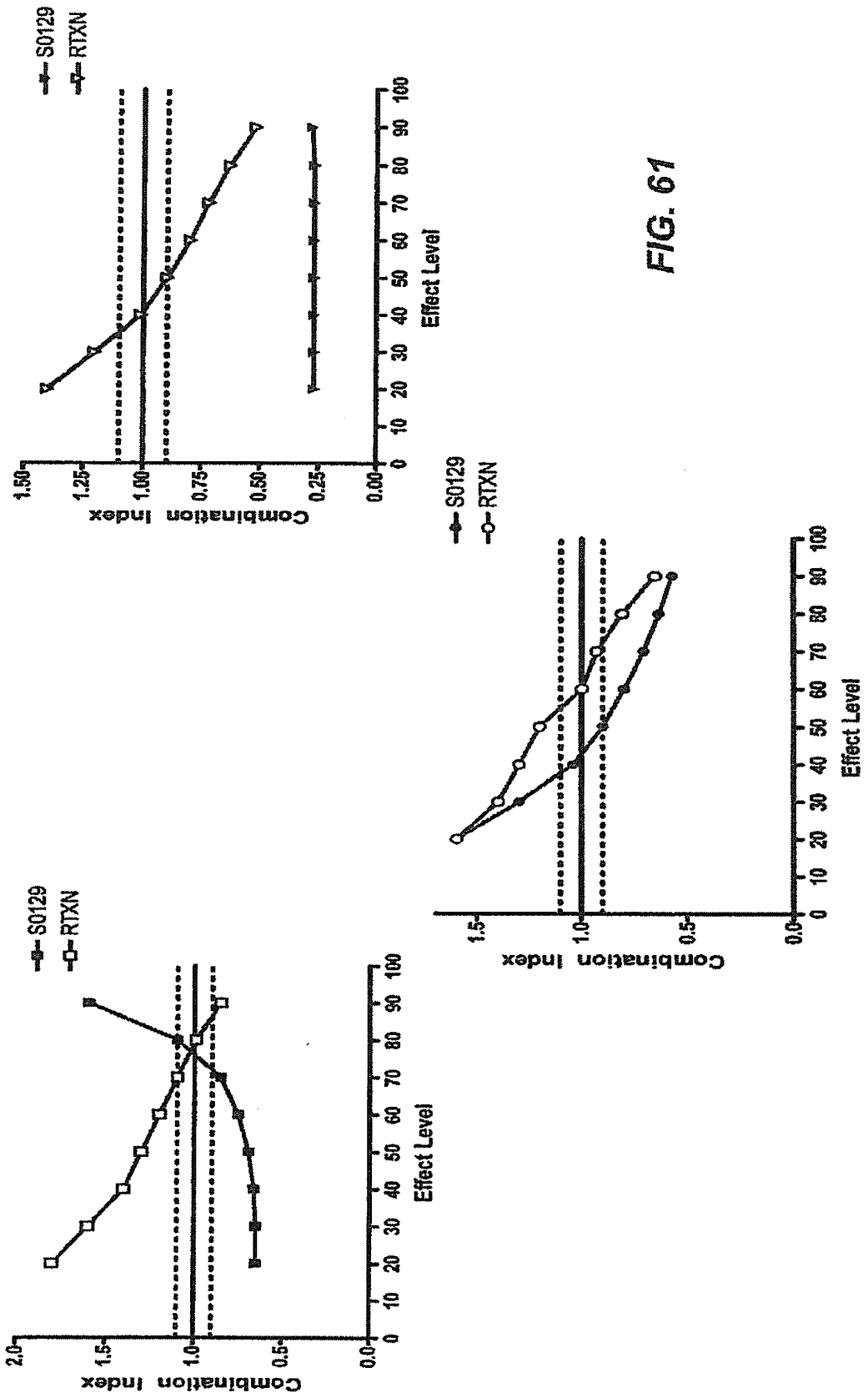

S0129
RTXN
Combination Index
Effect Level
0.0
0.5
1.0
1.5
2.0
0
10
20
30
40
50
60
70
80
90
100
0.00
0.25
0.50
0.75
1.00
1.25
1.50

FIG. 61