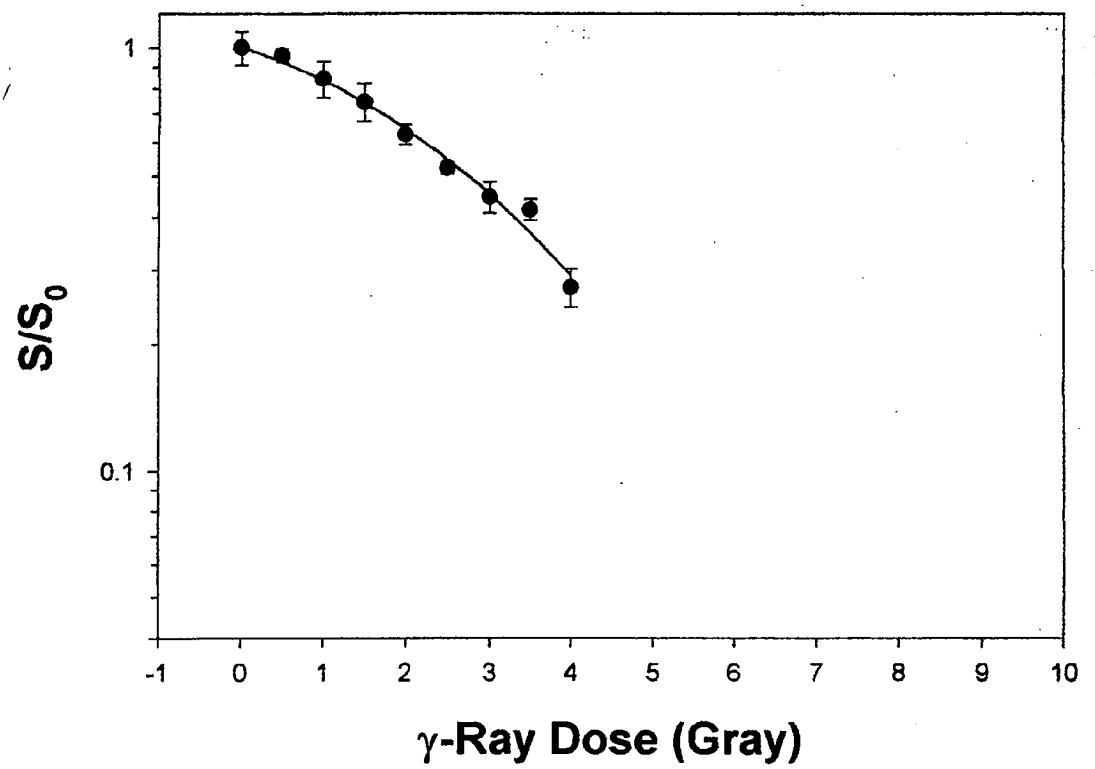
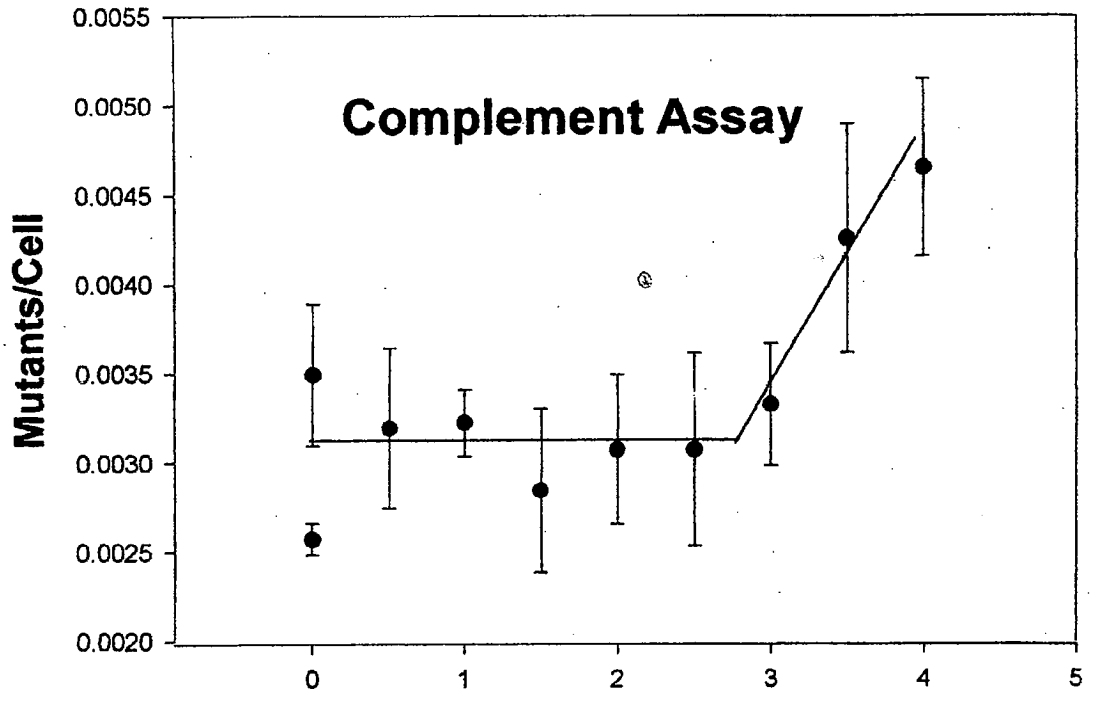
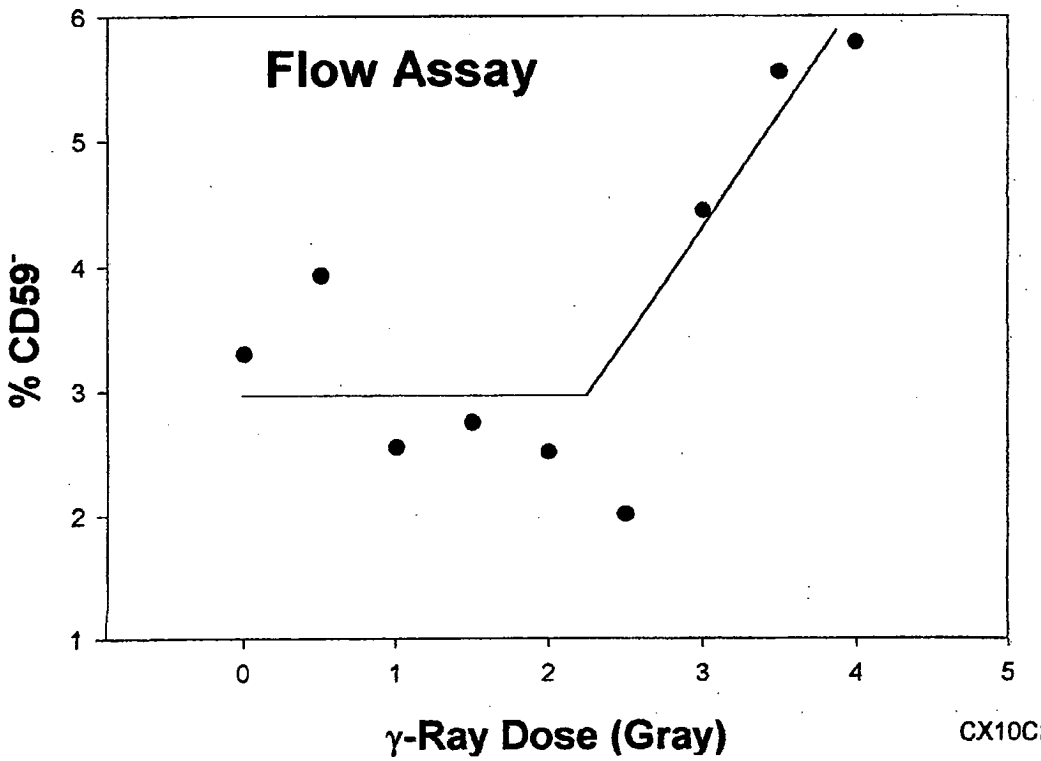
γ-Ray Survival of CX10
d0 = 6/15/00



Effect of γ-Rays on Mutation to CD59$^-$ in A_L-CX10 Cells Over-Expressing Connexin 43





CX10CD59mutants001106

B019844

CX10 Complement Assay Plated 10/31/00

Sample #	Col no C'						Col + C'						Mutant Colonies	PE no C'	std	PE + C'	std	PEC'/ PE no C'	Muants/cell	Std
1	50	52	60	44	44	58	62	65						0.540	0.0529	0.493	0.0924	0.9136	0.00257	8.600E-05
2	44	39	48	39	49		70	67	91	84	80	82		0.437	0.0451	0.453	0.0551	1.0382	0.00349	3.966E-04
3	35	46	45	55	51	49	71	87	72	98	70	84		0.420	0.0608	0.503	0.0503	1.1984	0.00319	4.472E-04
4	159	169	153	152	146	179	224	250	258	247	249	226		1.603	0.0808	1.503	0.0379	0.9376	0.00322	1.856E-04
5	43	42	36	38	61	40		76	65	48	62	69		0.403	0.0379	0.450	0.1389	1.1157	0.00284	4.608E-04
6	46	45	46	36	32	33	56	62	46	54	64	68		0.457	0.0058	0.380	0.0721	0.8321	0.00307	4.180E-04
7	36	36	36	34	46	22	61	58	77	49	49	62		0.360	0.0000	0.387	0.0643	1.0741	0.00307	5.362E-04
8	56	38	54	70	47	47	110	87	102	89	85	94		0.493	0.0987	0.570	0.1179	1.1554	0.00332	3.415E-04
9	45	46	61	57	28	50	99	116	126	81	101	98		0.507	0.0896	0.487	0.1801	0.9605	0.00425	6.434E-04
10	57	58	37	46	39	45	90	85	110	85	103	94		0.507	0.1185	0.407	0.0473	0.8026	0.00465	4.984E-04
																		1.00282		
																		Average	0.00310	

National® Brand 42-384 Made in U.S.A.

CX10 Mutations & Survivals

CX10	Gy	Survival Plate	Survival Exp.	Mutants Plate	Mutants Exp
1,2	0,0	100x2	70x2	1.5×10^5	10^5
3	0.5	100	70	1.5×10^5	10^5
4	1	150	70	2×10^5	10^5
5	1.5	200	70	2×10^5	10^5
6	2	200	70	2×10^5	10^5
7	2.5	250	70	2.5×10^5	10^5
8	3	300	90	3.3×10^5	10^5
9	3.5	600		5×10^5	10^5
10	4	1000	70	10^6	10^5

Need 3.1×10^6 cells — F12FC8 - F3S was not HI

2 P100's - subconfl farmed 6/9/00 — $185/4 \times 5 \times 10^4 = 2.3 \times 10^6$/ml

$\times 6\text{ml} = 1.4 \times 10^7$ Added 0.9ml → 2e6/ml

2e6 $\xrightarrow{0.115}$ 1.15e5 $\xrightarrow{0.1}$ 500 $\xrightarrow{15}$ 300 $\xrightarrow{17.5}$ 150 $\xrightarrow{26.7}$ 125 $\xrightarrow{24}$ 100 $\xrightarrow{15.75}$ 75 $\xrightarrow{13.3}$ 50

↓2	↓2	↓2	↓2	↓2	↓2	↓2
1000	600	300	250	200	150	100
x3	x3	x3	x3	x6	x3	x9
P35s						

P100's: 10ml F12FC8

2e6 $\xrightarrow{0.5}$ 10^6 x1

2e6 $\xrightarrow{0.075}$ 1.5e5 x3

2e6 $\xrightarrow{0.25}$ 5e5 x1

2e6 $\xrightarrow{0.1}$ 2e5 x3

2e6 $\xrightarrow{0.165}$ 3.3e5 x1

2e6 $\xrightarrow{0.125}$ 2.5e5 x1

Plated 6/15/00 11:00 AM

Irrad ~ 4:30 pm

6/20	Confl?			3ml	Doublings
1	~60%	$100/4 \times 5 \times 10^4 = 1.3 \times 10^6$	$\xrightarrow{0.2}$ 2.6×10^5	3.8×10^6	4.6
2	"	$168/4 \times 5 \times 10^4 = 2.1 \times 10^6$	$\xrightarrow{0.2}$ 4.2×10^5	6.3×10^6	5.4
3	"				
4	"				
5	"				
6	"				
7	"				
8	~70%				
9	~80%				
10	"				

Replated all @ 0.2ml/P100 in F12FC8 (~10^6)

B019846

CX10 Mutations & Survivals cont

P100's

6/23/00	All subconf	Fewer cells @ higher dose			Total cells x 3ml	doublings	~ total dbl
1	94/4 x 5x10⁴ = 1.2x10⁶	0.41 → 5x10⁵			3.6x10⁶	1.8	6.4
2	129/4 x 5x10⁴ = 1.6x10⁶	0.31 →			4.8x10⁶	2.3	7.7
3	119/4 x 5x10⁴ = 1.5x10⁶	0.35 →			4.5x10⁶	2.2	
4	111/4 x 5x10⁴ = 1.4x10⁶	0.36 →			4.2x10⁶	2.1	
5	102/4 x 5x10⁴ = 1.3x10⁶	0.38 →			3.9x10⁶	2.0	
6	128/4 x 5x10⁴ = 1.6x10⁶	0.31 →			4.8x10⁶	2.3	
7	78/4 x 5x10⁴ = 9.8x10⁵	0.51 →			2.9x10⁶	1.5	
8	42/9 x 5x10⁴ = 2.3x10⁵	2.17 →			6.9x10⁵	0	
9	59/4 x 5x10⁴ = 7.4x10⁵	0.68 →			2.2x10⁶	1.1	
10	38/4 x 5x10⁴ = 4.8x10⁵	1.04 →			1.4x10⁶	0.49	

6/27 Almost conf. Harvested in 1.5ml trip + 1.5ml PBS-A. Cent. Resusp in freezing medium 1.5ml F12FCI8 + 8% DMSO.

					x 3ml		
1	174/4 x 5x10⁴ =	2.2x10⁶			6.6x10⁶	2.2	8.6
2	172/4	2.2x10⁶			6.6x10⁶	2.2	9.9

10/18 Thaw, add to 2ml F12FCI8, cent., resusp in 10ml same. Transfer to P100s. may have lost ~ 50%

10/23/00 Replate: Harvest w. EDTA Rinse 2x w. 1ml PBS-A, 1.5ml EDTA, 1 min @ 37° into 1.5ml F12FCI8

					x 3ml	doublings	
Jumpers 1	244/4 x 5x10⁴ =	3.05x10⁶	0.33 → 10⁶		9.15e6	3.19	11.8
" 2	244/4 x 5x10⁴ =	3.05x10⁶	0.33 →		9.15e6	3.19	11.8
" 3	268/4	3.35	0.30 →		10.05e6		
" 4	282/4	3.53	0.28 →		10.59e6		
better* 5	300/4	3.75	0.27 →		11.25e6		
" 6	255/4	3.19	0.31 →		9.57e6		
worn " **7	164/4	2.05	0.49 →		6.15e6		
" 8	155/4	1.94	0.52 →		5.82e6		
9	222/4	2.78	0.36 →		8.34e6		
10	203/4	2.54	0.39 →		7.62e6		

* 75 bonks

** 100 "

w. higher dose more dead cells but maybe due to harder mixing

TOPS FORM 3616
LITHO IN U.S.A.

		CX10 Plated	dO =	6/15	F+S	6/23						
0,0	1,2	100	64	66	80	78	71	75	Av	72.3	±	6.5
0.5	3	100	72	67	69							
1	4	150	86	102	87							
1.5	5	200	97	108	119							
2	6	200	94	85	92							
2.5	7	250	95	97	91							
3	8	300	91	93	106							
3.5	9	600	169	186	186							
4	10	1000	175	201	216							

B019848

TOPS FORM 3616
LITHO IN U.S.A.

10/27/00

1. Rinse 3x w. PBS-A. Add 1.5ml EBTA to ea. dish. Stet 1-2 min. Transfer to 1.5 ml DPBS. Count. Replate @ 10^6 cells/plate.
2. Remove 10^6 cells to conical tubes. Cent. Wash 2x w. cold FACS buffer
3. Resusp. pellet in remainder
4. Add 0.5ml blocking buffer to ea. tube
5. Incubate on ice 30 min.
6. Wash 2x w. FACS buffer
7. Add 15λ isotype control or labelled Ab to ea. tube. Incub. on ice 30-60 min
8. Wash w. cold FACS buffer 2x
9. Add 5 ml paraformald. Incubate on ice 15 min, then store in fridge until 11/1/00.
10. Cent, resusp. in 0.1ml FACS buffer. Stet until 11/1/00. Cent, asp. SN, add 1ml 0.5% paraformaldehyde. Stet in fridge until 11/6.

	$(\times 10^4)$/ml	ml for 1×10^6	cells/ml x 3 Total cells	Doublings	Total Dbl
0. Neg	98	1.02	2.94e6	1.56	13.4
1	145	0.69	4.35e6	2.12	13.9
2	186	0.54	5.55e6		
3	186	0.56	5.4e6		
4	90	1.11	2.7e6		
5	80	1.25	2.40e6		
6	98	1.02	2.94e6		
7	121	0.83	3.63e6		
8	187	0.53	5.61x6		
9	164	0.61	4.92 x6		
10	156	0.64	4.68 x 6		
			Av (4.1 ± 1.22)e6		

B019849

TOPS FORM 3616
LITHO IN U.S.A.

10/31/00 Wash 2x w. PBS-A. Stet 2min EDTA Rm Temp. Transf. to 2ml F12FCI8

			CD59		HPRT		Replate
1	1.45×10^6	0.22 / 13 →	2.5×10^4 →	66.7	0.75 / 27 →	4×10^4	0.69 → 10^6
			↓2	↓1.5		↓5	
			5×10^4	100		2×10^5	
			×6 P60's	×6 P35's		×5 P100's	
2	1.68×10^6	0.19 / 13 →			0.64 →		0.59 →
3	1.60×10^6	0.20 / 13 →			0.675 →		0.62 →
4	0.43×10^6	0.75 / 13 →			2.51 →		~~2.3~~ → Remainder = 0.5ml
5	1.80×10^6	0.18 / 13 →			0.60 →		0.55 →
6	1.80×10^6	0.18 / 13 →			0.60 →		0.55 →
7	2.15×10^6	0.15 / 13 →			0.50 →		0.46 →
8	1.68×10^6	0.19 / 13 →			0.64 →		0.59 →
9	1.88×10^6	0.17 / 12 →			0.57 →		0.53 →
10	1.60×10^6	0.20 / 13 →			0.675 →		0.62 →

Incubate dishes 2 hrs. Add 5ml F12FCI8 containing

For HPRT: ~~3.50~~ ~~0.70~~ ml 6TG in ~~260~~ ~~52~~ ml F12FCI8. Add 5ml to ea. P100 x 50 @ ~2h

For CD59: ~~2.55~~ ~~2.72~~ ml C': Add 24λ to 30 P35's leaving ~~1.83~~ ~~2.0~~ ml C'

Add ~~0.34~~ ~~0.37~~ ml Ant S$_1$ to 2.0ml C'.

Add ~~35~~ ~~38~~λ of mixture to ea P60 x 60

Ran out @ 4 P60's of #1 have no C'-Ab.

11/2/00: Chg med on P35's to 2ml F12FCI9

Chg med on P60's to 5ml F12FCI9

Wed	11/8	F+S	P35's
Fri	11/10	F+S	P60's

B019850

Experiments: CY/10 + X-rays (Lenie exp)

Date: Oct. 31, 2000

Sample	Cell counting	$\bar{x} \times 10^4$/ml	stock susp. conc. $\times 10^5$/ml	Total in 4 ml.	HPRT 2×10^5 (in 8 µl)	HPRT 2.5×10^4 (ml)		(ml)
1	27, 31	29	14.5	5.8×10^6	~~138~~	0.75	~~17~~	34 0,22
2	24, 43	33.5	16.8	6.7×10^6	~~119~~	0.64	~~15~~	~~80~~ 0.1
3	34, 30	32	16.0	6.4×10^6	~~125~~	0.675	~~16~~	~~52~~ 0,2
4	10, 7	8,5	4,3 !	1.7×10^6	~~465~~	!2.51	~~58~~	~~116~~ 0.5
~~5~~	38, 34	36	18.0	7.2×10^6	~~144~~	0.60	14	~~28~~ 0,1
~~6~~	30, 42	36	18.0	7.2×10^6	~~144~~	0,60	14	28 0,1
~~7~~	37, 49	43	21,5	$8.6 \times$		0.50		0,1
~~8~~	26, 41	34	16.8	6.7		0.64		0,1
~~9~~	30, 45	38	18.8	7.5		0.57		0,1
10	27, 37	32	16.0	6.4		0.675		0.20

TOPS FORM 3616
LITHO IN U. S. A.

11/3/00 Harvest w. EDTA & replate

						Total Cells in 3ml
1	124/4	X 5×10^4 =	1.55e6	0.32 →	5×10^5	4.65×10^6
2	113		1.41 e6	0.35 →		4.23
3	146		1.83 e6	0.28 →		5.49
4	132		1.65e6	0.31 →		4.95
5	126		1.58 e6	0.32 →		4.74
6	85		1.06e6	0.47 →		3.18
7	80, 96		1.10 e6	0.46 →		3.30
8	105		1.31e6	0.38 →		3.93
9	149		1.86e6	0.27 →		5.58
10	99		1.24e6	0.41 →		3.72
S_1^-	178/4 (OG)	X 1×10^4 =	0.445e6	1.125 →		1.34

B019852

TOPS FORM 3616
• LITHO IN U. S. A.

CX10 C' assay - Plated 10/31/00, F+S 11/10/00

	Only counted colonies ~ > 1mm					
1	62	65	no C' →			
2	70	67	91	84	80	82
3	71	87	72	98	70	84
4	224	250	258	847	249	226
5	C	76	65	48	62	69
6	56	62	46	54	64	68
7	61	58	77	49	49	62
8	110	87	102	89	85	94
* 9	99	116	126	>81	>101	98
10	90	85	110	85	103	94

P35 Clusters

	No C' →			C'		
four tracks 1	50	52	60	44	44	58
2	44	39	48	39	49	C
3	35	46	45	55	51	49
4	159	169	153	152	146	179
5	43	42	36	38	61	40
6	46	45	46	36	32	33
7	36	36	36	54	46	22
8	56	38	54	70	47	47
9	45	46	61	57	28	50
10	57	58	37	46	39	45

11/13/00 CX10 HPRT assay - Plated 10/31/00: There are almost no colonies on these dishes - ∴ discarded

CX10 - Replated 10/27, fed 11/3 discarded - not worth reading the rest

* ? Not well mixed - had an area of confluence

Procedure for direct labeled monoclonal antibody staining of A_L cells

1. To harvest cells: (NB There should be one dish for the negative control) Remove media and rinse several times with PBSA. Dissociate with EDTA (5 ml/100 mm plate). Incubate 1-2 minutes. Remove cells by pipetting gently with a Pasteur pipette. Add cells to a tube containing an equal volume of DPBS (with Ca++ and Mg++!). Count cells in the fluorescence microscope with hemocytometer in fluorescein diacetate (Sigma) to check viability*. 10^6 cells are needed for each sample.). 5m EDTA
2. Centrifuge for 5 min at slow speed.
3. Aspirate supernatant and re-suspend cells in cold FACS buffer* to give a cell concentration of 10^6 cells/ml. For each sample and controls, add 1 ml of cells to a separate, 15-ml conical tube. (From this point on, cells must be kept on ice!)
4. Repeat centrifugation for 5 minutes at 4 degrees C.
5. Aspirate supernatant as thoroughly as possible and gently tap the bottom of the tube to re-suspend the cells in the residual buffer.
6. Add 0.5 ml of blocking buffer* to each. Incubate on ice for 30 min.
7. Wash 2X in FACS buffer. Repeat 5.
8. Add labeled antibody* (20ul) or isotype control mixing well by pipetting up and down and incubate on ice for 30-60 min. 15
9. Wash twice in 2 ml cold FACS buffer. (Spin at slow speed for 5 minutes) 0.5ml
10. Slowly add 5 ml of cold 0.5% paraformaldehyde* to the pellet while ***gently*** vortexing.
11. Incubate on ice at least 15 minutes. Samples may be refrigerated overnight in paraformaldehyde.
12. Centrifuge for 5 min and re-suspend in 100 ul cold FACS buffer.
13. Pipette sample into tubes for flow cytometry. Keep samples refrigerated until flow is run. (up to 1 week is OK)

Recipes*:

1. Fluorescein diacetate: Dissolve in acetone at 2 mg/ml. Add 1-4 ul to 98ul PBS for working solution.
2. FACS buffer: 1% BSA, 10 mM Sodium Azide in DPBS.
3. Blocking buffer: 0.375ml mouse IgG in 7.125 ml FACS buffer. for 13+ tubes
4. 0.5 % Paraformaldehyde: Make fresh each day. Wear gloves and mix in the hood. Make 1% solution in water. Put in 65-degree water bath for about 1 hour to solubilize powder. Add 0.4N NaOH drop wise until the paraformaldehyde is completely dissolved. Add an equal volume of 2X PBS (pH 6.5) to give a final concentration of 0.5% paraformaldehyde.
5. The amount of antibody to use depends on the product and titration experiments. For H19 PE labeled (Pharmingen cat# 33865X, page 37) – add 20 ul 15

For each experiment:

1. Auto-fluorescence control: Unstained test cells.
2. Isotype control test cells
3. Negative control: A_L/S_1^R

Samples:

1. *Unstained, untreated, blocked, fixed*
2. Unirradiated, blocked, fixed – isotype control
3. A_L/S_1^R negative control
4. Remaining samples

TOPS FORM 3616
LITHO IN U. S. A.

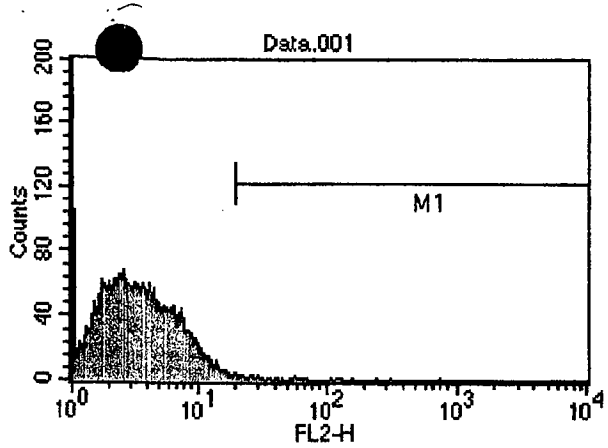
Do 5, 7.5, 10

			Not M1
1	unstained - untreated		99.35
2	ctrl - CD59+		
3	isotype - PE		97.1
4	neg - CD59 -		
5	12	CD59+	
6	3		
7	4		
8	5		
9	6		
10	7		
11	8		
12	9		
13	10		

14 mix #4 & #2 [1:1]

CHO → CD59 (human) (1° stain)

96.7
2.9
4.2% ← neg within CD59+

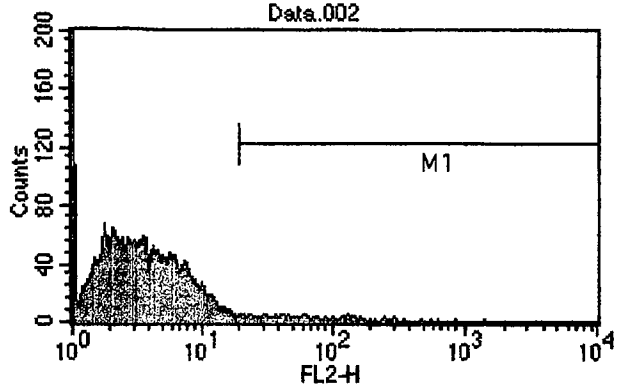


File: Data.001
Acquisition Date: 06-Nov-00
X Parameter: FL2-H (Log)

Log Data Units: Linear Values
Total Events: 10000

Marker	Left, Right	Events	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	4.32	3.31	209.98	3.12
M1	19, 9910	65	0.65	56.67	37.27	166.66	27.38

unstained - untreated

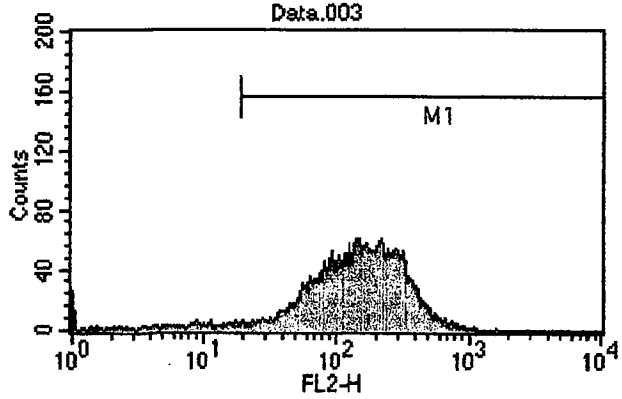


File: Data.002
Acquisition Date: 06-Nov-00
X Parameter: FL2-H (Log)

Log Data Units: Linear Values
Total Events: 10000

Marker	Left, Right	Events	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	5.98	3.59	359.28	3.25
M1	19, 9910	290	2.90	68.37	47.27	158.02	40.32

Isotype control

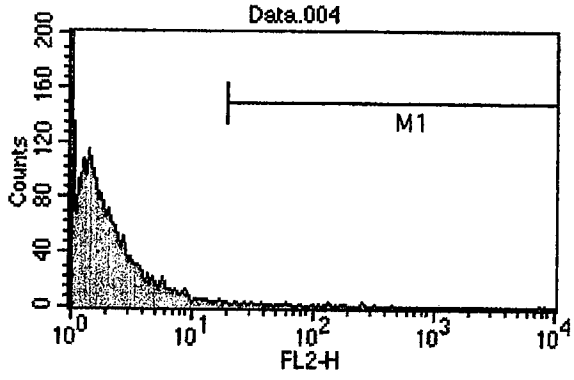


File: Data.003
Acquisition Date: 06-Nov-00
X Parameter: FL2-H (Log)

Log Data Units: Linear Values
Total Events: 10000

Marker	Left, Right	Events	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	175.80	130.70	72.16	147.22
M1	19, 9910	9670	96.70	181.52	145.04	68.92	152.61

$CD59^+$ = CX10

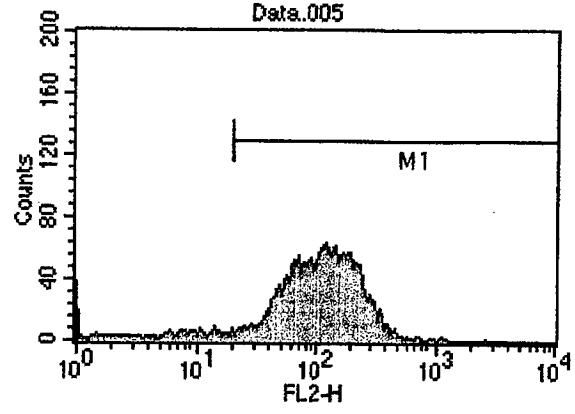


File: Data.004
Acquisition Date: 06-Nov-00
X Parameter: FL2-H (Log)

Log Data Units: Linear Values
Total Events: 10000

Marker	Left, Right	Events	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	4.35	1.97	2396.87	1.67
M1	19, 9910	81	0.81	253.84	58.38	448.21	39.95

Neg ctrl = A_L/S_1-

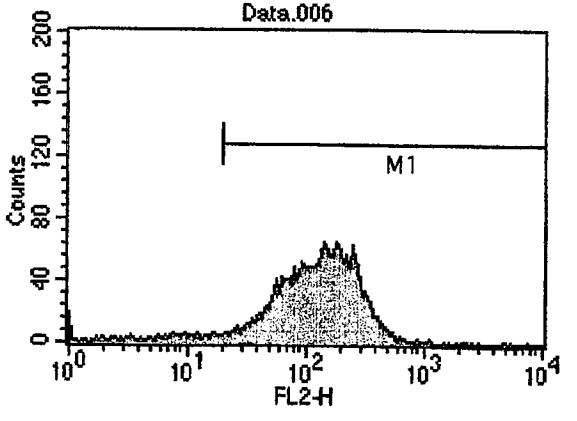


File: Data.005
Acquisition Date: 06-Nov-00
X Parameter: FL2-H (Log)

Log Data Units: Linear Values
Total Events: 10000

Marker	Left, Right	Events	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	121.78	90.33	72.85	103.66
M1	20, 9910	9428	94.28	128.69	106.78	67.34	109.41

CD59+: 0 dose

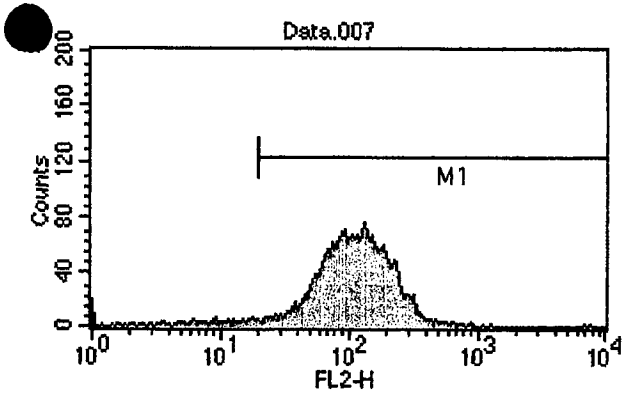


File: Data.006
Acquisition Date: 06-Nov-00
X Parameter: FL2-H (Log)

Log Data Units: Linear Values
Total Events: 10000

Marker	Left, Right	Events	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	147.96	110.24	101.87	125.21
M1	19, 9910	9607	96.07	153.66	123.69	98.31	129.80

0.564

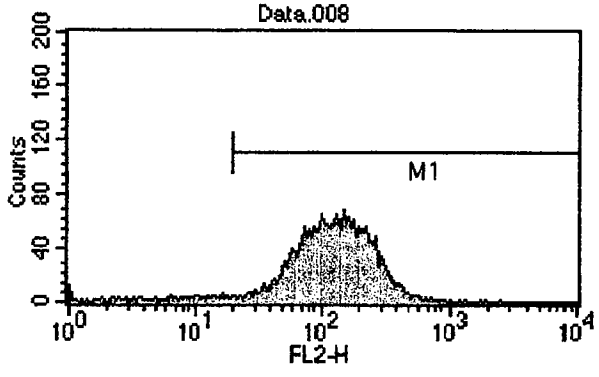


File: Data.007
Acquisition Date: 06-Nov-00
X Parameter: FL2-H (Log)

Log Data Units: Linear Values
Total Events: 10000

Marker	Left, Right	Events	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	127.16	102.41	95.99	108.43
M1	20, 9910	9745	97.45	130.23	109.61	93.78	111.40

1.0 Gy

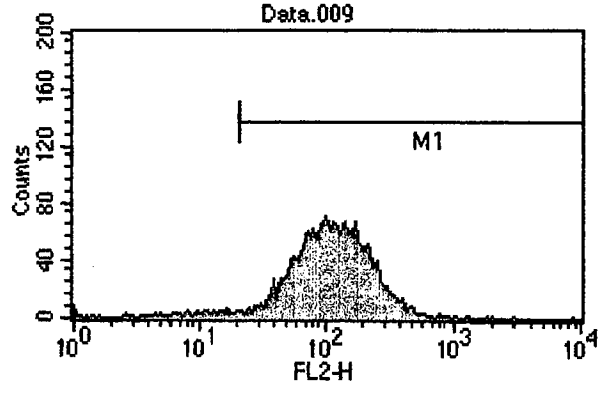


File: Data.008
Acquisition Date: 06-Nov-00
X Parameter: FL2-H (Log)

Log Data Units: Linear Values
Total Events: 10000

Marker	Left, Right	Events	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	138.14	109.23	70.90	115.48
M1	19, 9910	9725	97.25	141.77	117.70	68.33	118.64

1.5 Gy

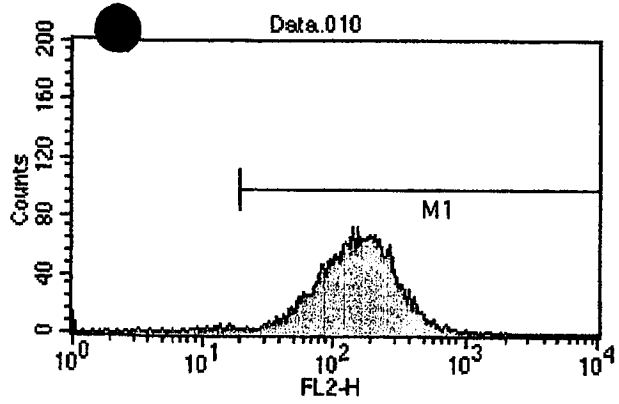


File: Data.009
Acquisition Date: 06-Nov-00
X Parameter: FL2-H (Log)

Log Data Units: Linear Values
Total Events: 10000

Marker	Left, Right	Events	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	126.68	101.20	94.59	105.54
M1	20, 9910	9749	97.49	129.65	107.58	92.48	107.46

2.0 Gy

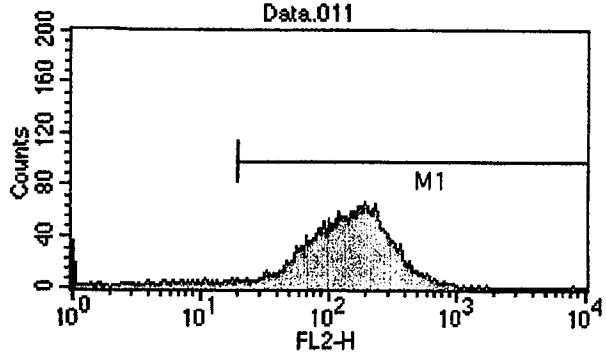


File: Data.010
Acquisition Date: 06-Nov-00
X Parameter: FL2-H (Log)

Log Data Units: Linear Values
Total Events: 10000

Marker	Left, Right	Events	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	166.70	133.49	68.38	143.30
M1	19, 9910	9799	97.99	169.92	141.60	66.43	144.60

2.5 Gy

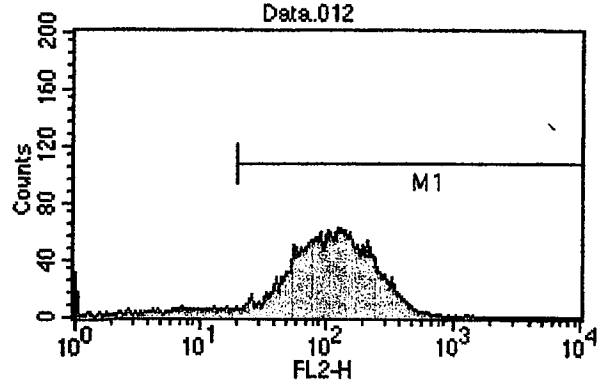


File: Data.011
Acquisition Date: 06-Nov-00
X Parameter: FL2-H (Log)

Log Data Units: Linear Values
Total Events: 10000

Marker	Left, Right	Events	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	163.63	119.93	85.78	137.00
M1	19, 9910	9555	95.55	170.86	137.73	81.60	142.02

3.0 Gy

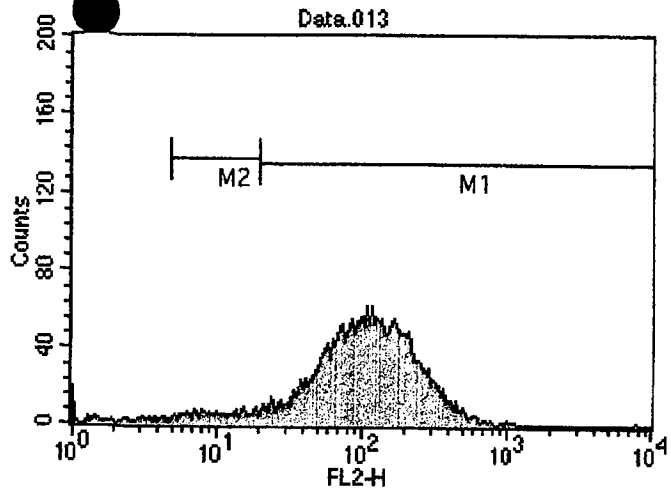


File: Data.012
Acquisition Date: 06-Nov-00
X Parameter: FL2-H (Log)

Log Data Units: Linear Values
Total Events: 10000

Marker	Left, Right	Events	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	124.34	91.73	73.83	101.82
M1	19, 9910	9445	94.45	131.14	107.07	68.57	107.46

3.5 Gy

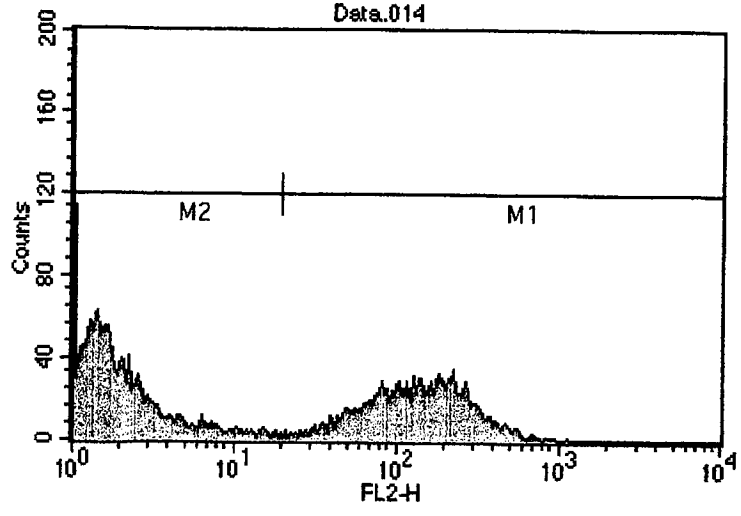


File: Data.013
Acquisition Date: 06-Nov-00
X Parameter: FL2-H (Log)

Log Data Units: Linear Values
Total Events: 10000

Marker	Left, Right	Events	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	130.76	94.09	94.99	105.54
M1	20, 9910	9421	94.21	138.23	110.04	89.81	111.40
M2	5, 20	451	4.51	11.24	10.44	37.62	10.55

4.064



File: Data.014
Acquisition Date: 06-Nov-00
X Parameter: FL2-H (Log)

Log Data Units: Linear Values
Total Events: 10000

Marker	Left, Right	Events	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	75.86	13.54	142.95	5.52
M1	20, 9910	4621	46.21	161.34	130.03	67.49	137.00
M2	1, 20	5379	53.79	2.42	1.94	95.20	1.65

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