

V79 COLONY FORMING ASSAY FOLLOWING FACS

Experiment Name: Cell separation by FACS and SF ($^3\text{HTdR}$ cluster, 50% labeling, five $^3\text{HTdR}$ conc.)

Exp. # 1;

Investigator: A. Bishayee

Date: 03/08/01

1. Set the rocker-roller at 37°C incubator with 5% CO₂, set the Coulter Counter, wash cells (from two 80-90% confluent 175 cm² flasks, subcultured 4-5 days before) with PBS, trypsinize cells, each resuspend in 7 ml MEMB, pool, pass five times through 5 or 10 cc syringe with 21 gauge needle, perform cell count by transferring 100 ul in Coulter cup containing 20 ml isotone (Coulter balanced electrolyte solution)
2. Dilute to ~2,000,000 cells/ml in MEMB [Actual count : 5,200,000 cells/ml]
3. Transfer 1 ml of cell suspension into two sets of tubes (7 tubes per set; Falcon plastic test tube, 17x100 mm)
4. Keep the tubes in the roller for 3-4 h at 37°C, 5% CO₂
5. Prepare MEMB containing radioactivity in hood
60 μl $^3\text{HTdR}$ (Stock : 1 $\mu\text{Ci}/\mu\text{l}$ on 02/05/01) + 3 ml MEMB
6. After 3-4 h, remove first set of tubes from roller and add MEMB with or without radioactivity according to Table below.

Date/Time: 03/08/01
3-00 pm

Date/Time: 03/08/01

7-00 pm

Tube #	$^3\text{HTdR}$ uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ $^3\text{HTdR}$ 20uCi/ml (ml)	CFDA in PBS (1 uM) (ml)
1	0	1.0	1.0	0	2
2	0	1.0	1.0	0	2
3	0.1	1.0	0.99	0.01	2
4	0.5	1.0	0.95	0.05	2
5	2	1.0	0.8	0.2	2
6	5	1.0	0.5	0.5	2
7	10	1.0	0	1	2

7. Add 1 ml of MEMB tubeReturn test tubes to roller for 14 h.
8. Next day, while test tubes are in roller label tubes (13 X 100 mm VWR glass test tube)

Date/Time: 03/08/01

7-30 pm

9. After ~14 h incubation period, remove tubes and centrifuge at 2000 rpm at 4°C for 10 min (precooled centrifuge).

Date/Time: 03/09/01; 8-30 a.m.

10. Remove buckets from centrifuge and carefully remove 150 µl of supernatant and place in prelabeled tubes.

11. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA

12. Centrifuge tubes for 10 min at 2000 rpm, 4°C

13. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA

14. Centrifuge tubes for 10 min at 2000 rpm, 4°C

15. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA

16. Centrifuge tubes for 10 min at 2000 rpm, 4°C

17. Decant supernatant, click tubes, vortex

18. Add 8 ml of PBS in each tube, vortex and transfer the content to 15-ml plastic centrifuge tube

19. Centrifuge tubes for 10 min at 2000 rpm, 4°C

20. Decant supernatant, click tubes, vortex

21. Add 2 ml of 1 µM CFDA in prewarmed PBS as per the Table and PBS in the remaining tubes.

22. Incubate all tubes at 37°C for 15 min.

23. Centrifuge tubes for 10 min at 2000 rpm, 4°C

24. Decant supernatant, click tubes, vortex, add 2 ml prewarmed MEMA

25. Incubate all tubes at 37°C for 30 min.

26. Centrifuge and decant the supernatant, suspend in 5 ml MEMA

27. Follow steps 11-24 for second set of tubes

28. Transfer the content of one tube from one set to the corresponding tube of another set

29. Centrifuge, decant the supernatant

30. Transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 µl) using 200 µl pipet tips

31. Again add 200 µl MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 µl)

32. Centrifuge tubes for 5 min at 1000 rpm, 4°C

30. Transfer tubes at 10°C for 72 h.

Date/Time: 03/09/01; 11-30 a.m.

33. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 µl wash MEMA and transfer the content to eight 15 ml tubes containing 10 ml PBS by using pasteur pipet

Date/Time: 03/09/01; 1-30 p.m.

34. Again add 200 µl PBS in microcentrifuge tubes, resuspend and transfer the cell suspensions in 15 ml tubes

35. Centrifuge the tubes for 10 min at 2000 rpm, 4°C (*precooled centrifuge*)
36. Decant supernatant, click tubes, vortex, pooled cells from corresponding tubes, centrifuge, decant the supernatant, resuspend in 2 ml PBS with, syringe and transfer aliquots for cell count (100 ul) and radioactivity count (50 ul)
37. Centrifuge, decant, resuspend in 1 ml PBS for each tube and transfer ~1ml in Falcon 12x75 mm polystyrene 6 ml tube, wrap the tubes with aluminium foil, put in ice and transfer for FACS study.
38. During sorting, collect both dye-positive and dye-negative cells in VWR 12x75 mm glass tube (pre-cooled in ice) containing 1 ml PBS with 100 U penicillin and 100 µg streptomycin (add 20 µl Pen-Strep from the commercial stock in 1 ml PBS to get the desired concentrations).
39. Transfer cells in PBS in 15-ml plastic centrifuge tube, add 7 ml of PBS, and centrifuge
40. Decant, vortex, resuspend in 1 ml of PBS, and transfer 100 µl for cell count
41. Transfer 300 µl in Falcon 12x75 mm polystyrene 6 ml tube for FACS analysis to check the purity of the sorted cells.
42. Dilute remaining cells (three 10-fold dilution by transferring 0.5 ml cells to 4.5 ml MEMA)
43. Plate required number of cells (200, 2000 or 20,000) in Falcon 60 mm tissue culture dish (in 4 ml total volume of MEMA).
44. Count colonies following a week.

Parameters

Date

3/8/01

Experiment No.

H-3/50%/FACs1

Investigator

A. Bishayee

Cell Line

V79

Modifier

None

Radionuclide

H-3

Half-life (days)

4500.45

Radiation Yield

1

Radiochemical

3HdThd

Manufacturer/Lot

NEN

Original Calibration Date/Time

2/15/01 0:00

Present Calibration Date/Time

3/8/01 0:00

Fraction of Cells Labeled

0.5

I-125=59,408, H-3=4500.45, Po-210=138,376, I-131=8.02
I-125=1.47, H-3=1.0, Po-210=1.0, I-131=8.02

Original Activity Concentration (MBq/ml)

Time Elapsed Since Original Calibration (d)

Present Activity Concentration (MBq/ml)

Liquid Scintillation Cocktail

Volume of LSC Cocktail (ml)

Volume/Type Counting Vial

Model of Counter

Counting Efficiency

Ecolume
6
6 ml plastic
Beckman
0.65

Activity Added (Date/Time)

Cells Washed (Date/Time)

Medium Tubes Counted (Date/Time)

Cell Tubes Counted (Date/Time)

Vol. Supernatant Counted (ul)

Vol. Suspension Counted Cell Activity (ul)

3/8/01 19:30
3/9/01 8:30
3/9/01 11:00
3/13/01 14:14 14:57
30
50

Time Elapsed Between Add and Wash (hr)
Time Elapsed Between Add and Count (hr)
Time Elapsed Between Wash and Count (hr)

103

Vol. Suspension Coupler (ul)

Coupler Manometer Volume (ul)

Average Coupler Background Counts

Coupler Calibration Parameter

Hemocytometer Count (Yes or No)?

100
50
2.333333333
400
No

Background
Coupler 1 Coupler 2 Coupler 3

SER: 6 ID: H3 HOWELL PRESET TIME: 1.00 FRI 09 MAR 2001 11:00
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR: N RS232: N
 H#: 1 ACC: N DCF: N RCM: N
 CHANNEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
 DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000
 HALF LIFE (DAYS): N

SAM	POS	CH	CPN	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	12.00	57.74	1.00	1.47	77.0	
2	**	2	9.00	66.67	1.00	3.04	75.0	
3	**	3	10.00	63.25	1.00	4.62	76.0	
4	**	4	11.00	60.30	1.00	6.18	76.0	
5	**	5	7.00	75.59	1.00	7.76	79.0	
6	**	6	8.00	70.71	1.00	9.33	74.0	
7	**	7	2299.00	4.17	1.00	10.90	77.0	
8	**	8	2278.00	4.19	1.00	12.53	79.0	
9	**	9	2413.00	4.07	1.00	14.15	79.0	
10	**	10	40634.74	1.99	0.95	15.73	78.0	
11	**	11	10910.53	1.96	0.95	17.24	78.0	
12	**	12	10894.74	1.97	0.95	18.77	77.0	
13	**	13	42052.00	1.95	0.25	19.57	79.0	
14	**	14	47168.00	1.84	0.25	20.37	80.0	
15	**	15	44984.00	1.89	0.25	21.17	81.0	
16	**	16	110426.66	1.55	0.15	21.87	83.0	
17	**	17	121740.00	1.81	0.10	22.58	83.0	
18	**	18	112953.33	1.54	0.15	23.28	81.0	
19	**	1	167833.33	1.26	0.15	24.04	78.0	
20	**	2	214013.33	1.12	0.15	24.76	77.0	
21	**	3	192173.33	1.18	0.15	25.47	76.0	

Am going,
No change this time
Clarey

TABLE-1

Expt. #: 1

Date/Time: 03/09/00; 11-00 a.m.

Tube #	Medium count for 30 ul (cpm)	Avg. cpm	dpm [cpm/0.65]	$\mu\text{Ci/ml (A)}$ on counting [dpm/66600]	$\mu\text{Ci/ml (A}_0)$ on addition [A/e ^{-λt}]
1		} 10			
2					
3		2320	3569	0.05	
4		10802	16619	0.25	
5		44724	68807	1.03	
6		115029	176968	2.65	
7		191329	294353	4.42	
8					
9					
10					

MediumActivity

Experiment: H-3/50%/FACS1
 Date: 3/8/2001

Tube #	1st	2nd	3rd	CPM Average	CPM corrected for control	DPM (CPM/y e)	At μ Ci/ml on counting	Ao μ Ci/ml at addition	Ao KBq/ml at addition
1	12	9	10	10	0	0	0	0	0
2	11	7	8	0	0	0	0	0	0
3	2299	2278	2413	2330	2321	3570	0.0536	0.0536	1.9835
4	10634	10910	10894	10813	10803	16620	0.2496	0.2496	9.2344
5	42052	47168	44984	44735	44725	68808	1.0332	1.0333	38.2304
6	110426	121740	112953	115040	115030	176969	2.6572	2.6575	98.3262
7	167833	214013	192173	191340	191330	294354	4.4197	4.4202	163.5463
8				#DIV/0!	0	0	0	0	0
9				#DIV/0!	0	0	0	0	0
10				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
11				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
12				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
13				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
14				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

[At/e-0.693t/T]

50 µl cells

PAGE: 1

COUNT ID: H3 HOWELL PRESET TIME: 1.00 TUE 13 MAR 2001 14:57
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR: N R5232: N
 NS: 1 AGC: N QCF: N RCM: N
 CHANNEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
 DATA CAL: CPM, UNKNOWN REPLICAS: 3: 1 NORM FACTOR: 0 1.00000
 HALF LIFE (DAYS): N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	9.00	66.67	1.00	1.42	76.0	
2	**	2	5.00	89.44	1.00	3.00	76.0	
3	**	3	13.00	55.47	1.00	4.62	73.0	
4	**	4	7.00	75.59	1.00	6.18	76.0	
5	**	5	9.00	66.67	1.00	7.76	74.0	
6	**	6	6.00	81.65	1.00	9.33	74.0	
7	**	7	842.00	6.59	1.00	10.95	73.0	
8	**	8	842.00	9.74	1.00	12.52	73.0	
9	**	9	363.00	10.50	1.00	14.08	75.0	
10	**	10	1309.00	5.53	1.00	15.65	74.0	
11	**	11	1267.00	5.62	1.00	17.28	75.0	
12	**	12	1407.00	5.33	1.00	18.85	75.0	
13	**	13	4252.00	3.07	1.00	20.47	77.0	
14	**	14	4621.00	2.94	1.00	22.10	73.0	
15	**	15	3123.00	3.58	1.00	23.67	74.0	
16	**	16	8303.00	2.19	1.00	25.23	80.0	
17	**	17	7380.00	2.34	1.00	26.87	78.0	
18	**	18	7426.00	2.32	1.00	28.44	79.0	
19	**	1	21660.00	1.91	0.50	29.55	90.0	
20	**	2	21438.00	1.93	0.50	30.61	89.0	
21	**	3	22735.55	1.98	0.45	31.62	92.0	

CellSuspension

Experiment: H-3/50%/FACS1
 Date: 03/08/01

Tube #	Suspension count (CPM)			CPM Average	CPM corrected for control	DPM (CPM/v e)	A _i μCi/ml on counting	A _o μCi/ml after uptake	A _o KBq/ml after uptake
	1st	2nd	3rd						
1	9	5	13	8	0	0	0.00000	0	0.0000
2	7	9	6	542	0	0	0.00000	0	0.0000
3	842	422	363	1328	534	822	0.00740	0.00741	0.2741
4	1309	1267	1407	1328	1320	2030	0.01829	0.01830	0.6770
5	4252	4621	3123	3999	3991	6139	0.05531	0.05534	2.0475
6	8303	7280	7428	7670	7662	11788	0.10620	0.10625	3.9314
7	21860	21438	22735	22011	22003	33851	0.30496	0.30512	11.2894
8				#DIV/0!	0	0	0.00000	0	0.0000
9				#DIV/0!	0	0	0.00000	0	0.0000
10				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
11				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
12				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
13				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
14				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

CoulterSurvival

Experiment: H-3/50%/FACS1
 Date/Time: 3/8/01

Tube #	Coulter count			Average	Cells/ml	Hemocytometer Count in Grid			
	1st	2nd	3rd			1st	2nd	3rd	4th
1	566	573	582	574	2285333				
2	619	645	634	633	2521333				
3	667	662	639	656	2614667				
4	630	619	636	628	2504000				
5	664	649	655	656	2614667				
6	533	522	519	525	2089333				
7	644	632	621	632	2520000				
8				#DIV/0!	#DIV/0!				
9				#DIV/0!	#DIV/0!				
10				#DIV/0!	#DIV/0!				
11				#DIV/0!	#DIV/0!				
12				#DIV/0!	#DIV/0!				
13				#DIV/0!	#DIV/0!				
14				#DIV/0!	#DIV/0!				

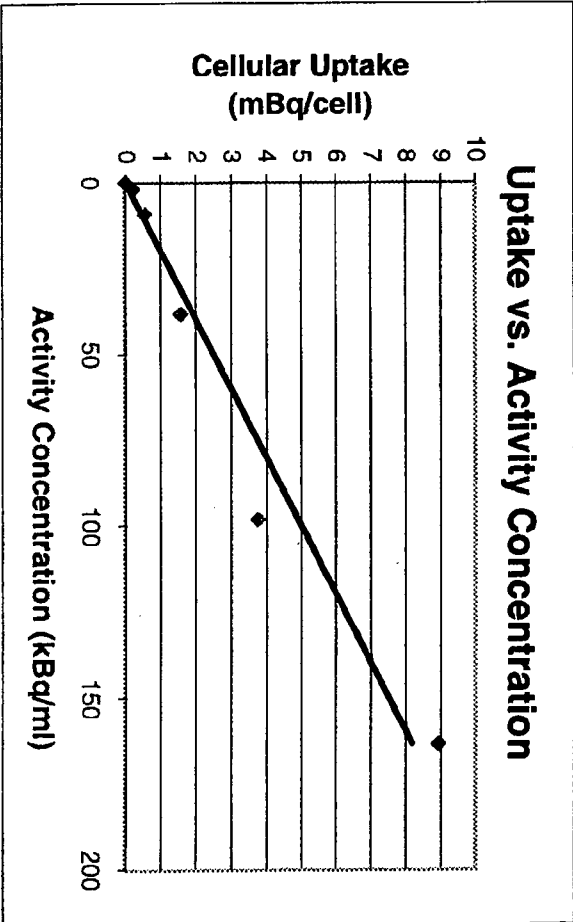
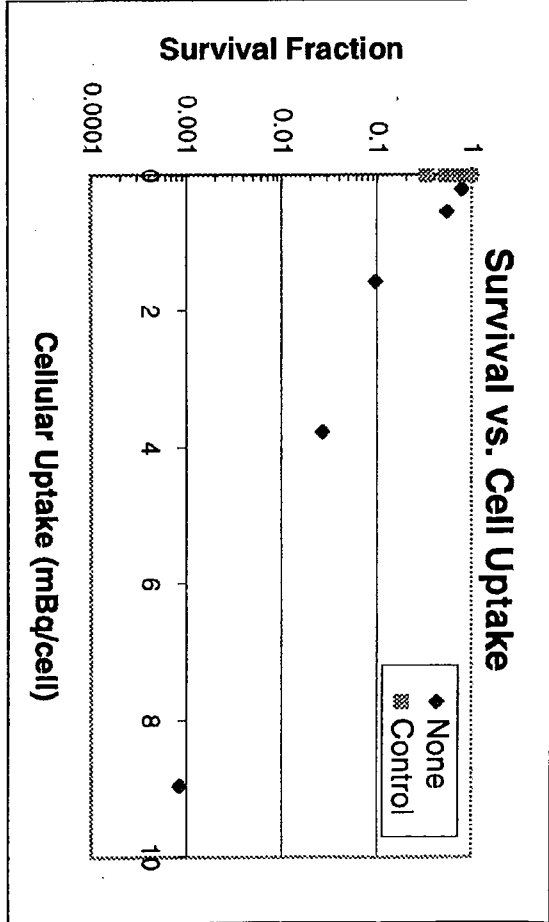
0.47
 0.58 } 0.59
 0.72
 0.60

Tube #	Predicted # Cells Seeded	Actual # Cells Seeded	Colony count			Average	PE (%)	SF	SF
			1st	2nd	3rd			Uncorrected	Corrected
1	200	229	93	86	111	94	39.251	1.00	1.0000
2	200	252	103	82	91				
3	200	261	79	73	68	73	28.047	0.7774	0.7146
4	200	250	51	60	43	51	20.501	0.5442	0.5223
5	2000	2615	85	90	95	90	3.442	0.0954	0.0877
6	2000	2039	20	25	30	25	1.197	0.0265	0.0305
7	20000	25200	6	8	10	8	0.032	0.0008	0.0008
8	200	#DIV/0!	112	109	89	97	0.48 #DIV/0!	1.0000	#DIV/0!
9	200	#DIV/0!	99	92	81				
10	200	#DIV/0!	92	99	89	93	#DIV/0!	0.9622	#DIV/0!
11	200	#DIV/0!	90	85	81	85	#DIV/0!	0.8797	#DIV/0!
12	200	#DIV/0!	59	66	78	68	#DIV/0!	0.6976	#DIV/0!
13	200	#DIV/0!	44	60	51	52	#DIV/0!	0.5326	#DIV/0!
14	200	#DIV/0!	23	40	33	32	#DIV/0!	0.3299	#DIV/0!

Experiment: 3/8/01
 Date/Time:

Tube #	Activity Conc. (KBq/ml)	Activity/Cell (mBq/cell)	Survival Uncorrected	Survival Corrected
1	0.000	0.000	1.0000	1.0000
2	0.000	0.000		
3	1.984	0.210	0.7774	0.7146
4	9.234	0.541	0.5442	0.5223
5	38.230	1.566	0.0954	0.0877
6	98.326	3.763	0.0265	0.0305
7	163.546	8.960	0.0008	0.0008
8	0.000	#DIV/0!	1.0000	#DIV/0!
9	0.000	#DIV/0!		
10	#DIV/0!	#DIV/0!	0.9622	#DIV/0!
11	#DIV/0!	#DIV/0!	0.8797	#DIV/0!
12	#DIV/0!	#DIV/0!	0.6976	#DIV/0!
13	#DIV/0!	#DIV/0!	0.5326	#DIV/0!
14	#DIV/0!	#DIV/0!	0.3299	#DIV/0!

Summary



Cell counting after sorting

03/12/01

MS = 50 μ l, background = 2

For dye(+) cells

Tube #	count	Avg.	Cell conc. (#/ml)	Dilution/ plated	vol. plated (μ l)
1	187, 165, 172	174	6,986.66	1:1000	290 (290)
2	111, 109, 119	113	452000	1:1000	440
3	250, 261, 243	251	1,005,333	1:1000	200
4	263, 249, 270	260	1,042,666	1:1000	190
5	240, 238, 227	235	940000	1:1000	210, 2100
6	281, 275, 290	268	1,074,666	1:1000	190, 1900
7	240, 238, 227	235	9,400.00	1:1000	212, 212

For dye(-)ve cells

1	160, 171, 181	170	682,666	1:1000	290
2	111, 120, 109	113	453,333	1:1000	440
3	241, 238, 229	236	944,000	1:1000	210
4	232, 241, 247	240	9,60,000	1:1000	210
5	243, 261, 251	251	1,006,666	1:1000	200
6	229, 231, 221	227	908,000	1:1000	220
7	241, 227, 251	229	918,666	1:1000	220, 2200

SORT

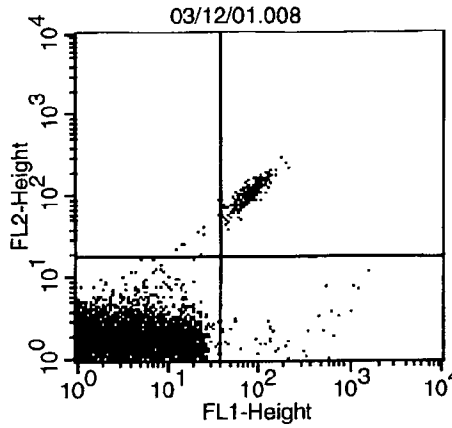
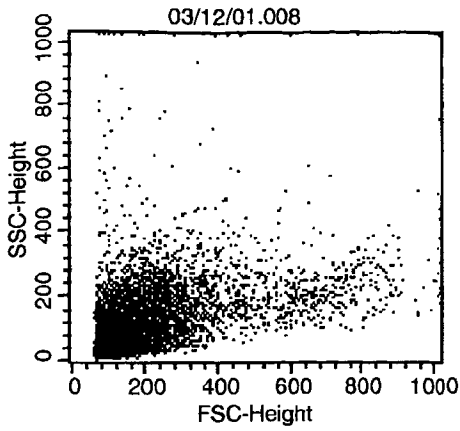
DATE: 3/12/2001

TIME: 2:30 - 5:00

INVESTIGATOR: J. Binkhayer

	LEFT SORT	RIGHT SORT	ABORT	FREQUENCY
	-	+		
TUBE 1	802016	778183	1.0	5.7
TUBE 2	502562	504220		
TUBE 3	1070349	1008857		
TUBE 4	1102569	1001092		
TUBE 5	1000859	1060345		
TUBE 6	1026537	961002		
TUBE 7	1000833	1007094		
TUBE 8				
TUBE 9				
TUBE 10				

After sorting



Quadrant Statistics

File: 03/12/01.008
 Sample ID: sort 2 negative
 Tube:
 Acquisition Date: 12-Mar-01
 Gated Events: 5160
 X Parameter: FL1-H FL1-Height (Log)
 Quad Location: 37, 18

Log Data Units: Linear Values
 Patient ID:
 Panel:
 Gate: No Gate
 Total Events: 5160
 Y Parameter: FL2-H FL2-Height (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	10	0.19	0.19	23.92	22.27	40.81	36.48
UR	213	4.13	4.13	83.58	79.61	115.79	109.29
LL	4898	94.92	94.92	5.49	3.41	2.09	1.91
LR	39	0.76	0.76	317.88	177.46	2.66	2.15

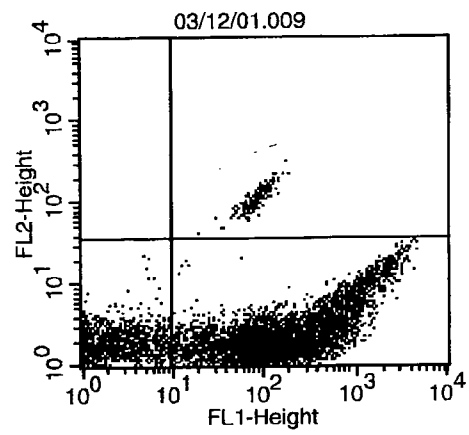
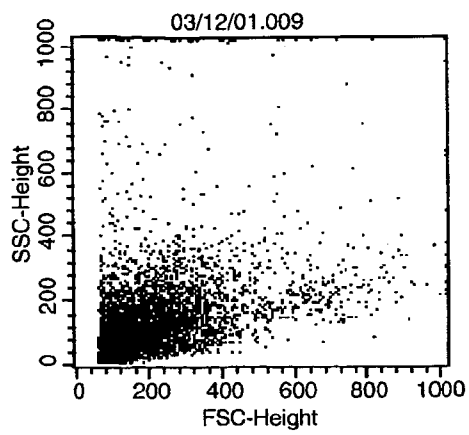
within dye(-)ve cells

5%

dye(+ve) cells

95%

dye(-)ve cells



Quadrant Statistics

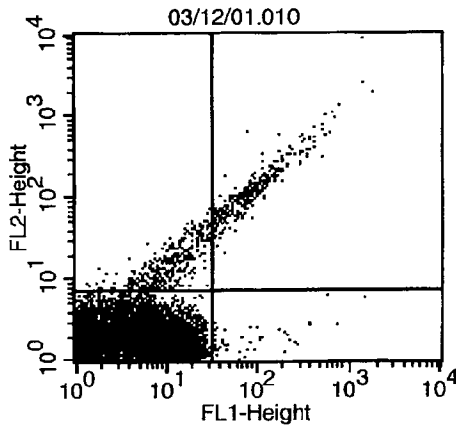
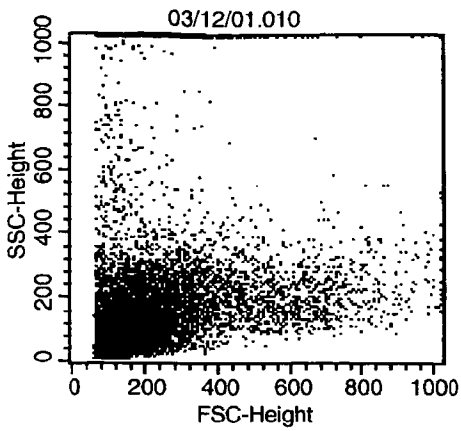
File: 03/12/01.009 Log Data Units: Linear Values
 Sample ID: sort 2 positive Patient ID:
 Tube: Panel:
 Acquisition Date: 12-Mar-01 Gate: No Gate
 Gated Events: 5100 Total Events: 5100
 X Parameter: FL1-H FL1-Height (Log) Y Parameter: FL2-H FL2-Height (Log)
 Quad Location: 9, 36

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	174	3.41	3.41	111.31	81.54	113.91	107.70
LL	953	18.69	18.69	3.03	2.42	2.13	1.94
LR	3973	77.90	77.90	369.82	166.14	3.18	2.41

81+31% pos
18.69% neg

within dye (+)ve cells

18% dye (-)ve cells
82% dye (+)ve cells

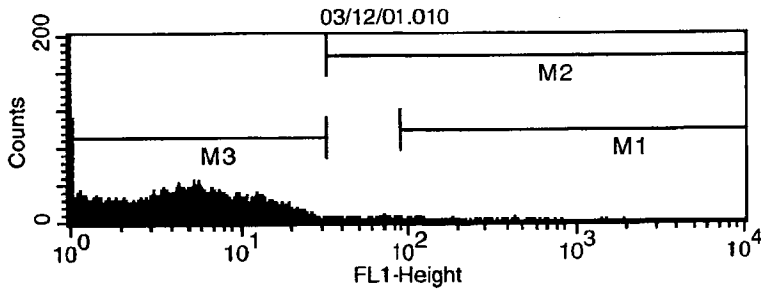


Quadrant Statistics

File: 03/12/01.010
 Sample ID: sort 7 negative
 Tube:
 Acquisition Date: 12-Mar-01
 Gated Events: 7875
 X Parameter: FL1-H FL1-Height (Log)
 Quad Location: 31, 7

Log Data Units: Linear Values
 Patient ID:
 Panel:
 Gate: No Gate
 Total Events: 7875
 Y Parameter: FL2-H FL2-Height (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	315	(+) 4.00	4.00	12.17	9.86	18.55	15.51
UR	297	3.77	3.77	132.71	90.52	214.44	126.88
LL	7219	91.67	91.67	6.12	4.27	2.09	1.92
LR	44	0.56	0.56	154.43	84.05	2.13	1.91



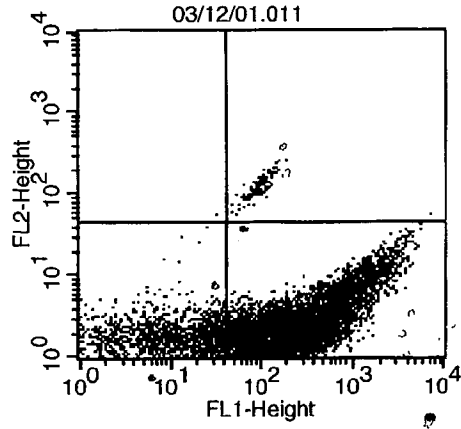
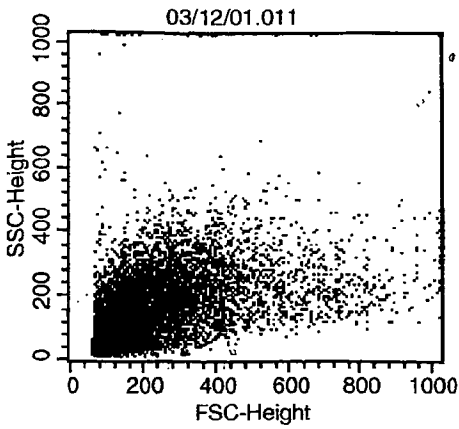
4% dye (+)ve

Histogram Statistics

File: 03/12/01.010
 Sample ID: sort 7 negative
 Tube:
 Acquisition Date: 12-Mar-01
 Gated Events: 7875
 X Parameter: FL1-H FL1-Height (Log)

Log Data Units: Linear Values
 Patient ID:
 Panel:
 Gate: No Gate
 Total Events: 7875

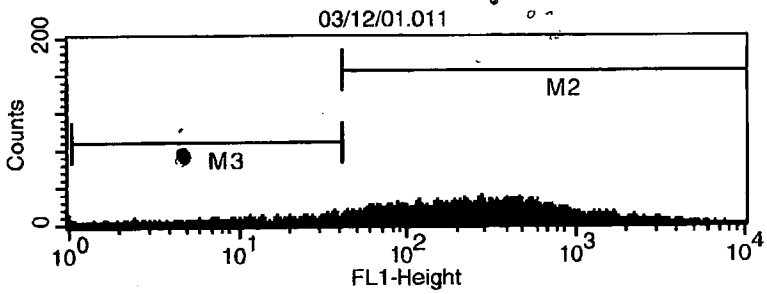
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	7875	100.00	100.00	11.97	5.04	405.00	4.87	1
M1	91, 9910	139	1.77	1.77	249.77	187.47	106.15	151.25	100
M2	32, 9910	336	4.27	4.27	137.06	91.06	142.49	79.86	70
M3	1, 32	7216	91.63	91.63	6.64	4.74	83.86	4.87	5



Quadrant Statistics

File: 03/12/01.011 Log Data Units: Linear Values
 Sample ID: sort 7 positive Patient ID:
 Tube: Panel:
 Acquisition Date: 12-Mar-01 Gate: No Gate
 Gated Events: 7365 Total Events: 7365
 X Parameter: FL1-H FL1-Height (Log) Y Parameter: FL2-H FL2-Height (Log)
 Quad Location: 40, 45

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.01	0.01	29.96	29.96	55.73	55.73
UR	101	-1.37	1.37	162.39	92.11	122.18	116.38
LL	1183	16.06	16.06	15.63	10.52	2.09	1.84
LR	6080	-82.55	82.55	480.16	275.73	3.45	2.59



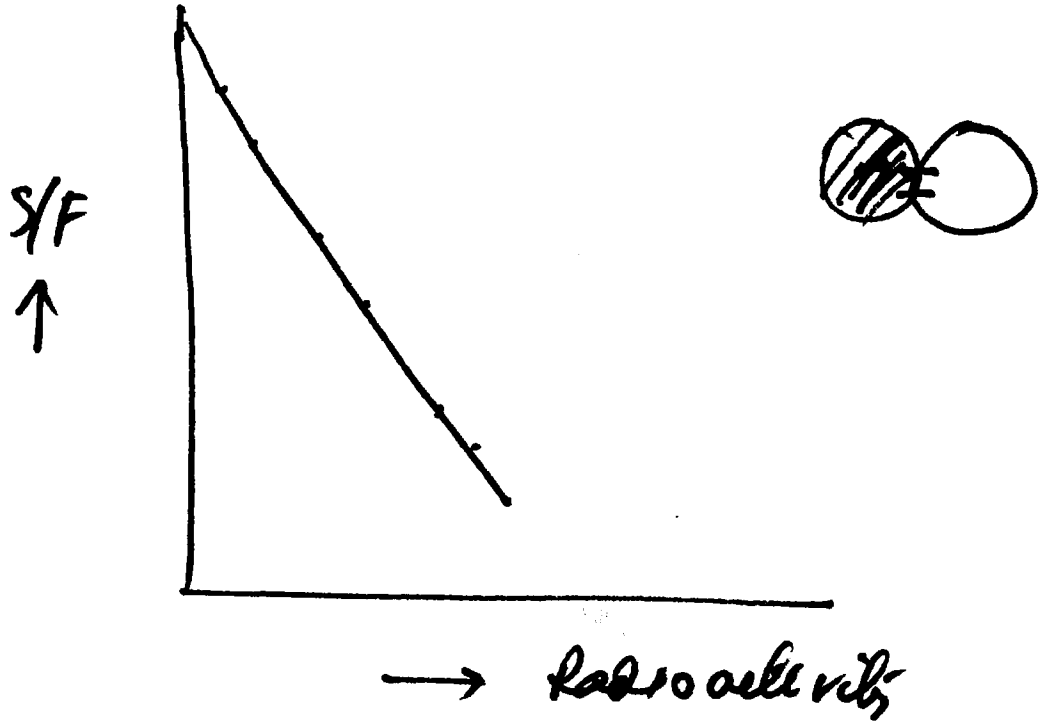
16% dye (-)ve cells

Histogram Statistics

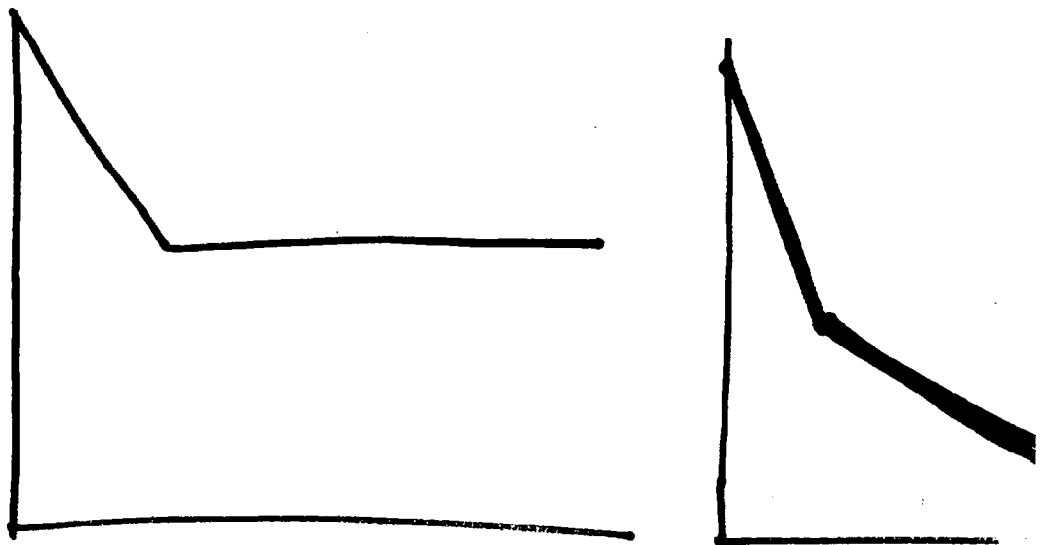
File: 03/12/01.011 Log Data Units: Linear Values
 Sample ID: sort 7 positive Patient ID:
 Tube: Panel:
 Acquisition Date: 12-Mar-01 Gate: No Gate
 Gated Events: 7365 Total Events: 7365
 X Parameter: FL1-H FL1-Height (Log)

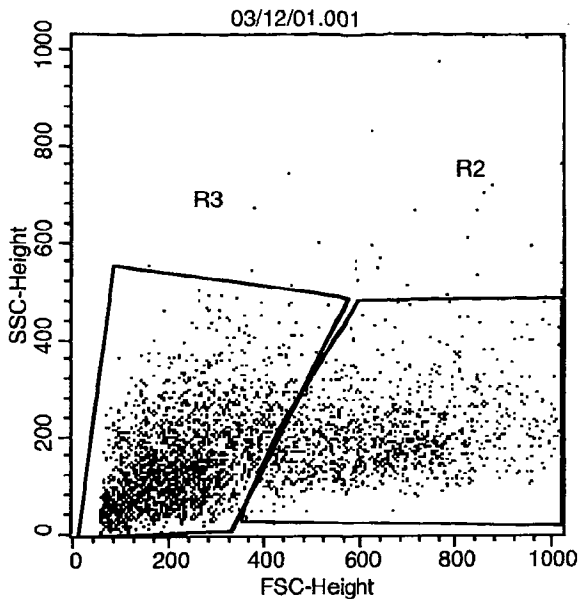
Marker	Left	Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1	9910	7365	100.00	100.00	401.13	160.70	148.14	201.69	264
M2	41	9910	6161	83.65	83.65	476.38	272.51	130.66	266.55	264
M3	1	41	1202	16.32	16.32	16.38	11.12	73.51	13.58	36

100%.



50%.

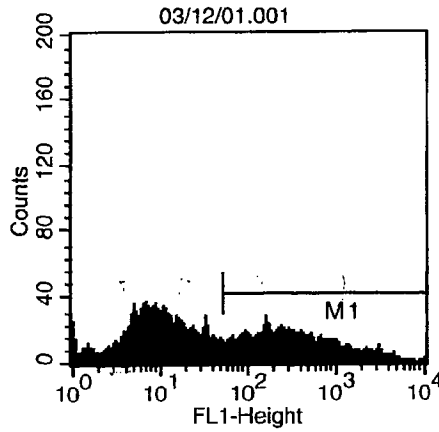
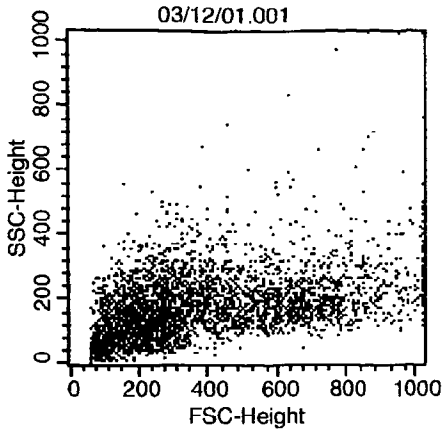




Region Statistics

File: 03/12/01.001
 Sample ID: isotype
 Tube:
 Acquisition Date: 12-Mar-01
 Gated Events: 10000
 X Parameter: FSC-H FSC-Height (Linear)
 Log Data Units: Linear Values
 Patient ID:
 Panel:
 Gate: No Gate
 Total Events: 10000
 Y Parameter: SSC-H SSC-Height (Linear)

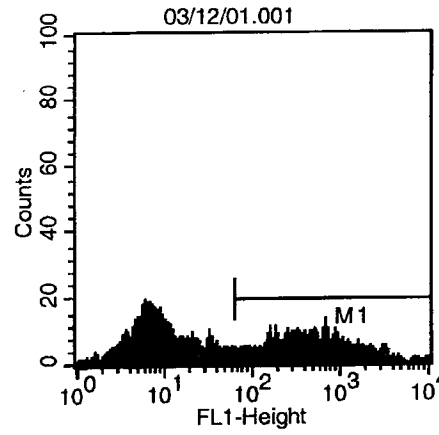
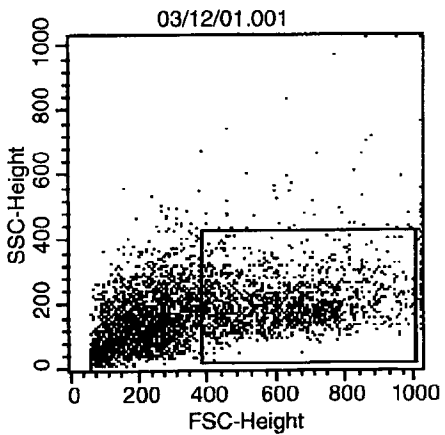
Region	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean	Px,Py
R1	3643	36.43	36.43	627.69	607.25	202.54	189.96	1, 2
R2	3438	34.38	34.38	670.33	649.90	200.85	187.21	1, 2
R3	6493	64.93	64.93	219.79	195.61	150.73	121.06	1, 2



Histogram Statistics

File: 03/12/01.001
 Sample ID: ~~isotype~~
 Tube:
 Acquisition Date: 12-Mar-01
 Gated Events: 10000
 X Parameter: FL1-H FL1-Height (Log)
 Log Data Units: Linear Values
 Patient ID:
 Panel:
 Gate: No Gate
 Total Events: 10000

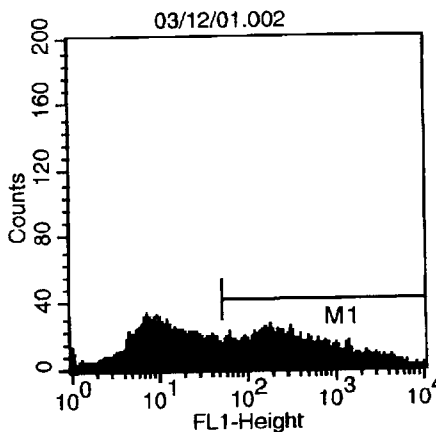
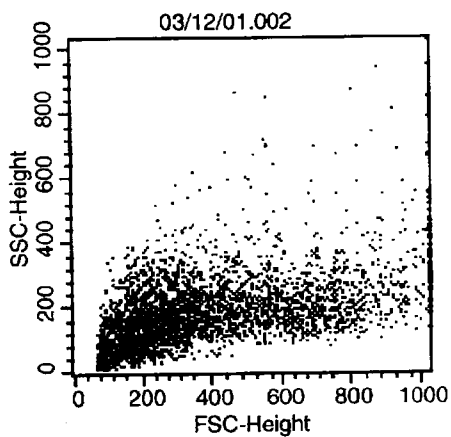
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak	Ch
All	1, 9910	10000	100.00	100.00	224.39	39.29	249.79	25.03		6
M1	51, 9910	4152	41.52	41.52	521.93	290.44	149.04	261.80		145



Histogram Statistics

File: 03/12/01.001
 Sample ID: ~~isotype~~ ()
 Tube:
 Acquisition Date: 12-Mar-01
 Gated Events: 3643
 X Parameter: FL1-H FL1-Height (Log)
 Log Data Units: Linear Values
 Patient ID:
 Panel:
 Gate: G1
 Total Events: 10000

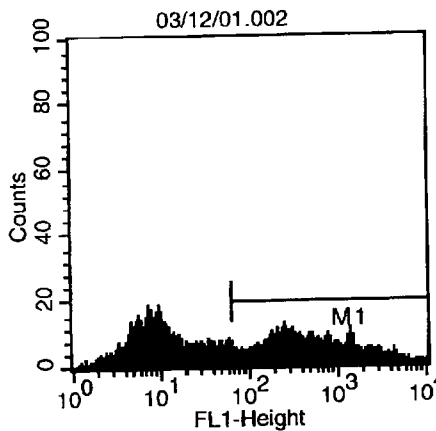
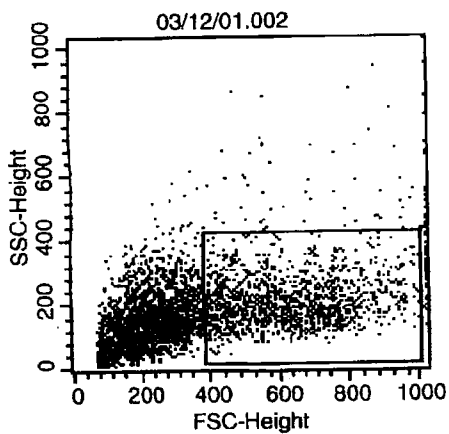
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak	Ch
All	1, 9910	3643	100.00	36.43	286.09	44.75	221.19	24.14		5
M1	63, 9910	1538	42.22	15.38	660.43	405.92	127.22	383.69		620



Histogram Statistics

File: 03/12/01.002 Log Data Units: Linear Values
 Sample ID: 2 Patient ID:
 Tube: Panel:
 Acquisition Date: 12-Mar-01 Gate: No Gate
 Gated Events: 10000 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log)

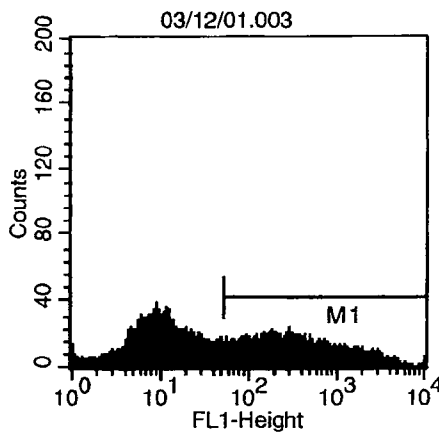
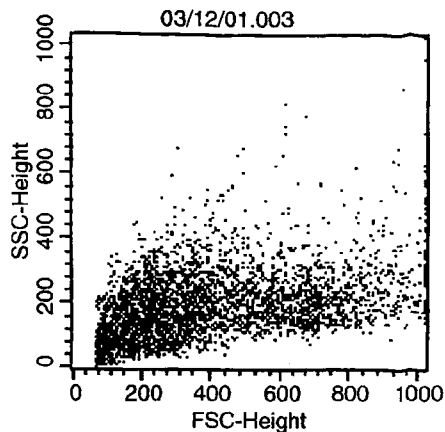
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak	Ch
All	1, 9910	10000	100.00	100.00	276.01	51.72	242.72	39.95		6
M1	51, 9910	4673	46.73	46.73	573.00	289.43	155.58	243.62		171



Histogram Statistics

File: 03/12/01.002 Log Data Units: Linear Values
 Sample ID: 2 Patient ID:
 Tube: Panel:
 Acquisition Date: 12-Mar-01 Gate: G1
 Gated Events: 3594 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log)

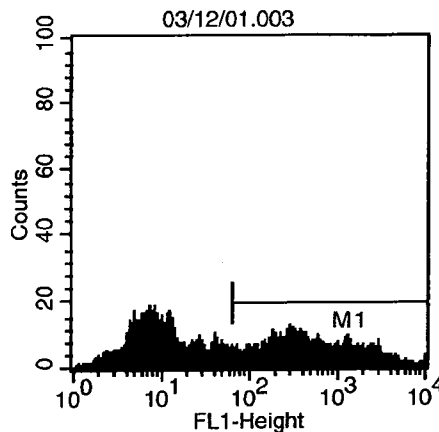
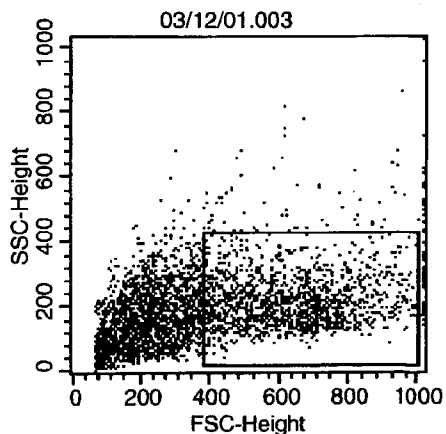
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak	Ch
All	1, 9910	3594	100.00	35.94	342.18	54.65	224.87	38.72		6
M1	63, 9910	1608	44.74	16.08	746.22	413.78	135.86	347.55		232



Histogram Statistics

File: 03/12/01.003 Log Data Units: Linear Values
 Sample ID: 3 Patient ID:
 Tube: Panel:
 Acquisition Date: 12-Mar-01 Gate: No Gate
 Gated Events: 10000 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log)

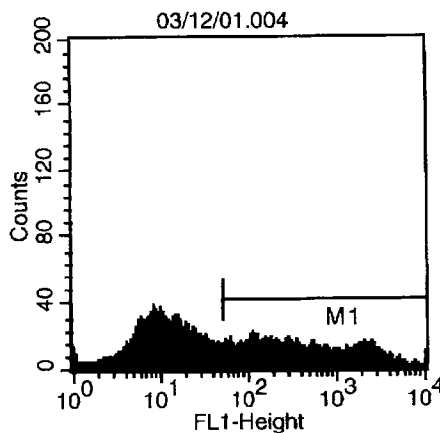
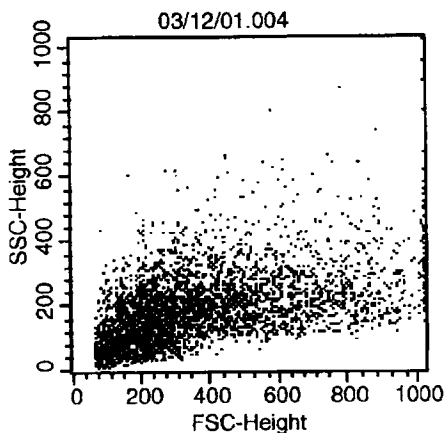
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak	Ch
All	1, 9910	10000	100.00	100.00	286.70	43.49	243.53	33.98		8
M1	51, 9910	4522	45.22	45.22	616.84	311.14	151.99	271.39		266



Histogram Statistics

File: 03/12/01.003 Log Data Units: Linear Values
 Sample ID: 3 Patient ID:
 Tube: Panel:
 Acquisition Date: 12-Mar-01 Gate: G1
 Gated Events: 3918 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log)

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak	Ch
All	1, 9910	3918	100.00	39.18	371.06	52.74	233.44	34.29		6
M1	63, 9910	1744	44.51	17.44	816.69	433.90	141.10	368.47		259

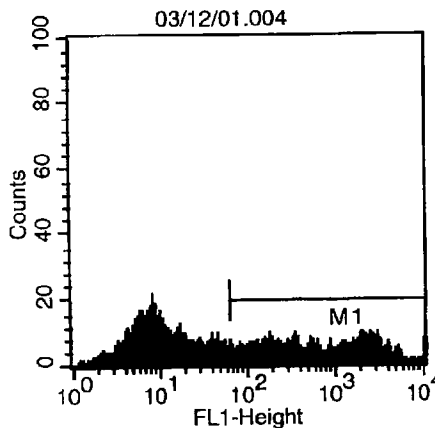
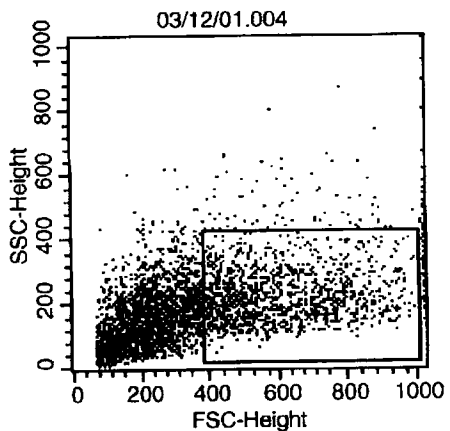


Histogram Statistics

File: 03/12/01.004
 Sample ID: 4
 Tube:
 Acquisition Date: 12-Mar-01
 Gated Events: 10000
 X Parameter: FL1-H FL1-Height (Log)

Log Data Units: Linear Values
 Patient ID:
 Panel:
 Gate: No Gate
 Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10000	100.00	100.00	389.31	53.50	233.87	32.78	7
M1	51, 9910	4454	44.54	44.54	855.78	387.56	141.62	329.29	105

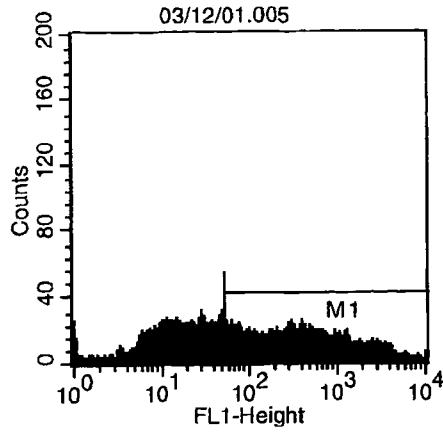
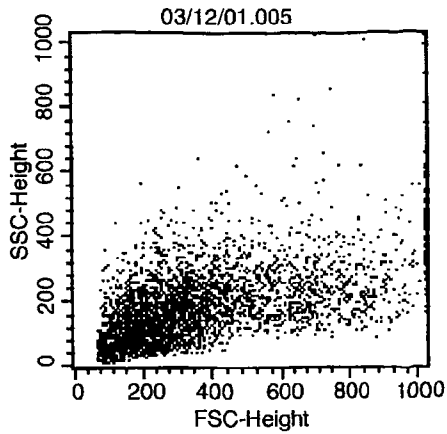


Histogram Statistics

File: 03/12/01.004
 Sample ID: 4
 Tube:
 Acquisition Date: 12-Mar-01
 Gated Events: 3591
 X Parameter: FL1-H FL1-Height (Log)

Log Data Units: Linear Values
 Patient ID:
 Panel:
 Gate: G1
 Total Events: 10000

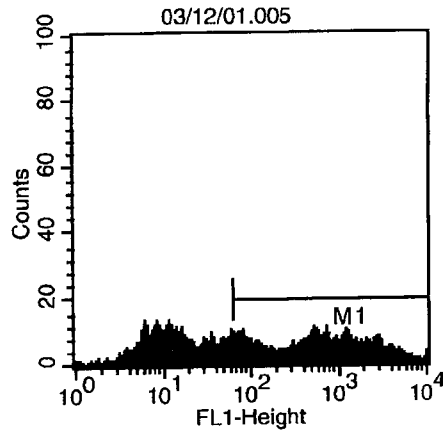
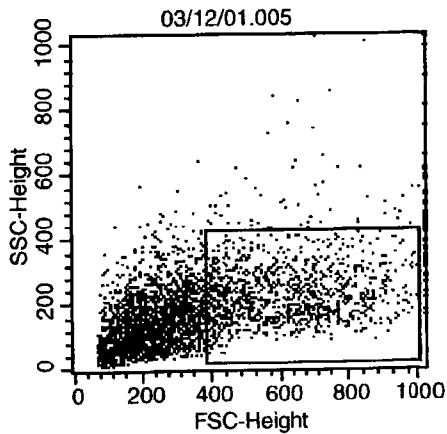
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	3591	100.00	35.91	480.27	54.88	223.54	29.96	7
M1	63, 9910	1525	42.47	15.25	1111.79	542.44	127.88	509.37	9910



Histogram Statistics

File: 03/12/01.005 Log Data Units: Linear Values
 Sample ID: 5 Patient ID:
 Tube: Panel:
 Acquisition Date: 12-Mar-01 Gate: No Gate
 Gated Events: 10000 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log)

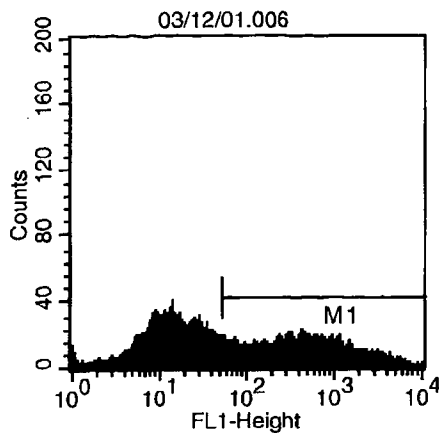
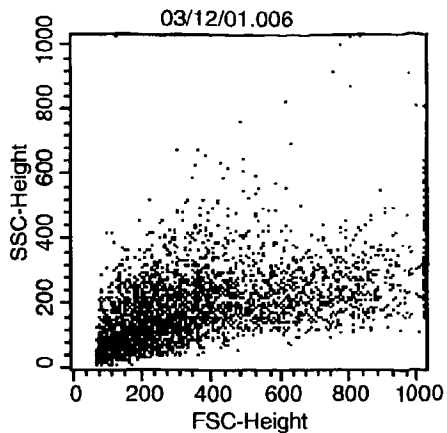
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10000	100.00	100.00	426.17	82.85	218.34	66.71	27
M1	51, 9910	5460	54.60	54.60	764.70	351.51	151.00	330.77	60



Histogram Statistics

File: 03/12/01.005 Log Data Units: Linear Values
 Sample ID: 5 Patient ID:
 Tube: Panel:
 Acquisition Date: 12-Mar-01 Gate: G1
 Gated Events: 3183 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log)

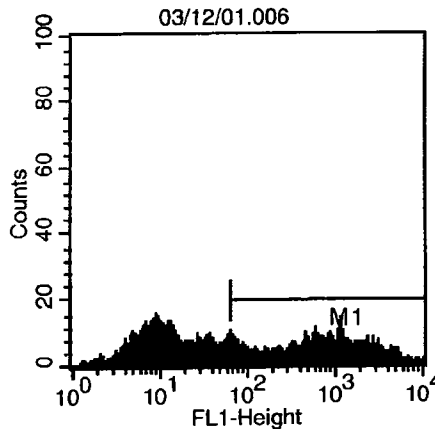
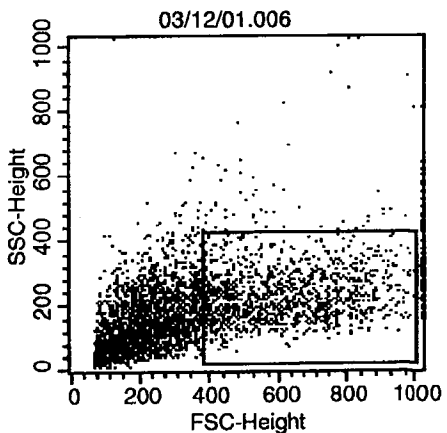
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	3183	100.00	31.83	578.51	94.58	192.00	72.99	5
M1	63, 9910	1685	52.94	16.85	1076.32	538.94	124.79	567.42	465



Histogram Statistics

File: 03/12/01.006 Log Data Units: Linear Values
 Sample ID: 6 Patient ID:
 Tube: Panel:
 Acquisition Date: 12-Mar-01 Gate: No Gate
 Gated Events: 10000 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log)

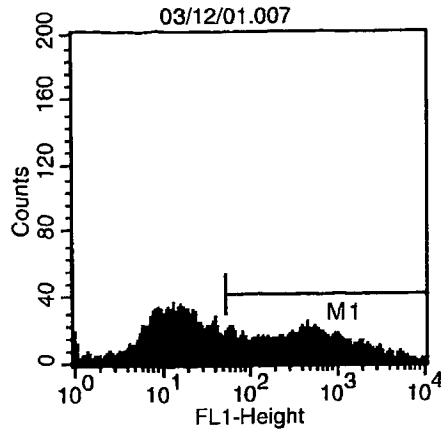
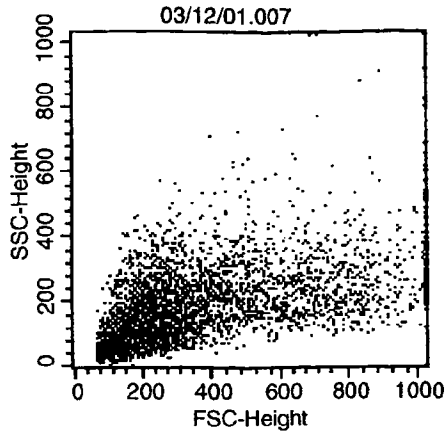
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10000	100.00	100.00	366.37	64.78	219.51	41.42	13
M1	51, 9910	4717	47.17	47.17	757.79	389.11	137.21	395.96	395



Histogram Statistics

File: 03/12/01.006 Log Data Units: Linear Values
 Sample ID: 6 Patient ID:
 Tube: Panel:
 Acquisition Date: 12-Mar-01 Gate: G1
 Gated Events: 3330 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log)

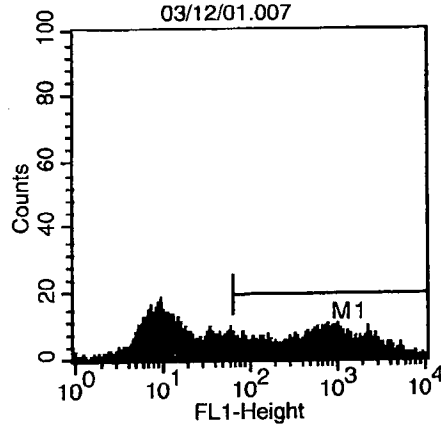
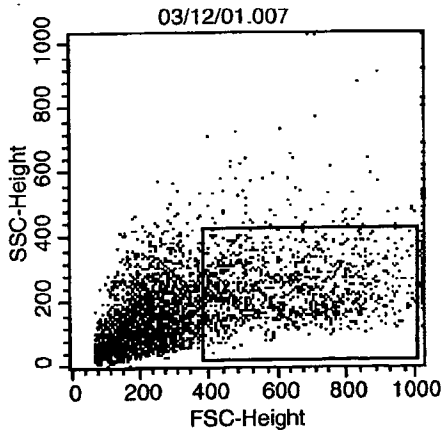
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	3330	100.00	33.30	470.39	68.21	194.20	41.42	8
M1	63, 9910	1480	44.44	14.80	1037.59	601.03	109.83	679.25	1045



Histogram Statistics

File: 03/12/01.007 Log Data Units: Linear Values
 Sample ID: 7 Patient ID:
 Tube: Panel:
 Acquisition Date: 12-Mar-01 Gate: No Gate
 Gated Events: 10000 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log)

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak	Ch
All	1, 9910	10000	100.00	100.00	322.26	58.24	233.21	36.85		12
M1	51, 9910	4492	44.92	44.92	696.34	354.37	143.84	349.12		410



Histogram Statistics

File: 03/12/01.007 Log Data Units: Linear Values
 Sample ID: 7 Patient ID:
 Tube: Panel:
 Acquisition Date: 12-Mar-01 Gate: G1
 Gated Events: 3361 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log)

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak	Ch
All	1, 9910	3361	100.00	33.61	434.87	67.22	202.70	41.79		9
M1	63, 9910	1502	44.69	15.02	952.20	533.55	117.64	585.57		685

wipe test for faes machine

PAGE: 1

US 6 10:43 HOWELL PRESET TIME: 1.00 TUE 13 MAR 2001 15:30
 SAMPLE: 177A: 1 CYCLE REPEAT: 1 SCR:N RS232:N
 CH 1: 100% BCF:N RCM:N
 CHANNEL CALL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
 DATA CALD: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000
 HALF LIFE(DAYS):N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	16.00	50.00	1.00	1.48	72.0	
2	**	2	10.00	63.25	1.00	3.06	75.0	
3	**	3	14.00	53.45	1.00	4.68	76.0	
4	**	4	12.00	57.74	1.00	6.31	78.0	
5	**	5	10.00	63.25	1.00	7.88	79.0	
6	**	6	15.00	51.64	1.00	9.45	76.0	
7	**	7	10.00	63.25	1.00	11.08	76.0	
8	**	8	10.00	63.25	1.00	12.71	74.0	
9	**	9	5.00	89.44	1.00	14.33	77.0	
10	**	10	11.00	60.30	1.00	15.90	73.0	
11	**	11	16.00	50.00	1.00	17.48	75.0	
12	**	12	5.00	89.44	1.00	19.11	74.0	
13	**	13	46.00	29.49	1.00	20.75	91.0	
14	**	14	10.00	63.25	1.00	22.32	80.0	
15	**	15	12.00	57.74	1.00	23.90	79.0	
16	**	16	29254.29	1.98	0.35	24.85	2.0	

Sample 1-14

back → Standard