

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. # 2;

Investigator: A. Bishayee

Date: 03/19/01

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 : 4,560,000 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 Date/Time: 03/19/01;
3-00 pm
5. Prepare MEMB containing radioactivity in hood
175 μl $^3\text{HTdR}$ (Stock : 1 $\mu\text{Ci}/\mu\text{l}$ on 2/15/01) + 3.5 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: 03/19/00;
6-45 pm

Tube #	$^3\text{HTdR}$ uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ $^3\text{HTdR}$ 50uCi/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	5	1.0	0.8	0.2	2
4	10	1.0	0.6	0.4	2
5	15	1.0	0.4	0.6	2
6	20	1.0	0.2	0.8	2
7	25	1.0	0	1	2

7. Add 1 ml of MEMB tube and return test tubes to roller for 14 h.

Date/Time: 03/19/01;

8. Next day, while test tubes are in roller label tubes (13 X 100 mm VWR glass test tube)

7-00 pm

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

Date/Time: 03/20/09; 9-15 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: 03/20/09; 12-00

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: 03/23/09; 1-00 pm.

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 ^{only} ~~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.

colony count was not possible due to contamination

30 ml medium

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 WED 21 MAR 2001 13:21
 SAMPLE REPEAT: 1 DATE: 20010321 TIME: 1321
 1: 1 600.00 20.00 IN RCM: N
 2: 1 600.00 20.00 IN RCM: N
 3: 1 600.00 20.00 IN RCM: N
 4: 1 600.00 20.00 IN RCM: N
 5: 1 600.00 20.00 IN RCM: N
 6: 1 600.00 20.00 IN RCM: N
 7: 1 600.00 20.00 IN RCM: N
 8: 1 600.00 20.00 IN RCM: N
 9: 1 600.00 20.00 IN RCM: N
 10: 1 600.00 20.00 IN RCM: N
 11: 1 600.00 20.00 IN RCM: N
 12: 1 600.00 20.00 IN RCM: N
 13: 1 600.00 20.00 IN RCM: N
 14: 1 600.00 20.00 IN RCM: N
 15: 1 600.00 20.00 IN RCM: N
 16: 1 600.00 20.00 IN RCM: N
 17: 1 600.00 20.00 IN RCM: N
 18: 1 600.00 20.00 IN RCM: N
 19: 1 600.00 20.00 IN RCM: N
 20: 1 600.00 20.00 IN RCM: N
 21: 1 600.00 20.00 IN RCM: N
 DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000
 HALF LIFE (DAYS): N

SAN	POS	CH	CPM	ZSIGX	TIME	EL TIME	AVG HW	ERR
1	**	1	6.00	81.65	1.00	1.42	74.0	
2	**	2	8.00	70.71	1.00	2.99	75.0	
3	**	3	8.00	70.71	1.00	4.61	75.0	
4	**	4	11.00	60.30	1.00	6.18	75.0	
5	**	5	14.00	53.45	1.00	7.73	76.0	
6	**	6	8.00	70.71	1.00	9.37	76.0	
7	**	7	121370.00	1.82	0.10	10.08	76.0	
8	**	8	124253.33	1.46	0.15	10.78	76.0	
9	**	9	123490.00	1.80	0.10	11.49	75.0	
10	**	10	223666.66	1.09	0.15	12.21	74.0	
11	**	11	218670.00	1.35	0.10	12.93	75.0	
12	**	12	199830.00	1.41	0.10	13.63	74.0	
13	**	13	349420.00	0.87	0.15	14.37	77.0	
14	**	14	336546.66	0.89	0.15	15.09	74.0	
15	**	15	366633.31	0.85	0.15	15.82	77.0	
16	**	16	456599.97	0.76	0.15	16.55	75.0	
17	**	17	427866.66	0.79	0.15	17.28	75.0	
18	**	18	453126.66	0.77	0.15	18.02	75.0	
19	**	1	586746.62	0.67	0.15	18.82	75.0	
20	**	2	591453.31	0.67	0.15	19.56	76.0	
21	**	3	482326.66	0.74	0.15	20.30	73.0	

03/20/01 9:15

03/29/01 3-15

$$7 \text{ days} \times 24 = \frac{168}{6}$$

174

Some cells

PAGE: 1

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 TUE 27 MAR 2001 15:17
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
 H#: 1 ACC: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 CALC: CPM, UNKNOWN REPLICATES: 1- NORM FACTOR: 1.00000
 HALF LIFE(DAYS):N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	7.00	75.59	1.00	1.42	70.0	
2	**	2	13.00	55.47	1.00	3.00	77.0	
3	**	3	9.00	66.67	1.00	4.57	76.0	
4	**	4	13.00	55.47	1.00	6.19	75.0	
5	**	5	7.00	75.59	1.00	7.82	76.0	
6	**	6	17.00	48.51	1.00	9.44	80.0	
7	**	7	8214.00	2.21	1.00	11.02	73.0	
8	**	8	8885.00	2.12	1.00	12.64	74.0	
9	**	9	8694.00	2.14	1.00	14.22	73.0	
10	**	10	13629.33	1.98	0.75	15.58	72.0	
11	**	11	13921.33	1.96	0.75	16.95	74.0	
12	**	12	13273.75	1.94	0.80	18.37	73.0	
13	**	13	19696.36	1.92	0.55	19.54	74.0	
14	**	14	18292.73	1.99	0.55	20.69	74.0	
15	**	15	18570.91	1.98	0.55	21.87	74.0	
16	**	16	27795.00	1.90	0.40	22.87	77.0	
17	**	17	29280.00	1.98	0.35	23.77	78.0	
18	**	18	26455.00	1.94	0.40	24.72	75.0	
19	**	1	48160.00	1.82	0.25	25.65	80.0	
20	**	2	43832.00	1.91	0.25	26.51	80.0	
21	**	3	45676.00	1.87	0.25	27.37	80.0	
22	**	4	29573.33	1.90	0.38	28.40	1.0	

→ H-3 Standard

MediumActivity

Experiment: H-3/50%/FACSS2
 Date: 3/19/2001

Tube #	1st	2nd	3rd	CPM Average	CPM corrected for control	DPM (CPM/(y e))	At counting	Ao addition	Ao addition
1	6	8	8	9	0	0	0	0	0
2	11	14	8	9	0	0	0	0	0
3	121370	124253	123490	123038	123029	189275	2.8420	2.8427	105.1809
4	223666	218670	199830	214055	214046	329302	4.9445	4.9458	182.9948
5	349420	336546	366633	350866	350857	539780	8.1048	8.1070	299.9587
6	456599	427866	453126	445864	445855	685930	10.2992	10.3020	381.1749
7	586746	591453	482326	553508	553499	851537	12.7858	12.7893	473.2037
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!

CellsSuspension

Experiment: H-3/50%/FACCS2
Date: 03/19/01

Tube #	Suspension count (CPM)			Average CPM	CPM corrected for control	DPM (CPM/y e)	A _i μ Ci/ml on counting	A _o μ Ci/ml after uptake	A _o KBq/ml after uptake
	1st	2nd	3rd						
1	7	13	9	11	0	0	0.00000	0	0.0000
2	13	7	17	11	0	0	0.00000	0	0.0000
3	8214	8885	8694	8598	8587	13210	0.11901	0.11914	4.4083
4	13629	13921	13273	13608	13597	20918	0.18845	0.18866	6.9804
5	19696	18292	18570	18853	18842	28987	0.26115	0.26144	9.6732
6	27795	29280	26455	27843	27832	42819	0.38576	0.38619	14.2889
7	48160	43832	45676	45889	45878	70582	0.63587	0.63658	23.5536
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%/FACS2
Date/Time: 3/19/01

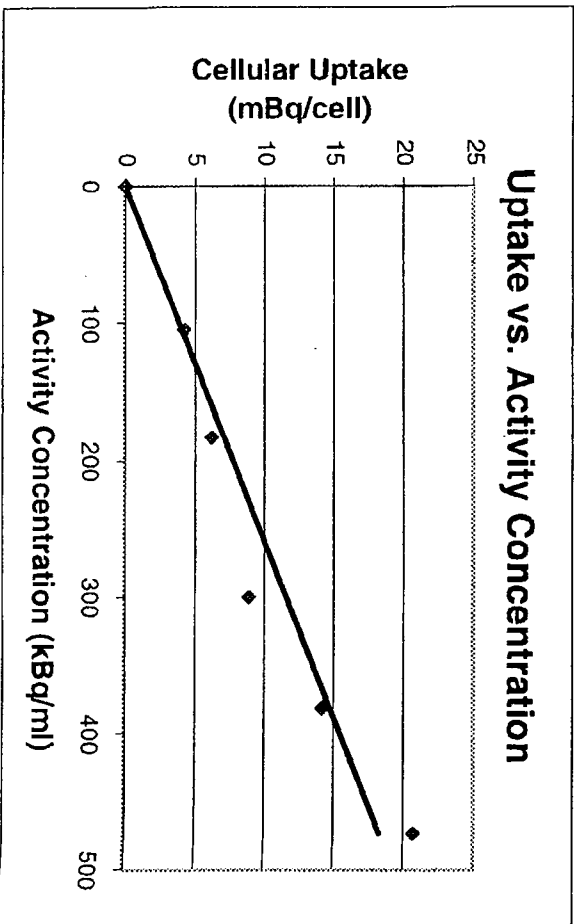
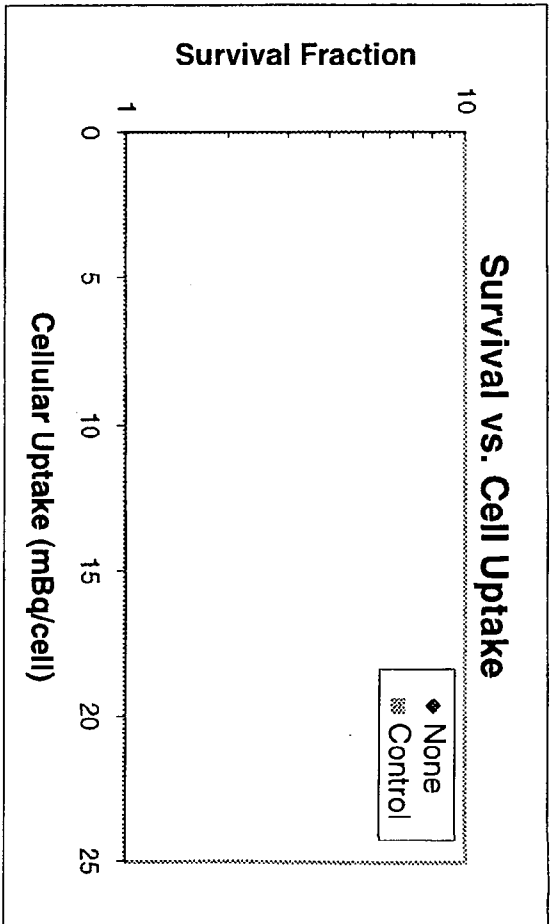
Tube #	Coulter count			Average	Cells/ml	Hemocytometer Count in Grid			
	1st	2nd	3rd			1st	2nd	3rd	4th
1	512	534	545	530	2110667				
2	555	523	539	539	2145333				
3	516	525	533	525	2088000				
4	577	566	553	565	2250667				
5	555	549	535	546	2174667				
6	511	509	495	505	2009333				
7	583	568	562	571	2273333				
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!				

Tube #	Predicted # Cells Seeded	Actual # Cells Seeded	1st	Colony count		Average	PE (%)	SF	SF
				2nd	3rd			Uncorrected	Corrected
1	200	211				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
2	200	215				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
3	200	209				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
4	200	225				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
5	200	217				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
6	200	201				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
7	2000	2273				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
8		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
9		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
10		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
11		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
12		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
13		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
14		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Experiment: 3/19/01
 Date/Time:

Tube #	Activity Conc. (KBq/ml)	Activity/Cell (mBq/cell)	Survival Uncorrected	Survival Corrected
1	0.000	0.000	#DIV/0!	#DIV/0!
2	0.000	0.000	#DIV/0!	#DIV/0!
3	105.181	4.223	#DIV/0!	#DIV/0!
4	182.995	6.203	#DIV/0!	#DIV/0!
5	299.959	8.896	#DIV/0!	#DIV/0!
6	381.175	14.223	#DIV/0!	#DIV/0!
7	473.204	20.722	#DIV/0!	#DIV/0!
8	0.000	#DIV/0!	#DIV/0!	#DIV/0!
9	0.000	#DIV/0!	#DIV/0!	#DIV/0!
10	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
11	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
12	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
13	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
14	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Summary



03/23/01 ⁵⁸

Cell count after sorting

MS = 500ul; Background = 25

Dye (-)ve cells

Tube #	collor count	Avg.	Cell conc. (#/ml)	# of cells plated (dil.)	vol. plated (ul)
1	1130, 1145, 1165	1146	458,666	200 (1:1000)	440
2	440, 421, 435	432	172800	200 (1:1000)	1155
3	1100, 1082, 1075	1085	434,266	200 (1:1000)	460
4	998, 1005, 1021	1008	403,200	200 (1:1000)	500
5	1217, 1178, 1169	1188	475200	200 (1:1000)	420
6	960, 982, 971	971	388400	200 (1:1000) 2000 (1:100)	515 515
7	982, 1015, 999	998	399466	200 (1:1000) 2000 (1:100)	500 500

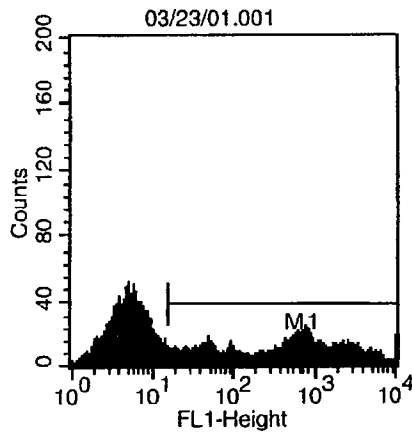
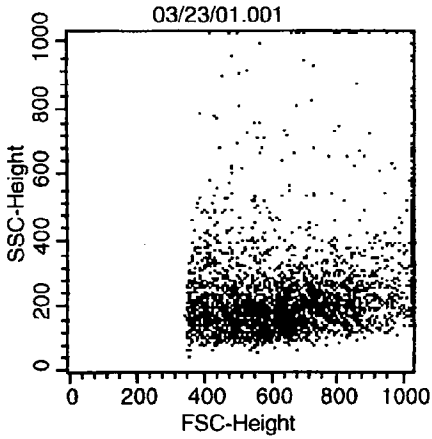
SORT

DATE: 3/23/2001

TIME: 2:23 - 3:45

INVESTIGATOR:

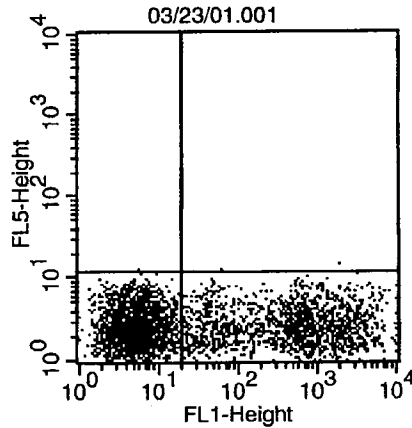
	LEFT SORT	RIGHT SORT	ABORT	FREQUENCY
	negative			
TUBE 1	501976		.13	2.3
TUBE 2	26461		.10	2.0
TUBE 3	501560		.30	5.0
TUBE 4	501057		.30	5.0
TUBE 5	500942		.30	(5.0)
TUBE 6	500938		.30	6.0
TUBE 7	501211		.30	6.0
TUBE 8				
TUBE 9				
TUBE 10				



Histogram Statistics

File: 03/23/01.001 Log Data Units: Linear Values
 Sample ID: control 1 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: No Gate
 Gated Events: 10000 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log)

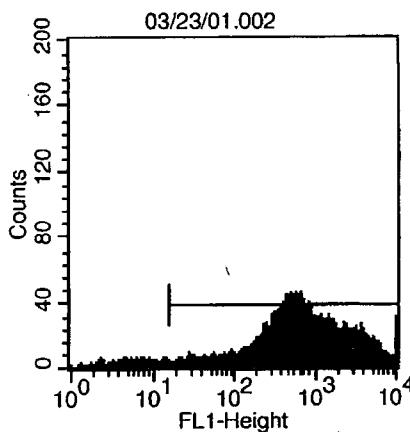
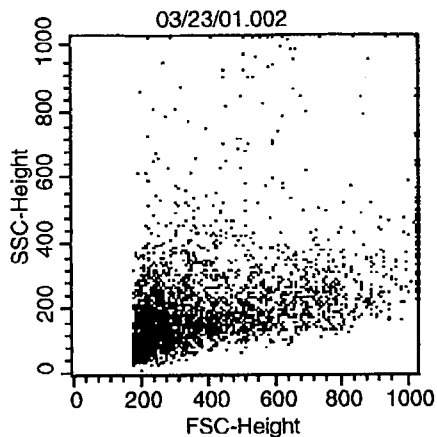
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10000	100.00	100.00	502.24	37.05	240.27	11.70	5
M1	16, 9910	4678	46.78	46.78	1067.02	355.84	148.59	509.37	9910



Quadrant Statistics

File: 03/23/01.001 Log Data Units: Linear Values
 Sample ID: control 1 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: No Gate
 Gated Events: 10000 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log) Y Parameter: FL5-H FL5-Height (Log)
 Quad Location: 19, 11

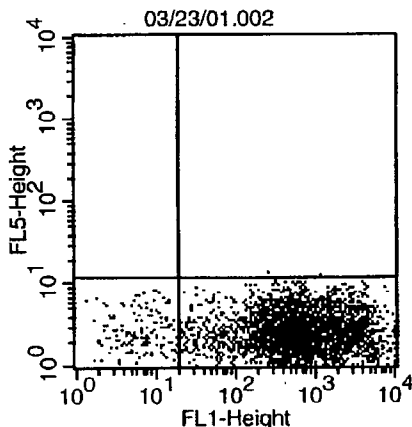
Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	3	0.03	0.03	9.23	8.11	12.12	12.12
UR	4	0.04	0.04	504.90	116.26	12.90	12.89
LL	5470	54.70	54.70	6.12	5.25	2.87	2.53
LR	4523	45.23	45.23	1102.56	394.08	2.89	2.55



Histogram Statistics

File: 03/23/01.002 Log Data Units: Linear Values
 Sample ID: control 2 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: No Gate
 Gated Events: 10000 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log)

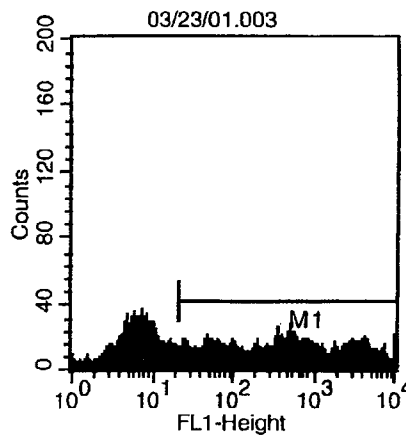
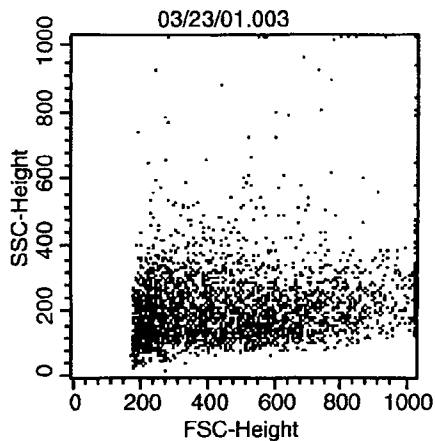
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10000	100.00	100.00	1193.15	528.94	131.65	582.94	9910
M1	16, 9910	9583	95.83	95.83	1244.76	642.50	127.30	615.27	9910



Quadrant Statistics

File: 03/23/01.002 Log Data Units: Linear Values
 Sample ID: control 2 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: No Gate
 Gated Events: 10000 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log) Y Parameter: FL5-H FL5-Height (Log)
 Quad Location: 19, 11

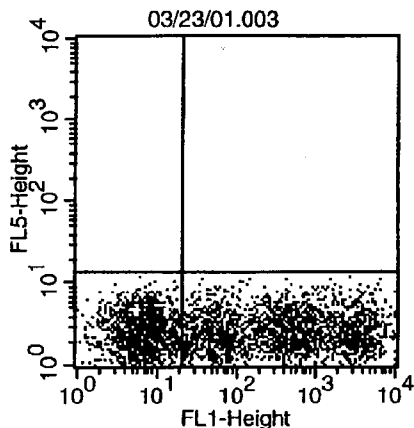
Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	3	0.03	0.03	632.58	513.97	12.50	12.48
LL	449	4.49	4.49	7.89	6.53	2.93	2.56
LR	9548	95.48	95.48	1249.07	650.38	2.88	2.54



Histogram Statistics

File: 03/23/01.003 Log Data Units: Linear Values
 Sample ID: 3 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: No Gate
 Gated Events: 10000 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log)

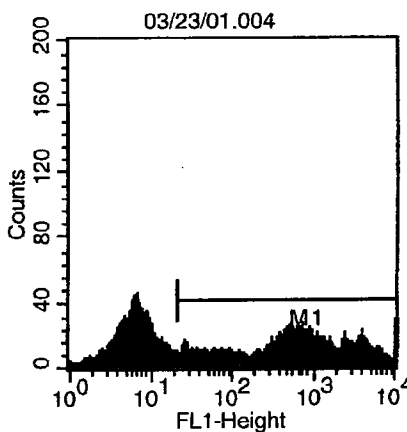
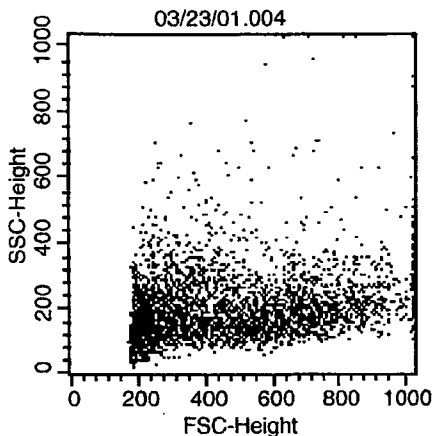
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10000	100.00	100.00	802.18	85.64	195.99	66.71	9910
M1	21, 9910	6189	61.89	61.89	1291.40	418.93	142.07	461.38	9910



Quadrant Statistics

File: 03/23/01.003 Log Data Units: Linear Values
 Sample ID: 3 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: No Gate
 Gated Events: 10000 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log) Y Parameter: FL5-H FL5-Height (Log)
 Quad Location: 21, 13

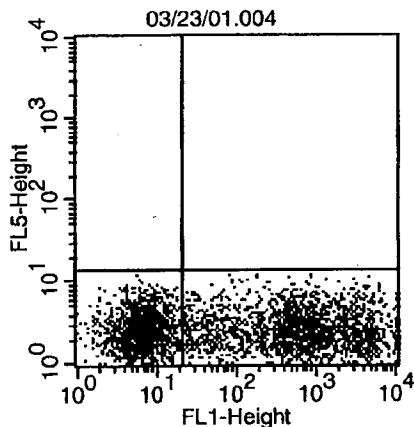
Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	0	0.00	0.00	***	***	***	***
LL	3805	38.05	38.05	7.67	6.49	2.92	2.57
LR	6195	61.95	61.95	1290.17	417.72	2.95	2.59



Histogram Statistics

File: 03/23/01.004 Log Data Units: Linear Values
 Sample ID: 4 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: No Gate
 Gated Events: 10000 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log)

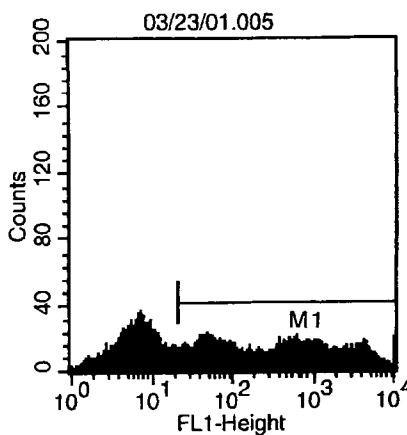
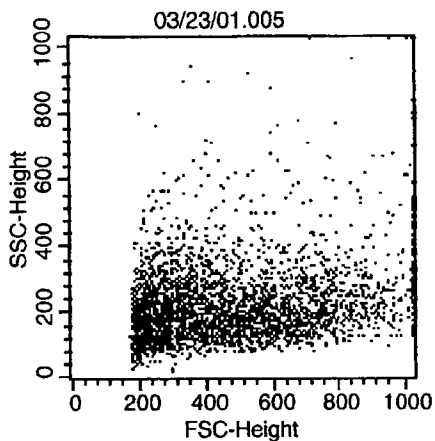
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch.
All	1, 9910	10000	100.00	100.00	796.76	85.24	195.61	71.69	9910
M1	21, 9910	5908	59.08	59.08	1343.52	515.48	136.86	588.21	9910



Quadrant Statistics

File: 03/23/01.004 Log Data Units: Linear Values
 Sample ID: 4 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: No Gate
 Gated Events: 10000 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log) Y Parameter: FL5-H FL5-Height (Log)
 Quad Location: 21, 13

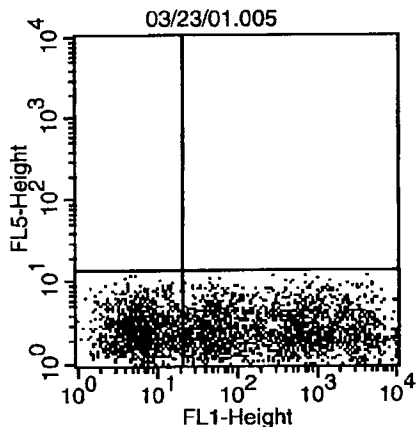
Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	0	0.00	0.00	***	***	***	***
LL	4087	40.87	40.87	7.33	6.33	2.88	2.53
LR	5913	59.13	59.13	1342.40	514.08	2.90	2.55



Histogram Statistics

File: 03/23/01.005 Log Data Units: Linear Values
 Sample ID: 5 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: No Gate
 Gated Events: 10000 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log)

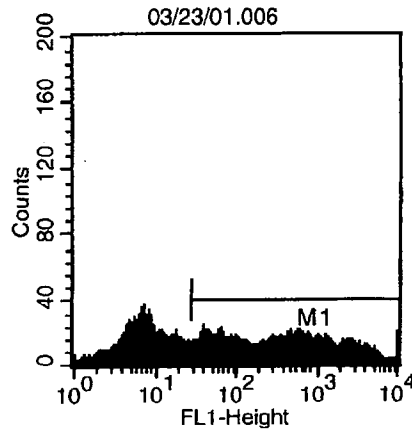
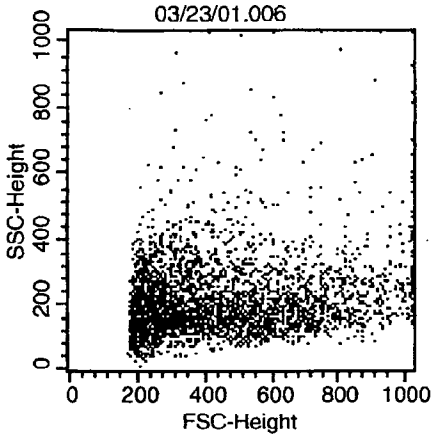
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10000	100.00	100.00	684.11	76.94	208.83	57.00	9910
M1	21, 9910	6302	63.02	63.02	1081.10	332.22	155.12	385.42	9910



Quadrant Statistics

File: 03/23/01.005 Log Data Units: Linear Values
 Sample ID: 5 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: No Gate
 Gated Events: 10000 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log) Y Parameter: FL5-H FL5-Height (Log)
 Quad Location: 21, 13

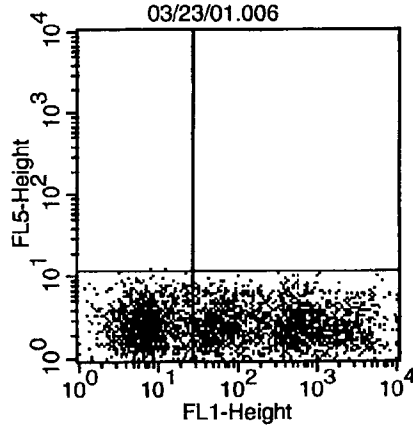
Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	0	0.00	0.00	***	***	***	***
LL	3690	36.90	36.90	7.55	6.34	2.94	2.59
LR	6310	63.10	63.10	1079.76	331.06	2.94	2.57



Histogram Statistics

File: 03/23/01.006 Log Data Units: Linear Values
 Sample ID: 6 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: No Gate
 Gated Events: 10000 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log)

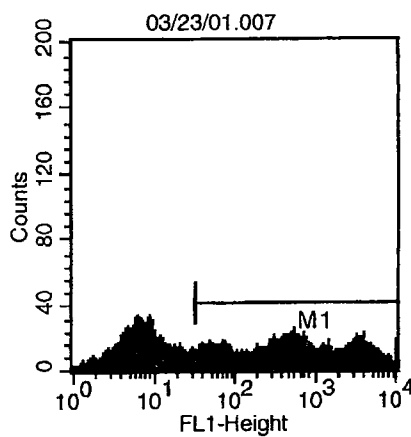
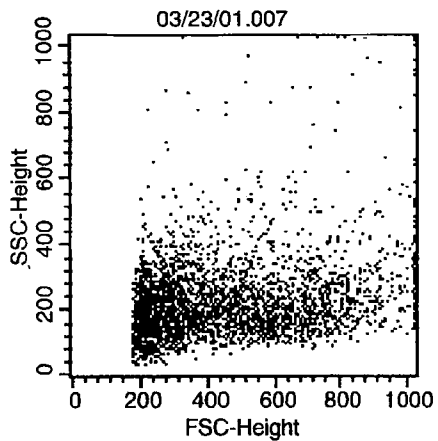
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10000	100.00	100.00	558.97	73.10	218.03	59.35	9910
M1	28, 9910	6073	60.73	60.73	914.58	325.48	159.35	349.12	9910



Quadrant Statistics

File: 03/23/01.006 Log Data Units: Linear Values
 Sample ID: 6 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: No Gate
 Gated Events: 10000 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log) Y Parameter: FL5-H FL5-Height (Log)
 Quad Location: 27, 11

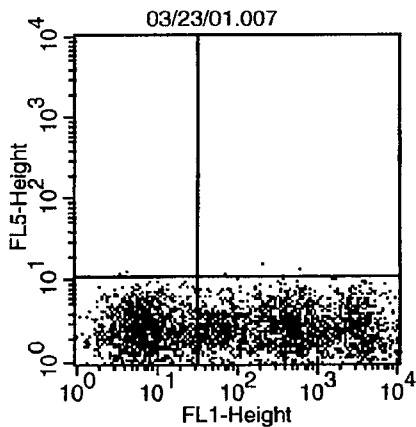
Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	4	0.04	0.04	9.67	9.35	12.28	12.27
UR	5	0.05	0.05	145.52	84.90	12.18	12.14
LL	3911	39.11	39.11	8.98	7.23	2.96	2.59
LR	6080	60.80	60.80	913.46	324.25	2.90	2.55



Histogram Statistics

File: 03/23/01.007 Log Data Units: Linear Values
 Sample ID: 7 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: No Gate
 Gated Events: 10000 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log)

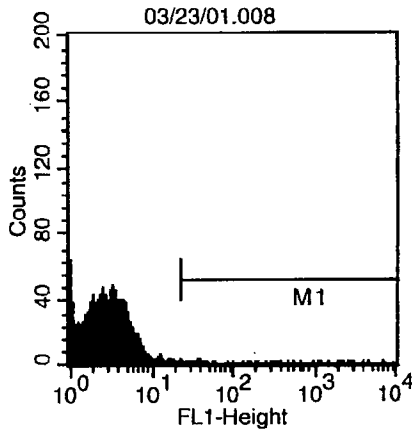
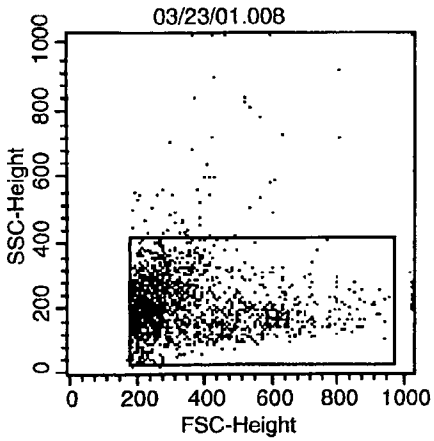
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak	Ch
All	1, 9910	10000	100.00	100.00	753.14	86.57	198.43	74.99		6
M1	32, 9910	5899	58.99	58.99	1270.23	482.44	139.38	474.00		9910



Quadrant Statistics

File: 03/23/01.007 Log Data Units: Linear Values
 Sample ID: 7 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: No Gate
 Gated Events: 10000 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log) Y Parameter: FL5-H FL5-Height (Log)
 Quad Location: 32, 11

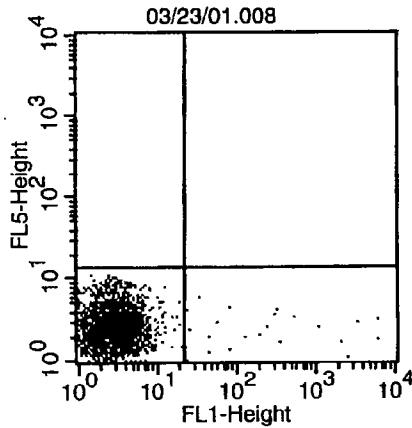
Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	4	0.04	0.04	4.48	4.07	11.84	11.84
UR	8	0.08	0.08	873.22	319.44	12.00	11.93
LL	4092	40.92	40.92	9.33	7.31	2.91	2.57
LR	5896	58.96	58.96	1269.72	481.60	2.94	2.59



Histogram Statistics

File: 03/23/01.008 Log Data Units: Linear Values
 Sample ID: sort 1 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: G1
 Gated Events: 5310 Total Events: 5505
 X Parameter: FL1-H FL1-Height (Log)

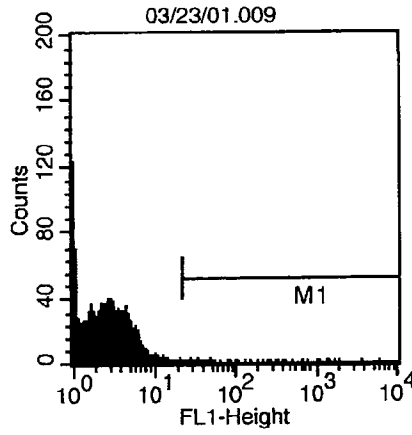
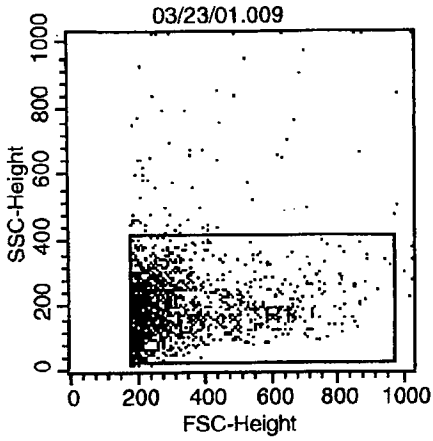
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	5310	100.00	96.46	13.74	3.00	1377.04	2.84	1
M1	22, 9910	72	1.36	1.31	774.59	178.47	186.28	147.22	36



Quadrant Statistics

File: 03/23/01.008 Log Data Units: Linear Values
 Sample ID: sort 1 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: G1
 Gated Events: 5310 Total Events: 5505
 X Parameter: FL1-H FL1-Height (Log) Y Parameter: FL5-H FL5-Height (Log)
 Quad Location: 22, 13

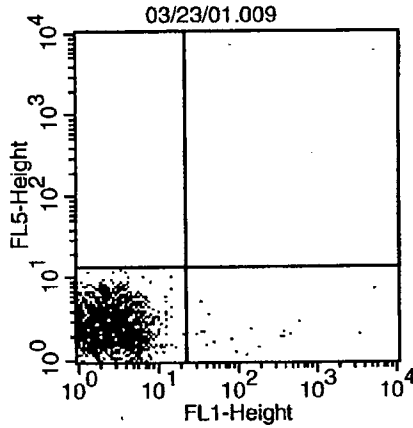
Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	0	0.00	0.00	***	***	***	***
LL	5238	98.64	95.15	3.28	2.83	2.97	2.60
LR	72	1.36	1.31	774.59	178.47	2.79	2.46



Histogram Statistics

File: 03/23/01.009 Log Data Units: Linear Values
 Sample ID: sort 7 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: G1
 Gated Events: 4829 Total Events: 5100
 X Parameter: FL1-H FL1-Height (Log)

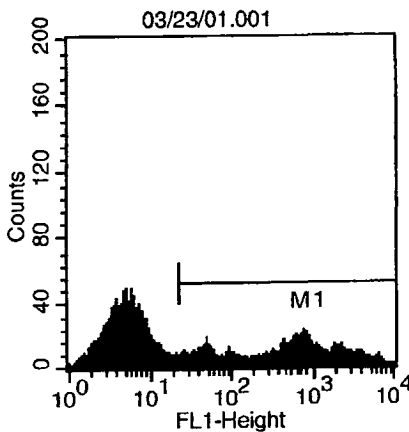
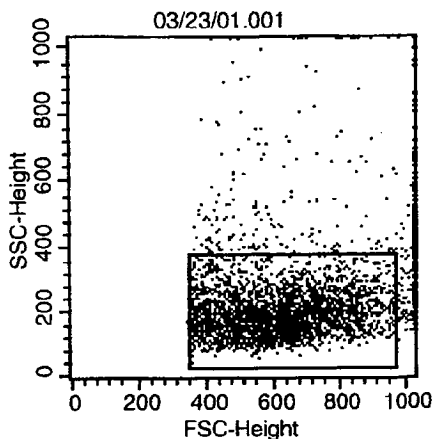
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	4829	100.00	94.69	8.42	2.80	1264.64	2.67	1
M1	22, 9910	57	1.18	1.12	448.70	137.24	196.61	95.60	26



Quadrant Statistics

File: 03/23/01.009 Log Data Units: Linear Values
 Sample ID: sort 7 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: G1
 Gated Events: 4829 Total Events: 5100
 X Parameter: FL1-H FL1-Height (Log) Y Parameter: FL5-H FL5-Height (Log)
 Quad Location: 22, 13

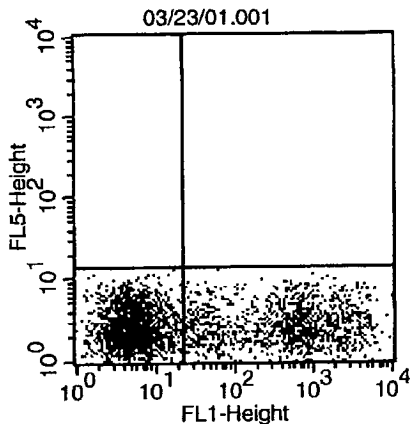
Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	0	0.00	0.00	***	***	***	***
LL	4771	98.80	93.55	3.16	2.67	2.98	2.61
LR	58	1.20	1.14	441.35	133.00	2.76	2.43



Histogram Statistics

File: 03/23/01.001 Log Data Units: Linear Values
 Sample ID: control 1 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: G1
 Gated Events: 8739 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log)

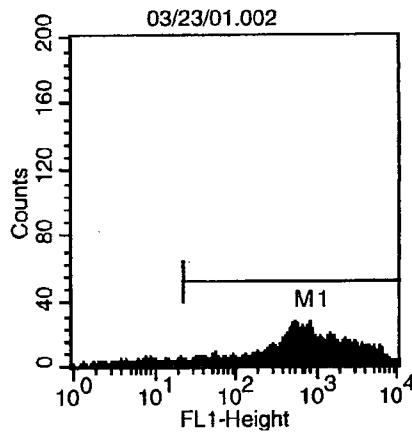
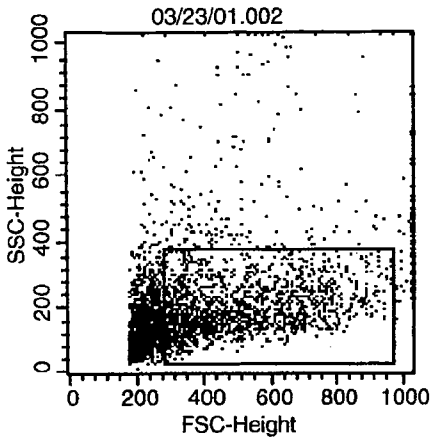
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	8739	100.00	87.39	409.52	31.70	233.77	9.47	5
M1	22, 9910	3656	41.84	36.56	970.37	394.82	132.38	532.80	723



Quadrant Statistics

File: 03/23/01.001 Log Data Units: Linear Values
 Sample ID: control 1 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: G1
 Gated Events: 8739 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log) Y Parameter: FL5-H FL5-Height (Log)
 Quad Location: 22, 13

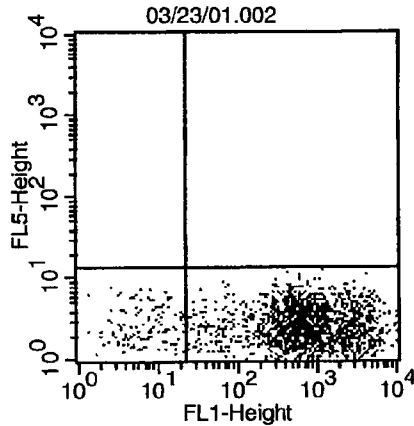
Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	0	0.00	0.00	***	***	***	***
LL	5076	58.08	50.76	6.10	5.16	2.87	2.53
LR	3663	41.92	36.63	968.56	392.66	2.90	2.56



Histogram Statistics

File: 03/23/01.002 Log Data Units: Linear Values
 Sample ID: control 2 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: G1
 Gated Events: 5044 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log)

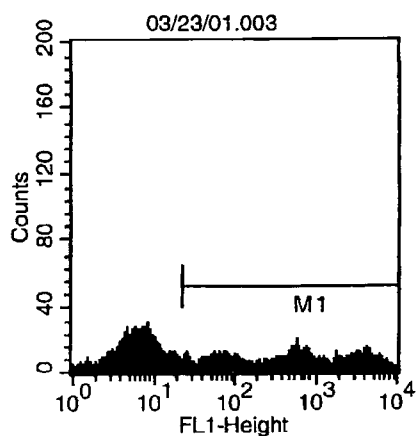
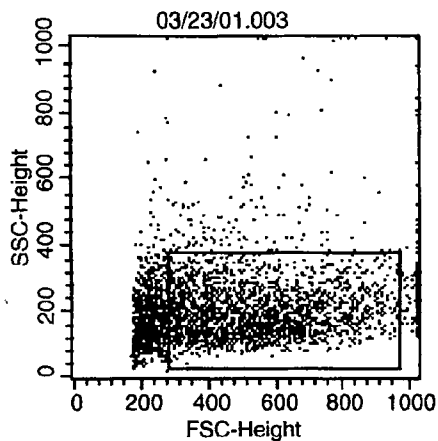
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	5044	100.00	50.44	1182.89	503.83	119.71	646.47	509
M1	22, 9910	4685	92.88	46.85	1272.90	701.50	112.34	704.14	509



Quadrant Statistics

File: 03/23/01.002 Log Data Units: Linear Values
 Sample ID: control 2 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: G1
 Gated Events: 5044 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log) Y Parameter: FL5-H FL5-Height (Log)
 Quad Location: 22, 13

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	1	0.02	0.01	245.82	245.82	13.34	13.34
LL	358	7.10	3.58	8.31	6.68	2.87	2.50
LR	4685	92.88	46.85	1272.85	701.14	2.87	2.52



Histogram Statistics

File: 03/23/01.003

Log Data Units: Linear Values

Sample ID: 3

Patient ID:

Tube:

Panel:

Acquisition Date: 23-Mar-01

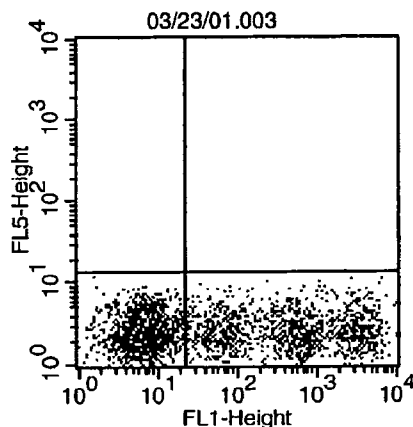
Gate: G1

Gated Events: 6387

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	6387	100.00	63.87	736.71	62.36	199.99	34.60	8
M1	22, 9910	3403	53.28	34.03	1376.13	462.61	129.98	542.47	547



Quadrant Statistics

File: 03/23/01.003

Log Data Units: Linear Values

Sample ID: 3

Patient ID:

Tube:

Panel:

Acquisition Date: 23-Mar-01

Gate: G1

Gated Events: 6387

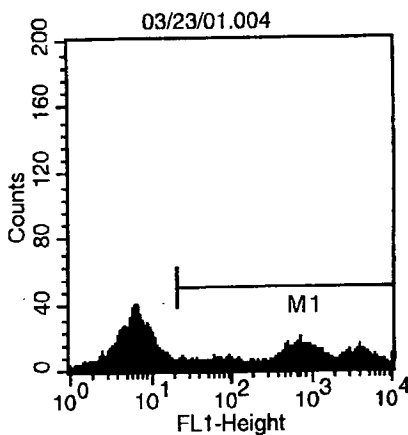
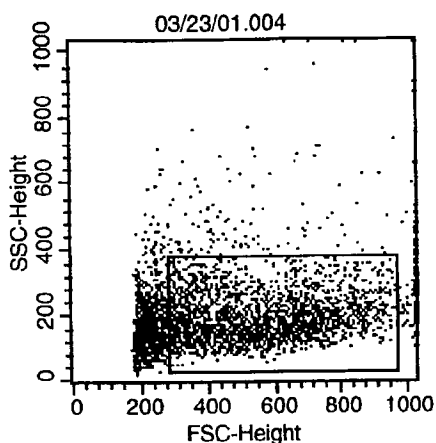
Total Events: 10000

X Parameter: FL1-H FL1-Height (Log)

Y Parameter: FL5-H FL5-Height (Log)

Quad Location: 22, 13

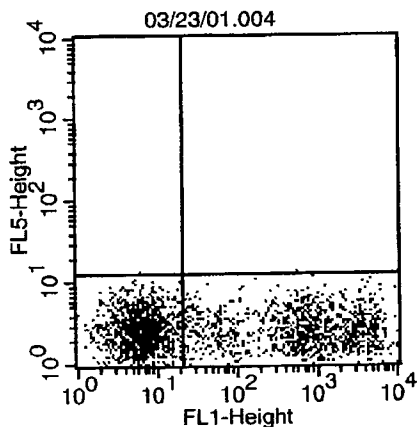
Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	0	0.00	0.00	***	***	***	***
LL	2980	46.66	29.80	7.49	6.33	2.90	2.54
LR	3407	53.34	34.07	1374.54	460.96	2.91	2.56



Histogram Statistics

File: 03/23/01.004 Log Data Units: Linear Values
 Sample ID: 4 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: G1
 Gated Events: 6381 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log)

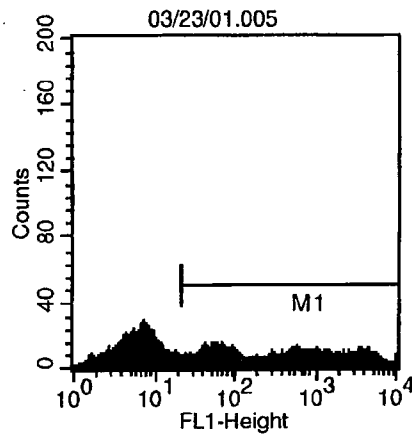
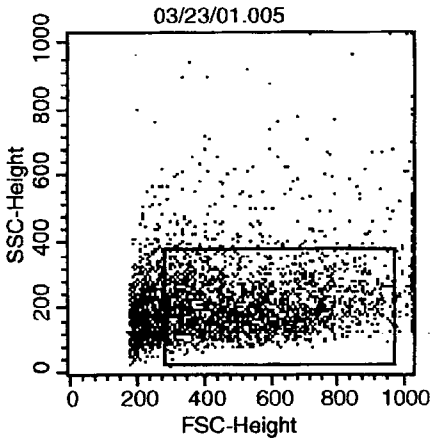
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	6381	100.00	63.81	708.92	54.25	208.52	15.96	6
M1	21, 9910	3067	48.06	30.67	1467.33	574.13	126.39	697.83	667



Quadrant Statistics

File: 03/23/01.004 Log Data Units: Linear Values
 Sample ID: 4 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: G1
 Gated Events: 6381 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log) Y Parameter: FL5-H FL5-Height (Log)
 Quad Location: 21, 12

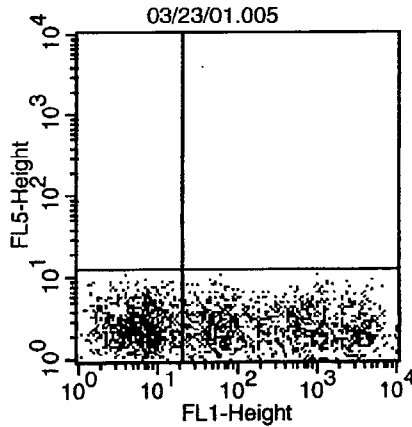
Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	2	0.03	0.02	5.71	5.70	12.75	12.75
UR	1	0.02	0.01	577.72	577.72	12.86	12.86
LL	3309	51.86	33.09	7.01	6.11	2.85	2.51
LR	3069	48.10	30.69	1466.21	572.27	2.88	2.54



Histogram Statistics

File: 03/23/01.005 Log Data Units: Linear Values
 Sample ID: 5 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: G1
 Gated Events: 6443 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log)

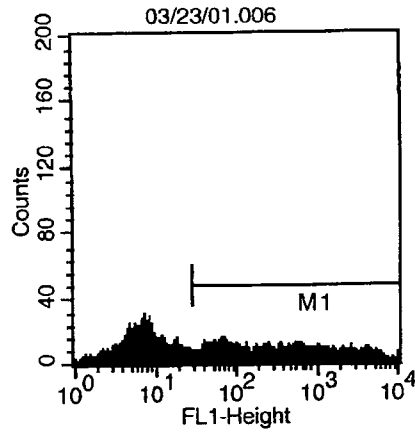
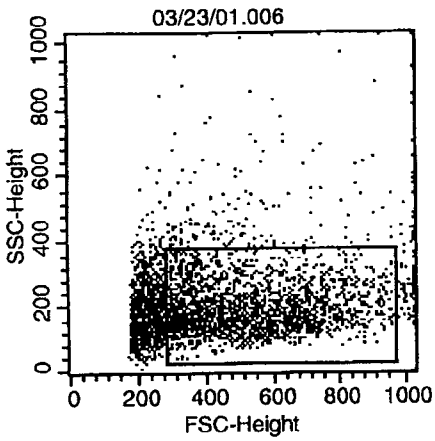
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	6443	100.00	64.43	622.06	57.38	215.06	40.68	7
M1	21, 9910	3606	55.97	36.06	1105.88	339.08	147.65	388.91	67



Quadrant Statistics

File: 03/23/01.005 Log Data Units: Linear Values
 Sample ID: 5 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: G1
 Gated Events: 6443 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log) Y Parameter: FL5-H FL5-Height (Log)
 Quad Location: 21, 12

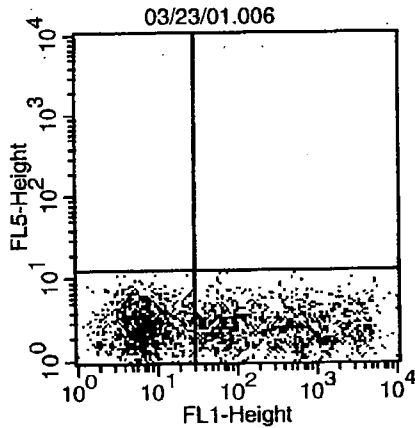
Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	1	0.02	0.01	1036.63	1036.63	12.52	12.52
LL	2834	43.99	28.34	7.06	5.99	2.89	2.55
LR	3608	56.00	36.08	1105.00	338.19	2.92	2.55



Histogram Statistics

File: 03/23/01.006 Log Data Units: Linear Values
 Sample ID: 6 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: G1
 Gated Events: 6194 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log)

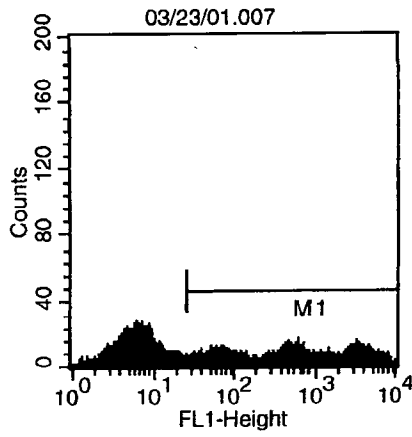
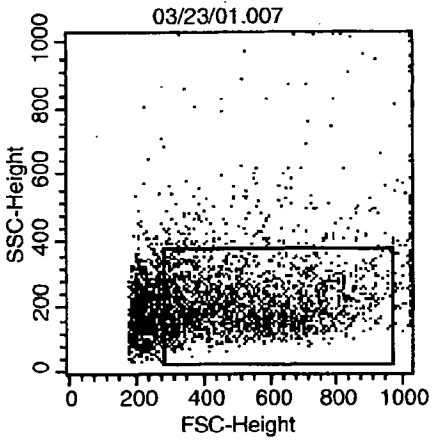
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	6194	100.00	61.94	482.14	52.26	228.35	29.42	7
M1	28, 9910	3300	53.28	33.00	897.75	316.92	153.77	324.88	63



Quadrant Statistics

File: 03/23/01.006 Log Data Units: Linear Values
 Sample ID: 6 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: G1
 Gated Events: 6194 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log) Y Parameter: FL5-H FL5-Height (Log)
 Quad Location: 29, 12

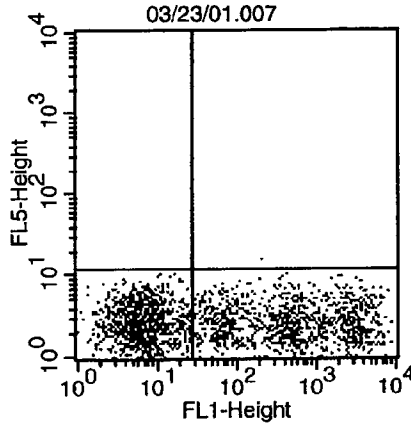
Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	1	0.02	0.01	6.49	6.49	12.75	12.75
UR	1	0.02	0.01	57.25	57.25	14.07	14.07
LL	2922	47.17	29.22	8.42	6.79	2.93	2.58
LR	3270	52.79	32.70	905.72	323.91	2.85	2.53



Histogram Statistics

File: 03/23/01.007 Log Data Units: Linear Values
 Sample ID: 7 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: G1
 Gated Events: 5993 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log)

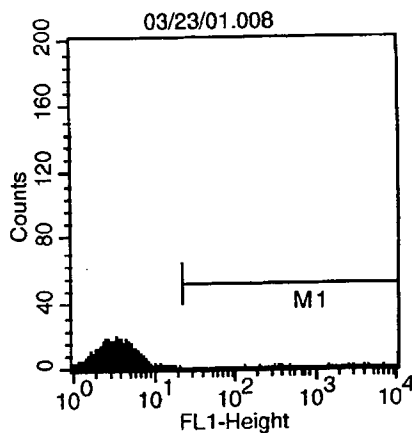
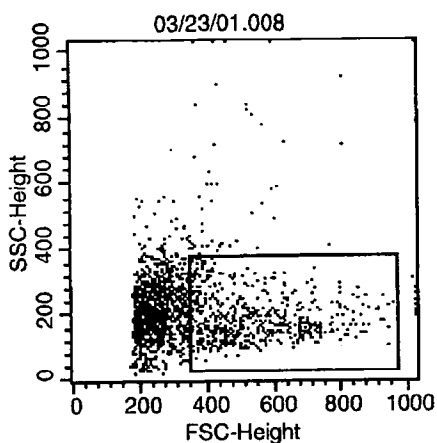
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	5993	100.00	59.93	673.60	59.12	203.99	35.23	6
M1	26, 9910	3114	51.96	31.14	1289.25	464.34	130.83	486.97	562



Quadrant Statistics

File: 03/23/01.007 Log Data Units: Linear Values
 Sample ID: 7 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: G1
 Gated Events: 5993 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log) Y Parameter: FL5-H FL5-Height (Log)
 Quad Location: 27, 11

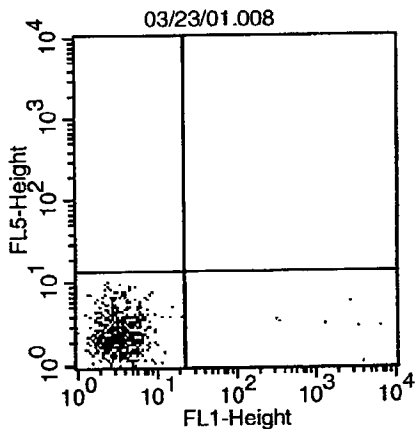
Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	2	0.03	0.02	3.77	3.75	11.97	11.97
UR	1	0.02	0.01	209.08	209.08	14.86	14.86
LL	2906	48.49	29.06	7.89	6.46	2.89	2.54
LR	3084	51.46	30.84	1301.47	477.12	2.90	2.56



Histogram Statistics

File: 03/23/01.008 Log Data Units: Linear Values
 Sample ID: sort 1 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: G1
 Gated Events: 1678 Total Events: 5505
 X Parameter: FL1-H FL1-Height (Log)

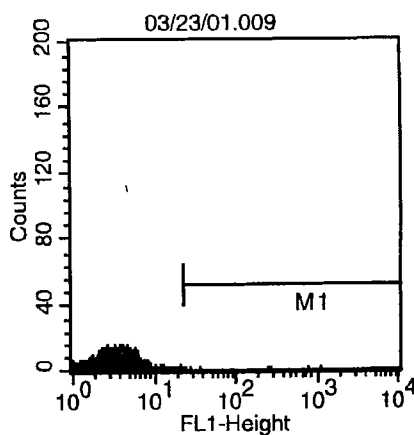
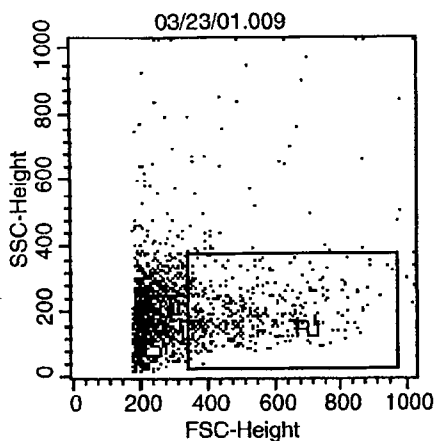
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	1678	100.00	30.48	20.31	3.53	1303.73	3.34	3
M1	22, 9910	13	0.77	0.24	2135.95	1028.06	103.75	1286.40	126



Quadrant Statistics

File: 03/23/01.008 Log Data Units: Linear Values
 Sample ID: sort 1 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: G1
 Gated Events: 1678 Total Events: 5505
 X Parameter: FL1-H FL1-Height (Log) Y Parameter: FL5-H FL5-Height (Log)
 Quad Location: 22, 13

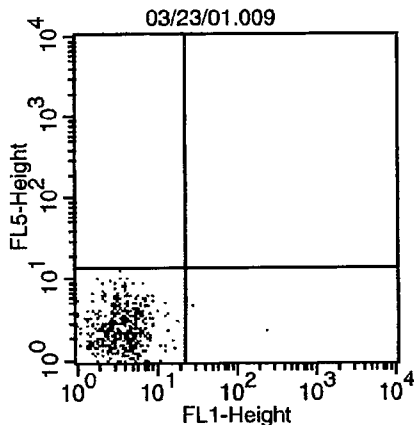
Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	0	0.00	0.00	***	***	***	***
LL	1665	99.23	30.25	3.79	3.38	2.94	2.56
LR	13	0.77	0.24	2135.95	1028.06	2.94	2.73



Histogram Statistics

File: 03/23/01.009 Log Data Units: Linear Values
 Sample ID: sort 7 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: G1
 Gated Events: 1355 Total Events: 5100
 X Parameter: FL1-H FL1-Height (Log)

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	1355	100.00	26.57	5.53	3.48	609.55	3.52	2
M1	22, 9910	7	0.52	0.14	319.01	135.42	117.16	232.91	22



Quadrant Statistics

File: 03/23/01.009 Log Data Units: Linear Values
 Sample ID: sort 7 Patient ID:
 Tube: Panel:
 Acquisition Date: 23-Mar-01 Gate: G1
 Gated Events: 1355 Total Events: 5100
 X Parameter: FL1-H FL1-Height (Log) Y Parameter: FL5-H FL5-Height (Log)
 Quad Location: 22, 13

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	0	0.00	0.00	***	***	***	***
LL	1348	99.48	26.43	3.90	3.42	2.97	2.58
LR	7	0.52	0.14	319.01	135.42	3.35	3.02

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Annex plus

Wipe test

PAGE: 1

USER: 6 ID:HS HOWELL PRESET TIME: 1.00 MON 26 MAR 2001 15:46
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
#: 1 ADC: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 CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR: 1.00000
HALF LIFE(DAYS):N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	13.00	55.47	1.00	1.43	77.0	
2	**	2	21.00	43.64	1.00	3.02	75.0	
3	**	3	13.00	55.47	1.00	4.66	78.0	
4	**	4	20.00	44.72	1.00	6.28	80.0	
5	**	5	163.00	15.67	1.00	7.97	92.0	
6	**	6	12.00	57.74	1.00	9.59	77.0	
7	**	7	15.00	51.64	1.00	11.23	76.0	
8	**	8	15.00	51.64	1.00	12.86	75.0	
9	**	9	20.00	44.72	1.00	14.49	72.0	
10	**	10	14.00	53.45	1.00	16.12	69.0	- background

0+2+11
+0