

V79 COLONY FORMING ASSAY FOLLOWING FACS

Experiment Name: Cell separation by FACS and SF (³HTdR cluster, 50% labeling, five ³HTdR conc.)

Exp. # 3;

Investigator: A. Bishayee

Date: 03/26/01

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
36 µl ³HTdR (Stock : 1 µCi/µl on 2/15/01) + 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: 03/26/01; 4:00 pm

Date/Time: 03/26/01; 7:15 pm

Tube #	³ HTdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ³ HTdR 12uCi/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	1	1.0	0.835	0.165	2
4	2	1.0	0.665	0.335	2
5	3	1.0	0.5	0.5	2
6	4	1.0	0.335	0.665	2
7	6	1.0	0	1	2

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

Date/Time: 03/26/01; 7:30 pm

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

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

Date/Time: 03/27/01; 9-30 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 ³⁰~~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: 03/27/01; 1-00 pm

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: 03/30/01; 12-45 pm

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

ERROR: timeout
OFFENDING COM

STACK:

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.

4/6/2001 7:00am initiate colony ~~count~~ ^{RNA} *[Signature]*
staining and counting

* ALL Dye positive colonies contaminated.
ALL Dye negative colonies not contaminated

1.2 - 167, 178
2.2 - 193, 204, 190
3.2 - 166, 152, 169
4.2 - 150, 132, 148
5.2 - 129, 135, 136
6.2 - 100, 127, 119
7.2 - 81, 75, 100

30 μ l medium

PAGE: 1

USER: 6 ID: H3 HOWELL PRESET TIME: 1.00 TUE 27 MAR 2001 10:04
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR: N RS232: N
 H#: 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: 0 1.00000
 HALF LIFE(DAYS): N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	59.00	26.04	1.00	1.45	76.0	
2	**	2	9.00	66.67	1.00	3.02	75.0	
3	**	3	12.00	57.74	1.00	4.59	77.0	
4	**	4	4.00	100.0	1.00	6.17	76.0	
5	**	5	8.00	70.71	1.00	7.73	76.0	
6	**	6	9.00	66.67	1.00	9.37	79.0	
7	**	7	30037.14	1.95	0.35	10.27	77.0	
8	**	8	*12.00	57.74	1.00	11.90	73.0	
9	**	9	**64890.00	1.76	0.20	12.65	85.0	
10	**	10	56870.00	1.88	0.20	13.46	78.0	
11	**	11	54540.00	1.91	0.20	14.27	77.0	
12	**	12	56435.00	1.88	0.20	15.02	78.0	
13	**	13	80786.66	1.82	0.15	15.72	76.0	
14	**	14	84560.00	1.78	0.15	16.43	78.0	
15	**	15	87933.33	1.74	0.15	17.13	77.0	
16	**	16	101253.33	1.62	0.15	17.83	77.0	
17	**	17	113006.66	1.54	0.15	18.53	76.0	
18	**	18	121893.33	1.48	0.15	19.23	78.0	
19	**	1	172990.00	1.52	0.10	20.00	74.0	
20	**	2	166430.00	1.55	0.10	20.71	74.0	
21	**	3	166500.00	1.27	0.15	21.42	76.0	

* Sample was not added
 ** Sample was added twice

MediumActivity

Experiment: H-3/50%/FACSS3
Date: 3/26/2001

Tube #	Medium count (CPM)	CPM Average	CPM corrected for control	DPM (CPM/e)	At μ Ci/ml on counting	Ao μ Ci/ml at addition [At/e-0.693/TT]	Ao KBq/ml at addition
1	59	17	0	0	0	0	0
2	4	31642	31626	48655	0.7305	0.7306	27.0329
3	30037	55948	55932	86048	1.2920	1.2921	47.8091
4	56870	84426	84410	129861	1.9499	1.9500	72.1516
5	80786	112051	112034	172360	2.5880	2.5882	95.7643
6	101253	168640	168623	259420	3.8952	3.8956	144.1358
7	172990						
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!

Southern

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 MON 02 APR 2001 09:41
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
 H#: 1 AQC: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
 H LIFE(DAYS):N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	9.00	66.67	1.00	1.43	70.0	
2	**	2	9.00	66.67	1.00	3.05	70.0	
3	**	3	5.00	89.44	1.00	4.68	69.0	
4	**	4	12.00	57.74	1.00	6.30	71.0	
5	**	5	10.00	63.25	1.00	7.93	71.0	
6	**	6	10.00	63.25	1.00	9.49	69.0	
7	**	7	2255.00	4.21	1.00	11.12	73.0	
8	**	8	2500.00	4.00	1.00	12.69	73.0	
9	**	9	2502.00	4.00	1.00	14.26	72.0	
10	**	10	4333.00	3.04	1.00	15.82	77.0	
11	**	11	4212.00	3.08	1.00	17.40	74.0	
12	**	12	3324.00	3.47	1.00	19.02	72.0	
13	**	13	3934.00	3.19	1.00	20.65	72.0	
14	**	14	4703.00	2.92	1.00	22.22	77.0	
15	**	15	3881.00	3.21	1.00	23.79	73.0	
16	**	16	6757.00	2.43	1.00	25.42	76.0	
17	**	17	8521.00	2.17	1.00	26.99	81.0	
18	**	18	6181.00	2.54	1.00	28.56	77.0	
19	**	1	8844.00	2.13	1.00	30.20	78.0	
20	**	2	12981.25	1.96	0.80	31.62	84.0	
21	**	3	11244.44	1.99	0.90	33.08	81.0	
22	**	4	29762.86	1.96	0.35	34.03	-1.0	

Standard →

CellSuspension

Experiment: H-3/50%/FACSS3
 Date: 03/26/01

Tube #	Suspension count (CPM)			CPM Average	CPM corrected for control	DPM (CPM/γ e)	A _i μCi/ml on counting	A _o μCi/ml after uptake	A _o KBq/ml after uptake
	1st	2nd	3rd						
1	9	9	5	9	0	0	0.00000	0	0.0000
2	12	10	10			0	0.00000	0	0.0000
3	2255	2500	2502	2419	2410	3707	0.03340	0.03340	1.2358
4	4333	4212	3324	3956	3947	6073	0.05471	0.05471	2.0242
5	3934	4703	3881	4173	4164	6405	0.05771	0.05771	2.1351
6	6757	8521	6181	7153	7144	10991	0.09901	0.09901	3.6635
7	8844	12981	11244	11023	11014	16944	0.15265	0.15265	5.6481
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%/FACS3
 Date/Time: 3/26/01

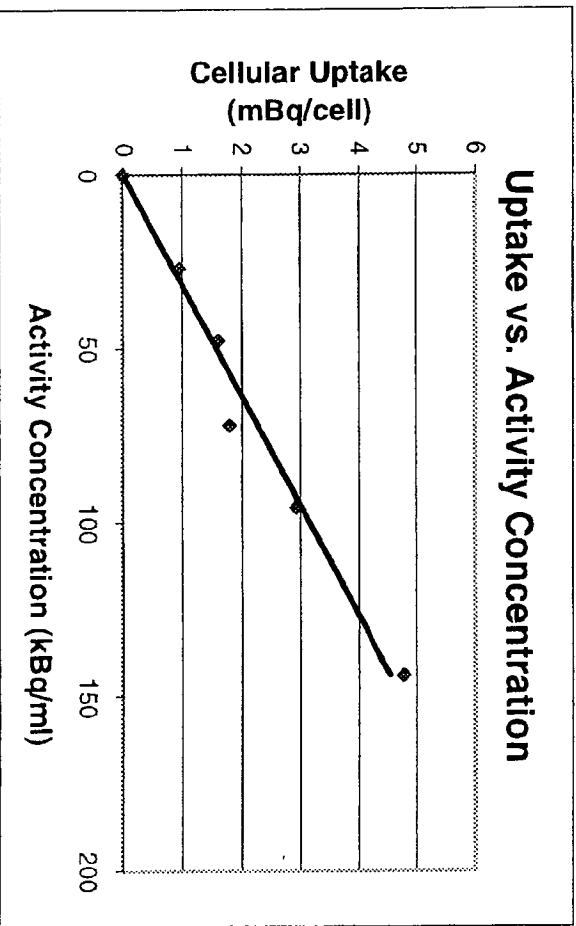
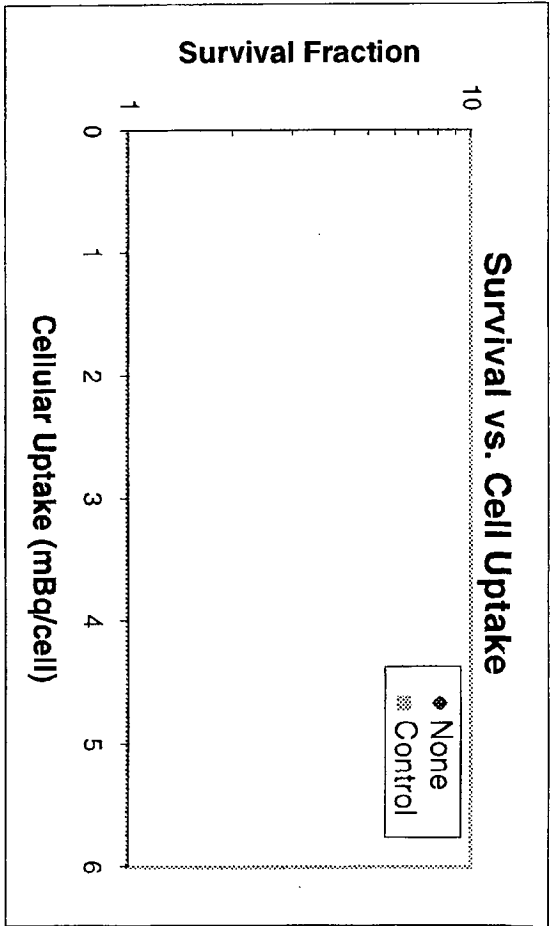
Tube #	Coulter count			Average	Cells/ml	Hemocytometer Count in Grid			
	1st	2nd	3rd			1st	2nd	3rd	4th
1	612	632	643	629	2505333				
2	633	621	654	636	2533333				
3	634	654	666	651	2594667				
4	635	619	644	633	2520000				
5	579	598	609	595	2370667				
6	599	641	642	627	2498667				
7	598	601	582	594	2364000				
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	Colony count		Average	PE (%)	SF	SF
			1st	2nd			3rd	Uncorrected
1	200	251					#DIV/0!	#DIV/0!
2	200	253					#DIV/0!	#DIV/0!
3	200	259					#DIV/0!	#DIV/0!
4	200	252					#DIV/0!	#DIV/0!
5	200	237					#DIV/0!	#DIV/0!
6	200	250					#DIV/0!	#DIV/0!
7	200	236					#DIV/0!	#DIV/0!
8		#DIV/0!					#DIV/0!	#DIV/0!
9		#DIV/0!					#DIV/0!	#DIV/0!
10		#DIV/0!					#DIV/0!	#DIV/0!
11		#DIV/0!					#DIV/0!	#DIV/0!
12		#DIV/0!					#DIV/0!	#DIV/0!
13		#DIV/0!					#DIV/0!	#DIV/0!
14		#DIV/0!					#DIV/0!	#DIV/0!

Experiment: 3/26/01
 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	27.033	0.953	#DIV/0!	#DIV/0!
4	47.809	1.606	#DIV/0!	#DIV/0!
5	72.152	1.801	#DIV/0!	#DIV/0!
6	95.764	2.932	#DIV/0!	#DIV/0!
7	144.136	4.778	#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



SORT

DATE: 3/30/01

TIME: 2:45 - 4:30

INVESTIGATOR:

	LEFT SORT	RIGHT SORT	ABORT	FREQUENCY
	+	-		
TUBE 1	201255	203487		
TUBE 2	563425	501016		
TUBE 3	500572	570786		
TUBE 4	528945	500562		
TUBE 5	522404	500873		
TUBE 6	571869	500629		
TUBE 7	555058	469014		
TUBE 8				
TUBE 9				
TUBE 10				

3/30/01

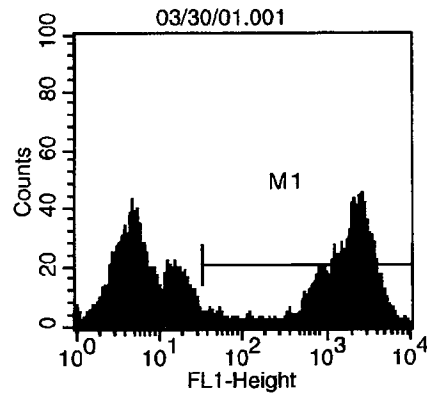
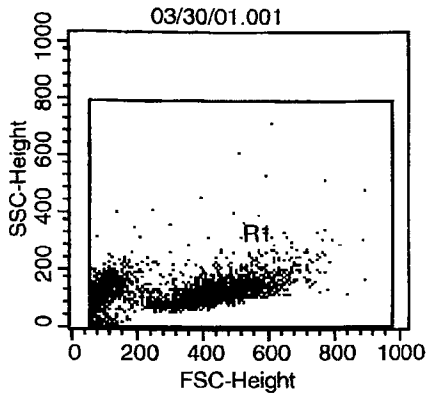
35

Cell count after sortingMS = 500 μ e; background = 21Dye(+)^{ve} cells

Tube #	Coulter Counts	Avg.	Cell Conc. (#/ml)	# of cells plated (dil)	Vol. Plated (μ e)
1	385, 395, 361	380	1.52133	200 (1:1000)	1315 420
2	1211, 1235, 1224	1223	4893.33	200 (1:1000)	410
3	1192, 1185, 1197	1191	4765.33	200 (1:1000)	420
4	1209, 1215, 1222	1215	4861.33	200 (1:1000)	411
5	1175, 1187, 1195	1185	4742.66	200 (1:1000)	420
				2000 (1:100)	420
6	1190, 1181, 1192	1187	4730.66	200 (1:1000)	420
				2000 (1:100)	420
7	1165, 1180, 1172	1172	4689.33	2000 (1:100)	425
				20,000 (1:10)	425

Dye(-)^{ve} cells

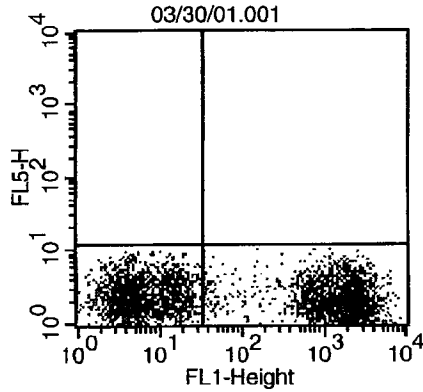
1	365, 380, 377	374	1496.00	200 (1:1000)	1335
2	1127, 1135, 1145	1135	4542.66	200 (1:1000)	440
3	1207, 1229, 1217	1217	4870.66	200 (1:1000)	410
4	1177, 1189, 1162	1176	4704.00	200 (1:1000)	425
5	1150, 1169, 1141	1153	4613.33	200 (1:1000)	433
6	1135, 1169, 1147	1150	4601.33	200 (1:1000)	435
7	1081, 1065, 1047	1064	4257.33	200 (1:1000)	470



Histogram Statistics

File: 03/30/01.001 Log Data Units: Linear Values
 Sample ID: control 1 Patient ID:
 Tube: Panel:
 Acquisition Date: 30-Mar-01 Gate: G1
 Gated Events: 9995 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	9995	100.00	99.95	981.64	99.16	131.47	54.25	2458
M1	32, 9910	5130	51.33	51.30	1905.05	1407.60	64.14	1826.92	2458

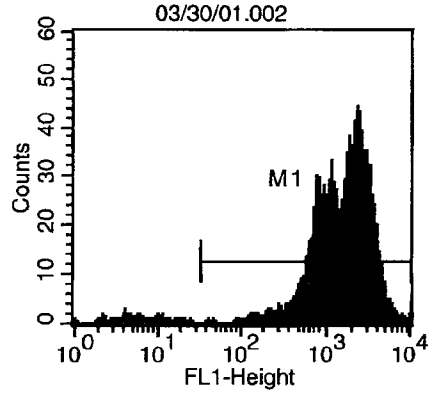
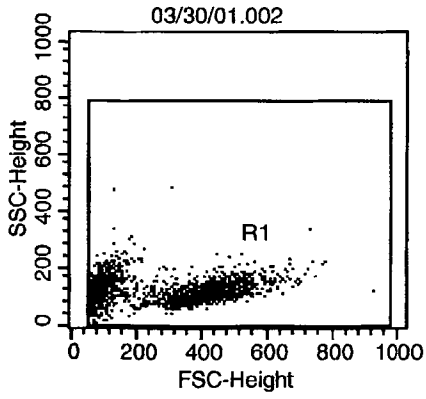


Quadrant Statistics

File: 03/30/01.001 Log Data Units: Linear Values
 Sample ID: control 1 Patient ID:
 Tube: Panel:
 Acquisition Date: 30-Mar-01 Gate: G1
 Gated Events: 9995 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log) Y Parameter: FL5-H (Log)
 Quad Location: 33, 12

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	4881	48.83	48.81	8.02	6.08	2.77	2.44
LR	5114	51.17	51.14	1910.91	1424.28	2.45	2.16

After pooling

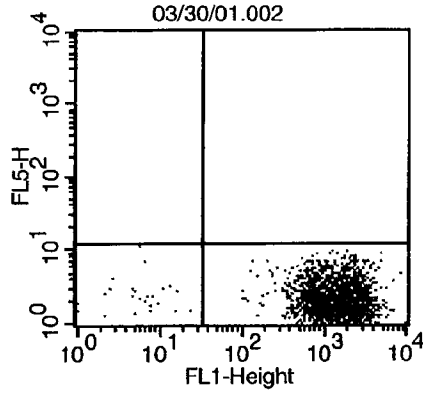


Histogram Statistics

File: 03/30/01.002
 Sample ID: left +
 Tube:
 Acquisition Date: 30-Mar-01
 Gated Events: 5067
 X Parameter: FL1-H FL1-Height (Log)

Log Data Units: Linear Values
 Patient ID:
 Panel:
 Gate: G1
 Total Events: 5070

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak	Ch
All	1, 9910	5067	100.00	99.94	1738.06	1341.26	61.30	1610.76	2035	
M1	32, 9910	4988	98.44	98.38	1765.47	1461.06	59.54	1640.00	2035	



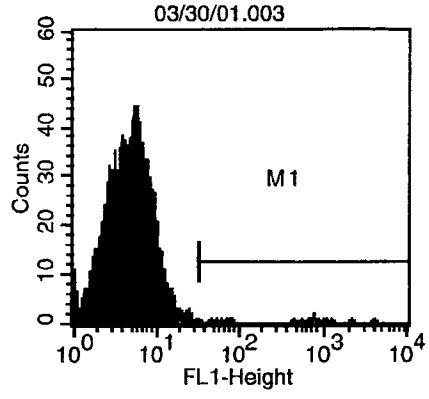
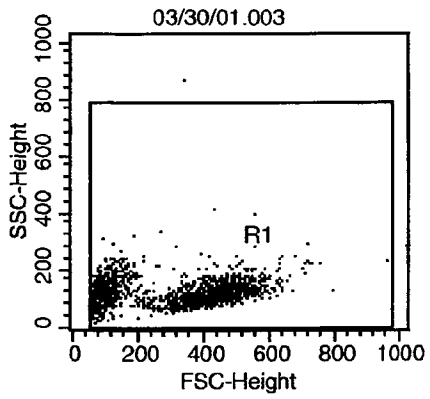
Quadrant Statistics

File: 03/30/01.002
 Sample ID: left +
 Tube:
 Acquisition Date: 30-Mar-01
 Gated Events: 5067
 X Parameter: FL1-H FL1-Height (Log)
 Quad Location: 33, 12

Log Data Units: Linear Values
 Patient ID:
 Panel:
 Gate: G1
 Total Events: 5070
 Y Parameter: FL5-H (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	1	0.02	0.02	1144.44	1144.44	12.30	12.30
LL	79	1.56	1.56	7.56	6.05	2.62	2.29
LR	4987	98.42	98.36	1765.59	1461.14	2.42	2.14

After parking

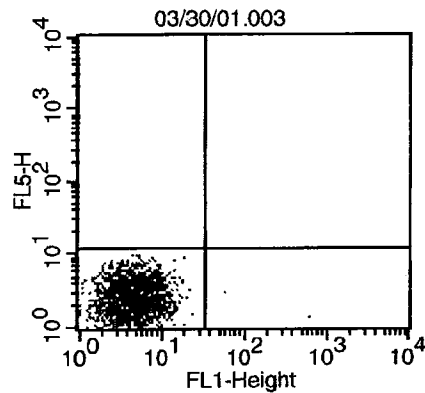


Histogram Statistics

File: 03/30/01.003
 Sample ID: right -
 Tube:
 Acquisition Date: 30-Mar-01
 Gated Events: 5068
 X Parameter: FL1-H FL1-Height (Log)

Log Data Units: Linear Values
 Patient ID:
 Panel:
 Gate: G1
 Total Events: 5070

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	5068	100.00	99.96	7.68	4.54	876.54	4.53	5
M1	32, 9910	17	0.34	0.34	754.24	346.91	121.50	626.43	710

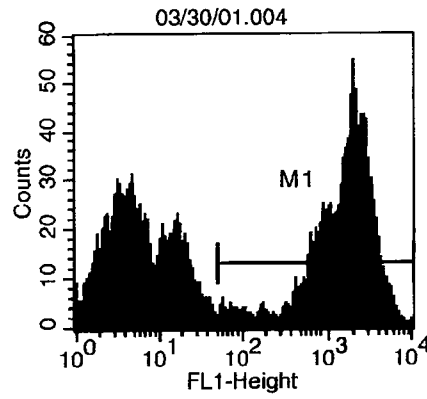
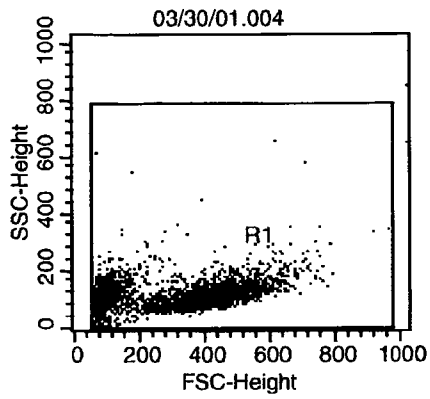


Quadrant Statistics

File: 03/30/01.003
 Sample ID: right -
 Tube:
 Acquisition Date: 30-Mar-01
 Gated Events: 5068
 X Parameter: FL1-H FL1-Height (Log)
 Quad Location: 33, 12

Log Data Units: Linear Values
 Patient ID:
 Panel:
 Gate: G1
 Total Events: 5070
 Y Parameter: FL5-H (Log)

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	5051	99.66	99.63	5.17	4.47	2.80	2.47
LR	17	0.34	0.34	754.24	346.91	2.53	2.21

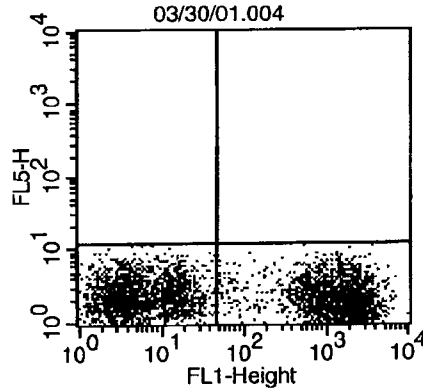


Histogram Statistics

File: 03/30/01.004
 Sample ID: control 2
 Tube:
 Acquisition Date: 30-Mar-01
 Gated Events: 9995
 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	9995	100.00	99.95	941.76	107.99	126.81	375.16	1762
M1	48, 9910	5344	53.47	53.44	1753.94	1353.65	63.73	1625.31	1762

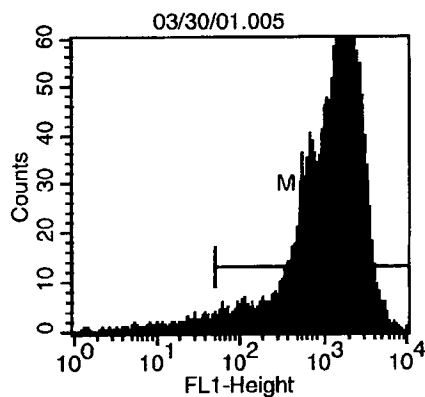
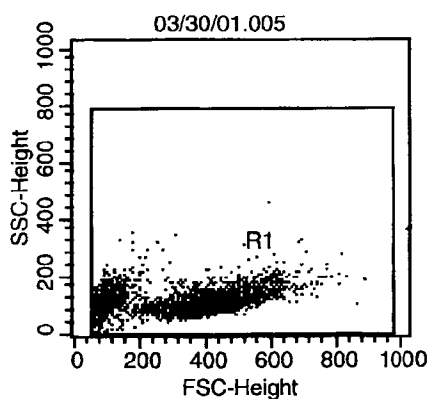


Quadrant Statistics

File: 03/30/01.004
 Sample ID: control 2
 Tube:
 Acquisition Date: 30-Mar-01
 Gated Events: 9995
 X Parameter: FL1-H FL1-Height (Log)
 Quad Location: 48, 12

Log Data Units: Linear Values
 Patient ID:
 Panel:
 Gate: G1
 Total Events: 10000
 Y Parameter: FL5-H (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	1	0.01	0.01	4.22	4.22	13.22	13.22
UR	0	0.00	0.00	***	***	***	***
LL	4650	46.52	46.50	8.55	5.91	2.84	2.49
LR	5344	53.47	53.44	1753.94	1353.65	2.49	2.19

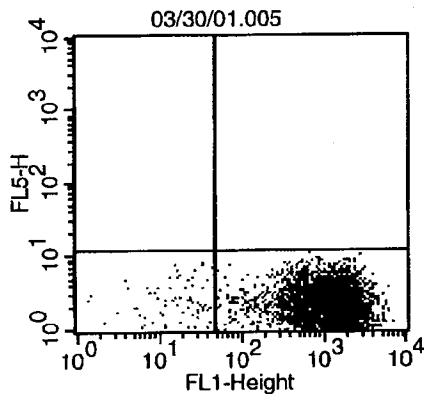


Histogram Statistics

File: 03/30/01.005
 Sample ID: 100%
 Tube:
 Acquisition Date: 30-Mar-01
 Gated Events: 9996
 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	9996	100.00	99.96	1397.07	1050.14	62.28	1321.58	1945
M1	48, 9910	9796	98.00	97.96	1425.13	1140.87	60.08	1345.57	1945

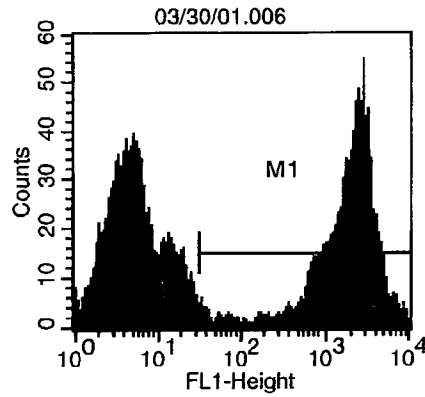
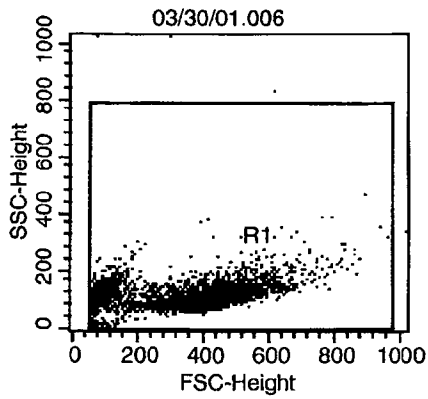


Quadrant Statistics

File: 03/30/01.005
 Sample ID: 100%
 Tube:
 Acquisition Date: 30-Mar-01
 Gated Events: 9996
 X Parameter: FL1-H FL1-Height (Log)
 Quad Location: 48, 12

Log Data Units: Linear Values
 Patient ID:
 Panel:
 Gate: G1
 Total Events: 10000
 Y Parameter: FL5-H (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	1	0.01	0.01	3718.03	3718.03	12.41	12.41
LL	200	2.00	2.00	22.73	18.13	2.98	2.62
LR	9795	97.99	97.95	1424.89	1140.73	2.47	2.18

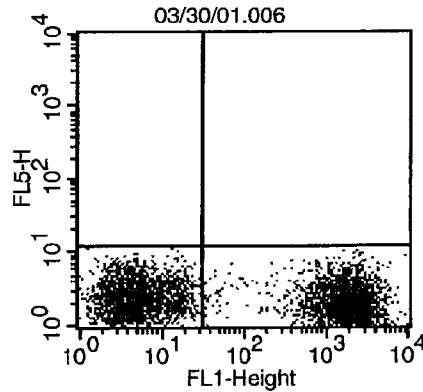


Histogram Statistics

File: 03/30/01.006
 Sample ID: 3
 Tube:
 Acquisition Date: 30-Mar-01
 Gated Events: 9988
 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	9988	100.00	99.88	998.49	83.98	133.77	22.98	2617
M1	30, 9910	4882	48.88	48.82	2035.89	1580.93	61.05	1919.57	2617

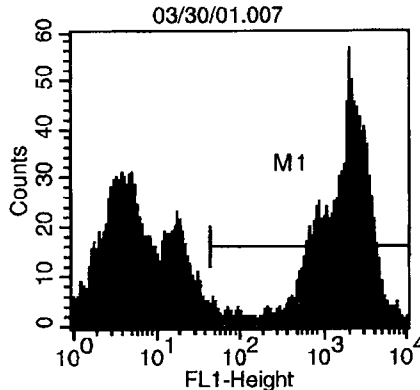
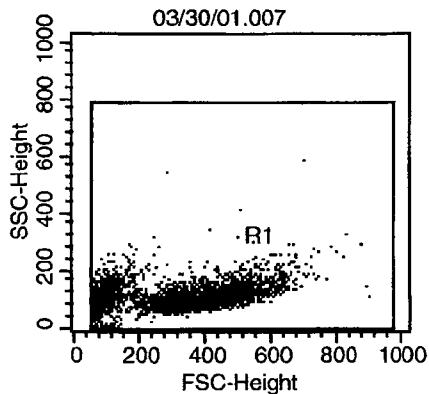


Quadrant Statistics

File: 03/30/01.006
 Sample ID: 3
 Tube:
 Acquisition Date: 30-Mar-01
 Gated Events: 9988
 X Parameter: FL1-H FL1-Height (Log)
 Quad Location: 31, 12

Log Data Units: Linear Values
 Patient ID:
 Panel:
 Gate: G1
 Total Events: 10000
 Y Parameter: FL5-H (Log)

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	5126	51.32	51.26	6.69	5.11	2.78	2.45
LR	4862	48.68	48.62	2044.14	1606.84	2.45	2.16

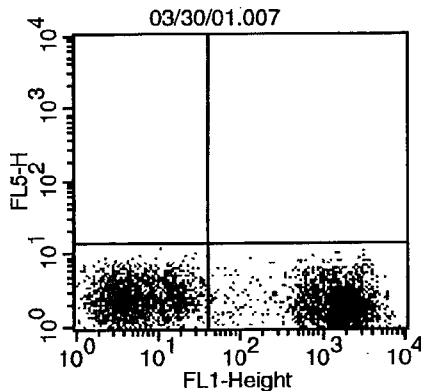


Histogram Statistics

File: 03/30/01.007
 Sample ID: 4
 Tube:
 Acquisition Date: 30-Mar-01
 Gated Events: 9993
 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	9993	100.00	99.93	963.26	100.42	128.89	199.89	1860
M1	42, 9910	5170	51.74	51.70	1854.25	1450.56	62.30	1762.36	1860



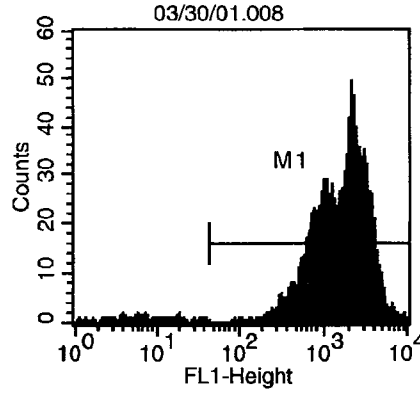
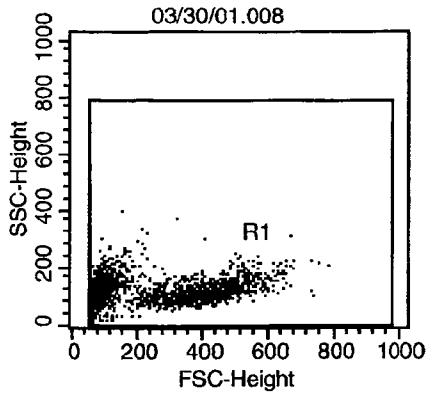
Quadrant Statistics

File: 03/30/01.007
 Sample ID: 4
 Tube:
 Acquisition Date: 30-Mar-01
 Gated Events: 9993
 X Parameter: FL1-H FL1-Height (Log)
 Quad Location: 41, 14

Log Data Units: Linear Values
 Patient ID:
 Panel:
 Gate: G1
 Total Events: 10000
 Y Parameter: FL5-H (Log)

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	4823	48.26	48.23	8.17	5.74	2.83	2.49
LR	5170	51.74	51.70	1854.25	1450.56	2.46	2.16

After sorting

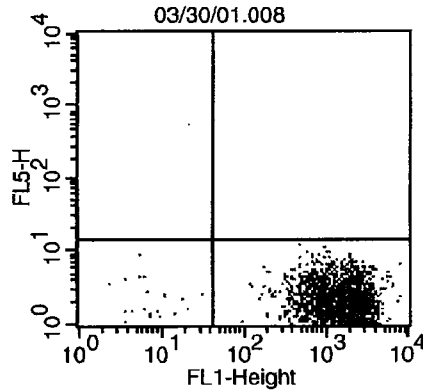


Histogram Statistics

File: 03/30/01.008
 Sample ID: sort 4 +
 Tube:
 Acquisition Date: 30-Mar-01
 Gated Events: 5190
 X Parameter: FL1-H FL1-Height (Log)

Log Data Units: Linear Values
 Patient ID:
 Panel:
 Gate: G1
 Total Events: 5190

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CY	Median	Peak Ch
All	1, 9910	5190	100.00	100.00	1715.64	1348.71	60.05	1596.34	1928
M1	42, 9910	5126	98.77	98.77	1736.93	1438.54	58.66	1625.31	1928



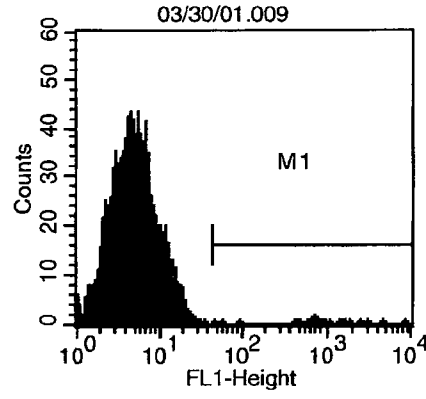
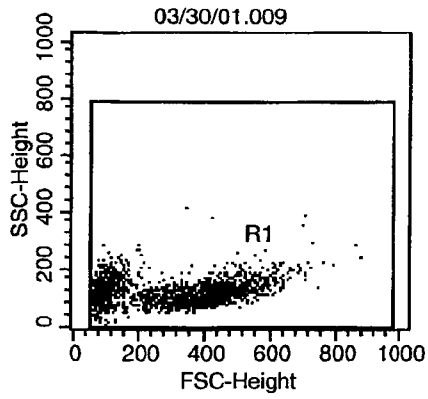
Quadrant Statistics

File: 03/30/01.008
 Sample ID: sort 4 +
 Tube:
 Acquisition Date: 30-Mar-01
 Gated Events: 5190
 X Parameter: FL1-H FL1-Height (Log)
 Quad Location: 41, 14

Log Data Units: Linear Values
 Patient ID:
 Panel:
 Gate: G1
 Total Events: 5190
 Y Parameter: FL5-H (Log)

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	64	1.23	1.23	10.31	7.71	2.66	2.37
LR	5126	98.77	98.77	1736.93	1438.54	2.48	2.18

After rising

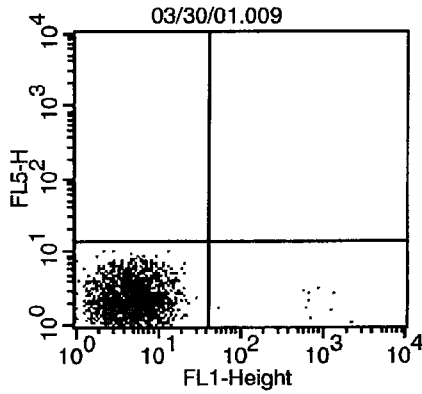


Histogram Statistics

File: 03/30/01.009
 Sample ID: sort 4 -
 Tube:
 Acquisition Date: 30-Mar-01
 Gated Events: 5277
 X Parameter: FL1-H FL1-Height (Log)

Log Data Units: Linear Values
 Patient ID:
 Panel:
 Gate: G1
 Total Events: 5280

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	5277	100.00	99.94	12.64	4.75	1157.19	4.53	4
M1	42, 9910	30	0.57	0.57	1265.48	706.89	118.72	720.15	637

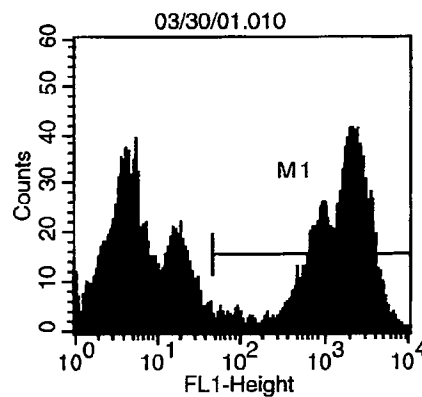
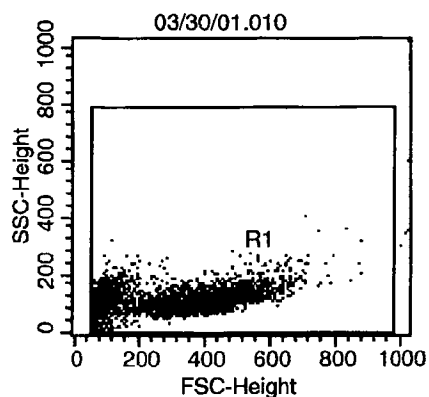


Quadrant Statistics

File: 03/30/01.009
 Sample ID: sort 4 -
 Tube:
 Acquisition Date: 30-Mar-01
 Gated Events: 5277
 X Parameter: FL1-H FL1-Height (Log)
 Quad Location: 41, 14

Log Data Units: Linear Values
 Patient ID:
 Panel:
 Gate: G1
 Total Events: 5280
 Y Parameter: FL5-H (Log)

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	5247	99.43	99.38	5.48	4.62	2.80	2.47
LR	30	0.57	0.57	1265.48	706.89	2.55	2.32

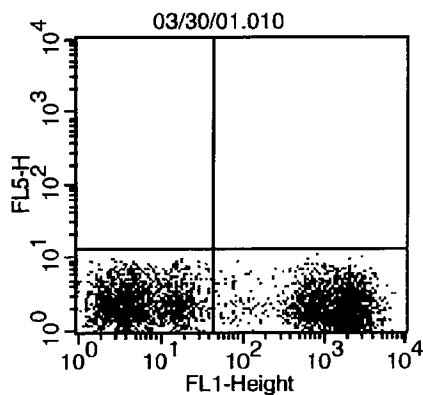


Histogram Statistics

File: 03/30/01.010
 Sample ID: 5
 Tube:
 Acquisition Date: 30-Mar-01
 Gated Events: 9990
 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	9990	100.00	99.90	871.01	92.55	131.47	88.57	1810	
M1	45, 9910	5095	51.00	50.95	1699.82	1319.65	63.61	1582.04	1810	

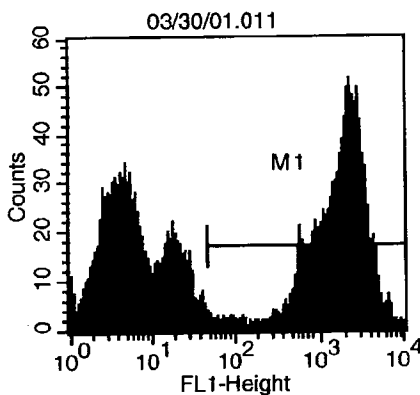
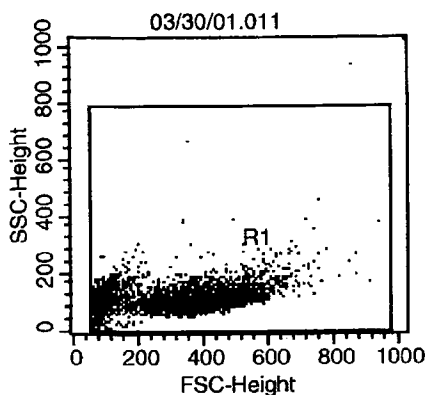


Quadrant Statistics

File: 03/30/01.010
 Sample ID: 5
 Tube:
 Acquisition Date: 30-Mar-01
 Gated Events: 9990
 X Parameter: FL1-H FL1-Height (Log)
 Quad Location: 45, 13

Log Data Units: Linear Values
 Patient ID:
 Panel:
 Gate: G1
 Total Events: 10000
 Y Parameter: FL5-H (Log)

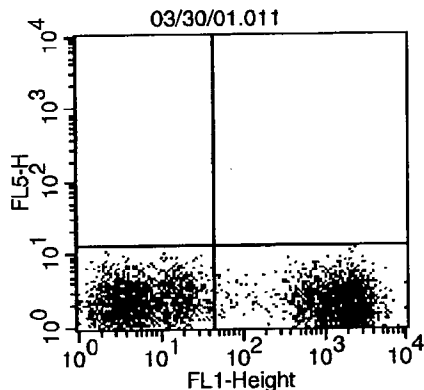
Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	0	0.00	0.00	***	***	***	***
LL	4895	49.00	48.95	8.33	5.82	2.84	2.50
LR	5095	51.00	50.95	1699.82	1319.65	2.49	2.19



Histogram Statistics

File: 03/30/01.011 Log Data Units: Linear Values
 Sample ID: 6 Patient ID:
 Tube: Panel:
 Acquisition Date: 30-Mar-01 Gate: G1
 Gated Events: 9995 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log)

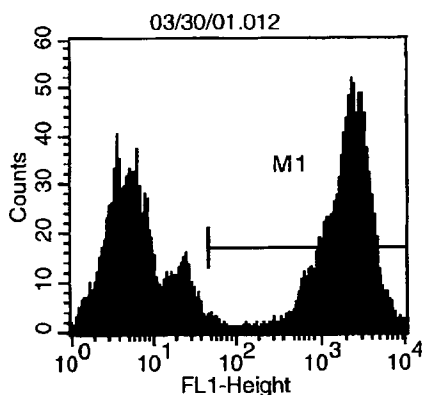
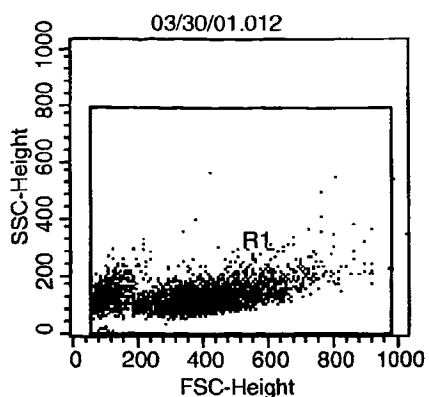
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9995	100.00	99.95	991.07	109.18	123.99	441.09	2053
M1	45, 9910	5279	52.82	52.79	1868.94	1501.44	59.23	1778.28	2053



Quadrant Statistics

File: 03/30/01.011 Log Data Units: Linear Values
 Sample ID: 6 Patient ID:
 Tube: Panel:
 Acquisition Date: 30-Mar-01 Gate: G1
 Gated Events: 9995 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log) Y Parameter: FL5-H (Log)
 Quad Location: 45, 13

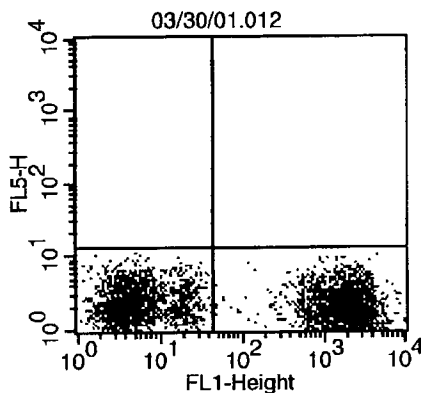
Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	0	0.00	0.00	***	***	***	***
LL	4716	47.18	47.16	8.39	5.81	2.80	2.46
LR	5279	52.82	52.79	1868.94	1501.44	2.45	2.16



Histogram Statistics

File: 03/30/01.012 Log Data Units: Linear Values
 Sample ID: 7 Patient ID:
 Tube: Panel:
 Acquisition Date: 30-Mar-01 Gate: G1
 Gated Events: 9992 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	9992	100.00	99.92	1169.46	128.43	110.83	685.39	2090
M1	45, 9910	5417	54.21	54.17	2150.51	1793.80	56.18	2016.91	2090



Quadrant Statistics

File: 03/30/01.012 Log Data Units: Linear Values
 Sample ID: 7 Patient ID:
 Tube: Panel:
 Acquisition Date: 30-Mar-01 Gate: G1
 Gated Events: 9992 Total Events: 10000
 X Parameter: FL1-H FL1-Height (Log) Y Parameter: FL5-H (Log)
 Quad Location: 45, 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	4575	45.79	45.75	7.86	5.66	2.77	2.44
LR	5417	54.21	54.17	2150.51	1793.80	2.42	2.13

Wipe test

ER: 6 ID:H3 HOWELL PRESET TIME: 1.00 TUE 03 APR 2001 14:14
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
H#: 1 AQC:N GCF:N RCM:N
CHANNEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIB: 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	9.00	66.67	1.00	1.47	73.0	
2	**	2	14.00	53.45	1.00	3.11	79.0	
3	**	3	13.00	55.47	1.00	4.73	73.0	
4	**	4	12.00	57.74	1.00	6.36	73.0	
5	**	5	6.00	81.65	1.00	7.92	74.0	
6	**	6	8.00	70.71	1.00	9.58	80.0	
7	**	7	14.00	53.45	1.00	11.21	75.0	
8	**	8	9.00	66.67	1.00	12.83	77.0	
9	**	9	7.00	75.59	1.00	14.46	76.0	
10	**	10	7.00	75.59	1.00	16.03	79.0	
11	**	11	11.00	60.30	1.00	17.61	71.0	Background