

### V79 COLONY FORMING ASSAY FOLLOWING FACS

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

**Exp. # 4;**

**Investigator:** A. Bishayee

**Date:** 04/02/01

1. Set the rocker-roller at 37°C incubator with 5% CO<sub>2</sub>, set the Coulter Counter, wash cells (from two 80-90% confluent 175 cm<sup>2</sup> 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 : 5,196,000 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% CO<sub>2</sub> Date/Time: 04/04/01
5. Prepare MEMB containing radioactivity in hood 3-30 pm  
 300 µl <sup>3</sup>HTdR (Stock : 4.7 µCi/µl on 2/15/01) + 4.7 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: 04/02/01

7-15 pm

NOTES

- all colonies contaminated  
no results

high concentration indicates focus is on unlabeled cells

Tube #	<sup>3</sup> HTdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ <sup>3</sup> HTdR 60uCi/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	10	1.0	0.667	0.333	2
4	15	1.0	0.5	0.5	2
5	20	1.0	0.334	0.666	2
6	25	1.0	0.167	0.833	2
7	30	1.0	0	1	2

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

Date/Time: 04/02/01

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

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: 04/03/01; 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 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: 04/03/01; 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: 04/06/01; 1-30 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 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.

30ul medium

SER: 6 ID: H3 HOWELL      PRESET TIME: 1.00      TUE 03 APR 2001 13:54  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR: N      RS232: N  
 H#: 1 ABC: 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	29- 1	1	10.00	63.25	1.00	1.43	78.0	
2	29- 2	1	12.00	57.74	1.00	3.00	73.0	
3	29- 3	1	12.00	57.74	1.00	4.56	73.0	
4	29- 4	1	12.00	57.74	1.00	6.13	76.0	
5	29- 5	1	7.00	75.59	1.00	7.76	74.0	
6	29- 6	1	10.00	63.25	1.00	9.33	74.0	
7	29- 7	1	255773.33	1.02	0.15	10.05	76.0	
8	29- 8	1	269713.31	0.99	0.15	10.77	76.0	
9	29- 9	1	283206.66	0.97	0.15	11.48	76.0	
10	29-10	1	426213.31	0.79	0.15	12.22	77.0	
11	29-11	1	405800.00	0.99	0.10	12.95	74.0	
12	29-12	1	362320.00	0.86	0.15	13.68	72.0	
13	29-13	1	444986.66	0.77	0.15	14.41	73.0	
14	29-14	1	486986.66	0.74	0.15	15.14	73.0	
15	29-15	1	470933.31	0.75	0.15	15.88	73.0	
16	29-16	1	581760.00	0.68	0.15	16.62	73.0	
17	29-17	1	611550.00	0.81	0.10	17.38	73.0	
18	29-18	1	563120.00	0.84	0.10	18.12	73.0	
19	**- 1	1	642200.00	0.64	0.15	18.93	73.0	
20	**- 2	1	678313.31	0.63	0.15	19.68	70.0	
21	**- 3	1	712860.00	0.61	0.15	20.44	71.0	

50 Mc cells

5

USER: 6 ID:H3 HOWELL      PRESET TIME: 1.00      MON 09 APR 2001 09:13  
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:  
DATA CALC: CPM, UNKNOWN REPLICATES: 1      NORM FACTOR: 1.00000  
HALF LIFE(DAYS):N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ET
1	**	1	9.00	66.67	1.00	1.42	70.0	
2	**	2	19.00	45.88	1.00	3.01	72.0	
3	**	3	6.00	81.65	1.00	4.58	71.0	
4	**	4	8.00	70.71	1.00	6.15	71.0	
5	**	5	8.00	70.71	1.00	7.72	73.0	
6	**	6	14.00	53.45	1.00	9.28	72.0	
7	**	7	17275.00	1.96	0.60	10.44	76.0	
8	**	8	17670.00	1.94	0.60	11.66	76.0	
9	**	9	17195.00	1.97	0.60	12.88	75.0	
10	**	10	24560.00	1.90	0.45	13.93	77.0	
11	**	11	26952.50	1.93	0.40	14.94	79.0	
12	**	12	25255.00	1.99	0.40	15.89	76.0	
13	**	13	29668.57	1.96	0.35	16.79	77.0	
14	**	14	33353.33	2.00	0.30	17.64	78.0	
15	**	15	30440.00	1.94	0.35	18.54	77.0	
16	**	16	37026.66	1.90	0.30	19.39	76.0	
17	**	17	37670.00	1.88	0.30	20.24	78.0	
18	**	18	39156.66	1.85	0.30	21.09	79.0	
19	**	1	47760.00	1.83	0.25	21.95	83.0	
20	**	2	53195.00	1.94	0.20	22.75	85.0	
21	**	3	53515.00	1.93	0.20	23.55	82.0	

MediumActivity

Experiment: H-3/50%/FACSA4  
Date: 4/2/2001

Tube #	Medium count (CPM)		3rd	CPM Average	CPM corrected for control	DPM (CPM/y e)	At $\mu$ Ci/ml on counting	Ao $\mu$ Ci/ml at addition	Ao KBq/ml at addition
1	10	12	12	11	0	0	0	0	0
2	12	7	10	11	0	0	0	0	0
3	255773	269713	283206	269564	269554	414698	6.2267	6.2273	230.4113
4	426213	405800	362320	398111	398101	612462	9.1961	9.1971	340.2918
5	444986	486986	470933	467635	467625	719422	10.8021	10.8032	399.7201
6	581760	611550	563120	585477	585466	900717	13.5243	13.5257	500.4498
7	642200	678313	712860	677791	677781	1042739	15.6567	15.6584	579.3591
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!

[Ave-0.693/tT]

Coulter count before sorting

MS = 50 ul ; Background = 2, 3, 2

Tube #	Coulter count
1	580, <del>565</del> , 571
2	572, 578, 561
3	527, 530, 544
4	566, 571, 561
5	572, 571, 565
6	554, 551, 569
7	533, 521, 511

8

cell counting after sorting

MS = 50 ul ; Background = 3, 4, 2

Dye (+) ve cells

Tube #	Counter count	Avg.	Cell conc	Dilution/cell plated	Vol. of cells plated (ul)
1	35, 39, 30	34.6	138666	1:1000 (200)	1.44
2	109, 99, 112	106	426666	1:1000 (200)	0.47
3	95, 89, 103	95	382666	1:100 (2000) 1:10 (20,000)	0.520 0.520
4	87, 97, 81	88	353333	1:100 (2000) 1:10 (20,000)	0.570 0.570
5	56, 51, 58	55	220000	1:100 (2000) 1:10 (20,000)	0.9 0.9
6	93, 101, 85	93	372000	1:100 (2000) 1:10 (20,000)	0.54 0.54
7	81, 76, 89	82	<del>48</del> 328000	1:100 (2000) 1:10 (20000)	0.61 0.61

Dye (-) ve cells

1	29, 35, 31	31	126666	1:1000 (200)	1.58
2	90, 98, 103	97	388000	1:1000 (200)	0.52
3	88, 95, 99	94	376000	1:1000 (200)	0.53
4	101, 95, 96	97	389333	1:1000 (200)	0.51
5	61, 55, 52	56	224000	1:1000 (200)	0.90
6	111, 101, 109	107	428000	1:1000 (200) 1:100 (2000)	0.47 0.47
7	115, 110, 119	114	458666	1:1000 (200) 1:100 (2000)	0.44 0.44



SORT

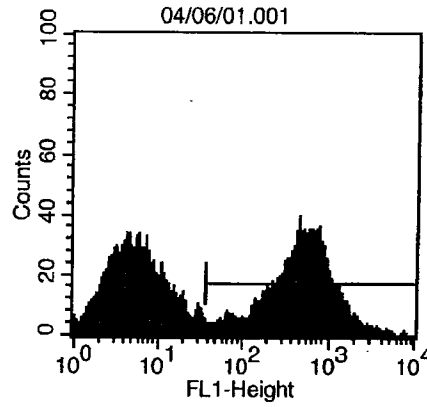
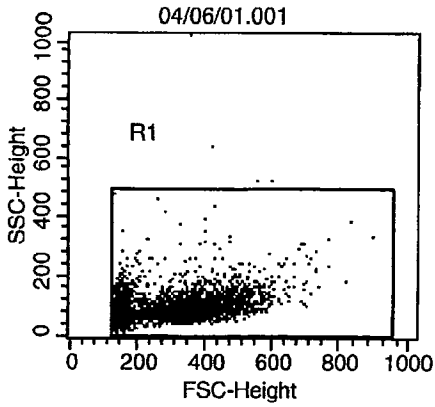
MAK 4/6/2001  
5:00pm

DATE: 2/6/01

TIME: 2:30 - 4:30

INVESTIGATOR:

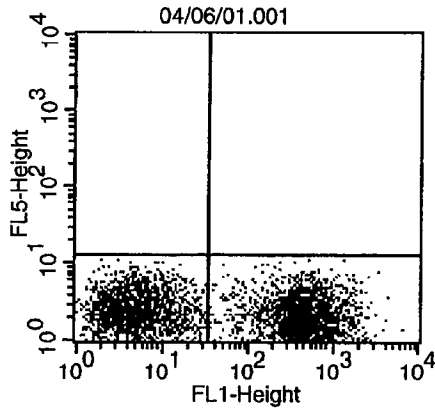
	LEFT SORT	RIGHT SORT	ABORT	FREQUENCY
	-	+		
TUBE 1	156845 <del>153068</del>	158110 <del>145394</del>		
TUBE 2	534311	500000		
TUBE 3	528307	500583		
TUBE 4	501212	516229		
TUBE 5	271059	261562		
TUBE 6	528909	501208		
TUBE 7	511297	500695		
TUBE 8				
TUBE 9				
TUBE 10				



Histogram Statistics

File: 04/06/01.001                      Log Data Units: Linear Values  
 Sample ID: control 1                      Patient ID:  
 Tube:    Panel:  
 Acquisition Date: 06-Apr-01              Gate: G1  
 Gated Events: 9989                        Total Events: 10000  
 X Parameter: FL1-H FL1-Height (Log)

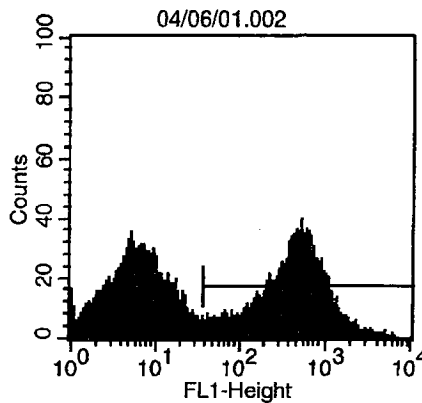
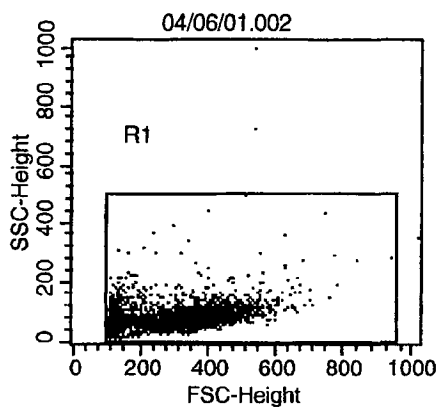
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9989	100.00	99.89	303.17	53.04	146.04	79.86	410
M1	35, 9910	5211	52.17	52.11	574.65	430.80	81.93	465.55	410



Quadrant Statistics

File: 04/06/01.001                      Log Data Units: Linear Values  
 Sample ID: control 1                      Patient ID:  
 Tube:    Panel:  
 Acquisition Date: 06-Apr-01              Gate: G1  
 Gated Events: 9989                        Total Events: 10000  
 X Parameter: FL1-H FL1-Height (Log)    Y Parameter: FL5-H FL5-Height (Log)  
 Quad Location: 34, 13

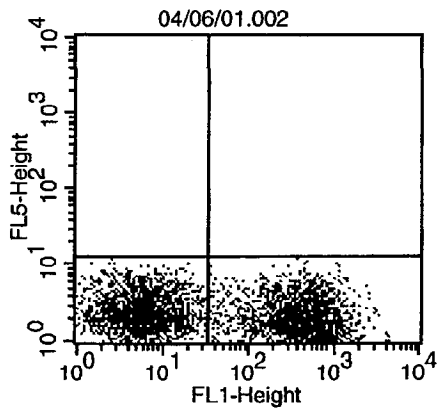
Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	0	0.00	0.00	***	***	***	***
LL	4775	47.80	47.75	7.08	5.40	2.77	2.45
LR	5214	52.20	52.14	574.34	430.17	2.38	2.10



Histogram Statistics

File: 04/06/01.002                      Log Data Units: Linear Values  
 Sample ID: control 2                      Patient ID:  
 Tube:    Panel:  
 Acquisition Date: 06-Apr-01              Gate: G1  
 Gated Events: 9994                          Total Events: 10000  
 X Parameter: FL1-H FL1-Height (Log)

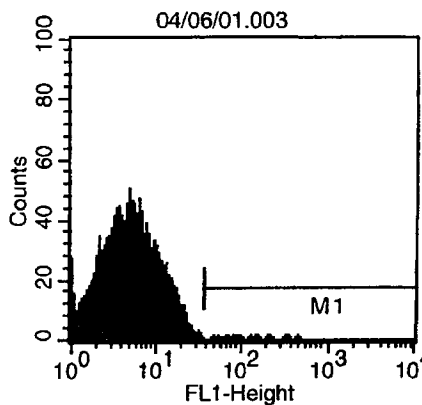
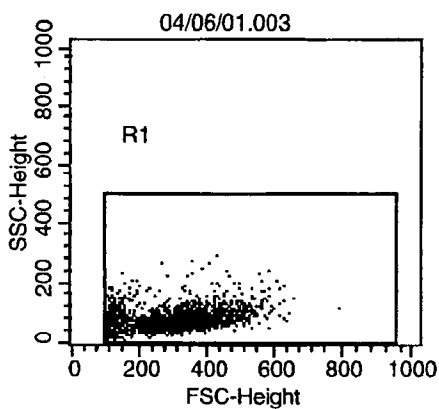
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak	Ch
All	1, 9910	9994	100.00	99.94	269.05	50.10	148.56	56.74	486	
M1	35, 9910	5173	51.76	51.73	512.75	375.37	84.00	417.92	486	



Quadrant Statistics

File: 04/06/01.002                      Log Data Units: Linear Values  
 Sample ID: control 2                      Patient ID:  
 Tube:    Panel:  
 Acquisition Date: 06-Apr-01              Gate: G1  
 Gated Events: 9994                          Total Events: 10000  
 X Parameter: FL1-H FL1-Height (Log)      Y Parameter: FL5-H FL5-Height (Log)  
 Quad Location: 34, 13

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	2	0.02	0.02	505.32	489.16	12.75	12.75
LL	4817	48.20	48.17	7.54	5.76	2.87	2.53
LR	5175	51.78	51.75	512.38	374.65	2.42	2.12

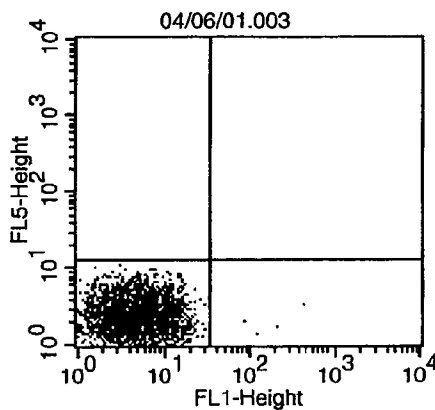


Histogram Statistics

File: 04/06/01.003  
 Sample ID: negative 2  
 Tube:  
 Acquisition Date: 06-Apr-01  
 Gated Events: 7110  
 X Parameter: FL1-H FL1-Height (Log)

Log Data Units: Linear Values  
 Patient ID:  
 Panel:  
 Gate: G1  
 Total Events: 7110

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	7110	100.00	100.00	6.40	4.85	149.96	4.74	4
M1	35, 9910	17	0.24	0.24	145.19	115.85	75.31	91.40	44

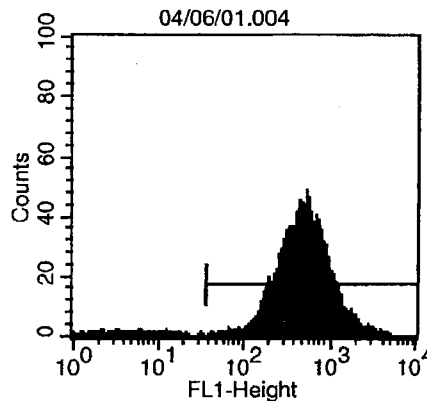
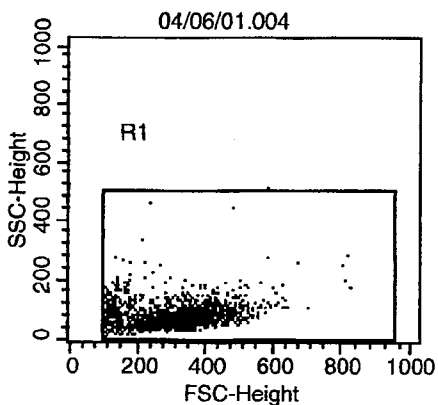


Quadrant Statistics

File: 04/06/01.003  
 Sample ID: negative 2  
 Tube:  
 Acquisition Date: 06-Apr-01  
 Gated Events: 7110  
 X Parameter: FL1-H FL1-Height (Log)  
 Quad Location: 34, 13

Log Data Units: Linear Values  
 Patient ID:  
 Panel:  
 Gate: G1  
 Total Events: 7110  
 Y Parameter: FL5-H FL5-Height (Log)

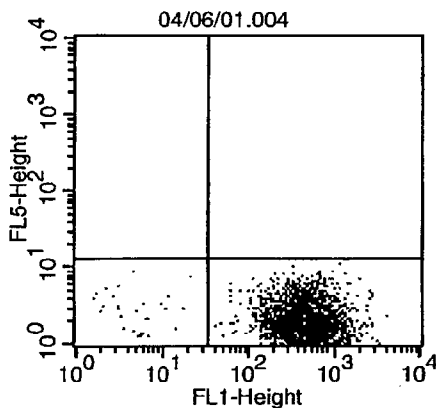
Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	0	0.00	0.00	***	***	***	***
LL	7093	99.76	99.76	6.07	4.82	2.73	2.41
LR	17	0.24	0.24	145.19	115.85	2.99	2.60



Histogram Statistics

File: 04/06/01.004                      Log Data Units: Linear Values  
 Sample ID: positive 2                    Patient ID:  
 Tube:    Panel:  
 Acquisition Date: 06-Apr-01            Gate: G1  
 Gated Events: 5922                        Total Events: 5925  
 X Parameter: FL1-H FL1-Height (Log)

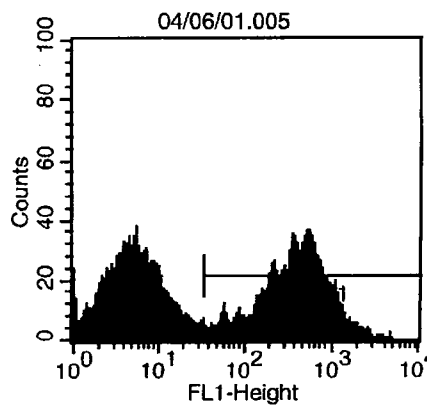
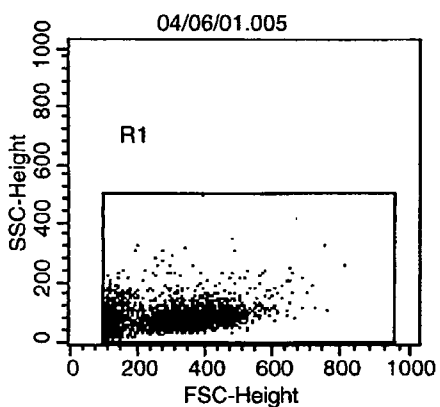
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	5922	100.00	99.95	540.28	421.11	72.55	449.10	465
M1	35, 9910	5832	98.48	98.43	548.52	451.05	70.97	453.16	465



Quadrant Statistics

File: 04/06/01.004                      Log Data Units: Linear Values  
 Sample ID: positive 2                    Patient ID:  
 Tube:    Panel:  
 Acquisition Date: 06-Apr-01            Gate: G1  
 Gated Events: 5922                        Total Events: 5925  
 X Parameter: FL1-H FL1-Height (Log)    Y Parameter: FL5-H FL5-Height (Log)  
 Quad Location: 34, 13

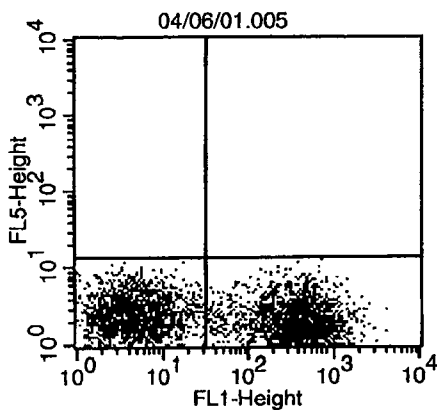
Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	0	0.00	0.00	***	***	***	***
LL	90	1.52	1.52	6.38	4.92	2.85	2.52
LR	5832	98.48	98.43	548.52	451.05	2.34	2.06



Histogram Statistics

File: 04/06/01.005                      Log Data Units: Linear Values  
 Sample ID: 3                              Patient ID:  
 Tube:  
 Acquisition Date: 06-Apr-01              Gate: G1  
 Gated Events: 9994                      Total Events: 10000  
 X Parameter: FL1-H FL1-Height (Log)

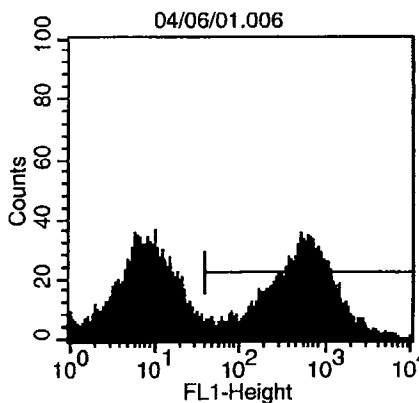
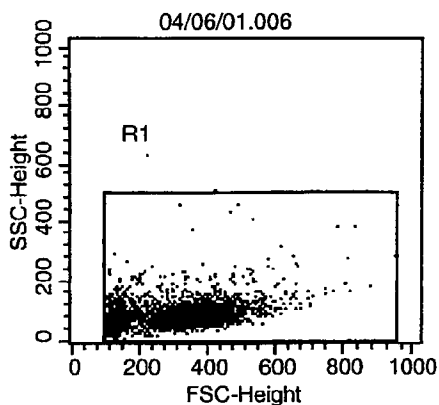
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak	Ch
All	1, 9910	9994	100.00	99.94	248.68	44.56	146.39	52.57		5
M1	33, 9910	5132	51.35	51.32	478.12	355.50	80.96	388.91		486



Quadrant Statistics

File: 04/06/01.005                      Log Data Units: Linear Values  
 Sample ID: 3                              Patient ID:  
 Tube:  
 Acquisition Date: 06-Apr-01              Gate: G1  
 Gated Events: 9994                      Total Events: 10000  
 X Parameter: FL1-H FL1-Height (Log)      Y Parameter: FL5-H FL5-Height (Log)  
 Quad Location: 32, 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	4853	48.56	48.53	6.45	4.96	2.83	2.48
LR	5141	51.44	51.41	477.34	354.01	2.38	2.09

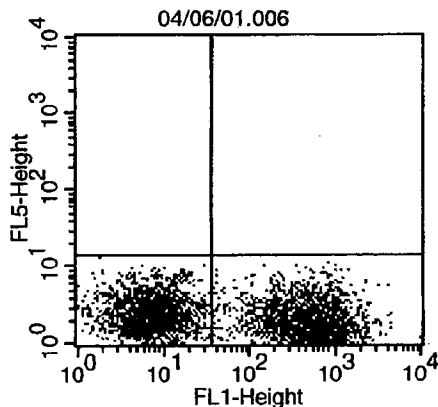


Histogram Statistics

File: 04/06/01.006  
 Sample ID: 4  
 Tube:  
 Acquisition Date: 06-Apr-01  
 Gated Events: 9997  
 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	9997	100.00	99.97	331.95	63.61	154.44	62.79	9
M1	38, 9910	5240	52.42	52.40	624.72	443.54	90.73	491.37	474

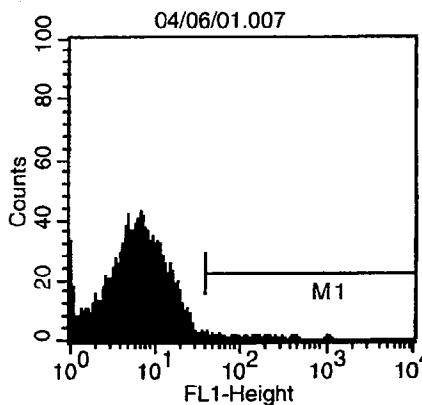
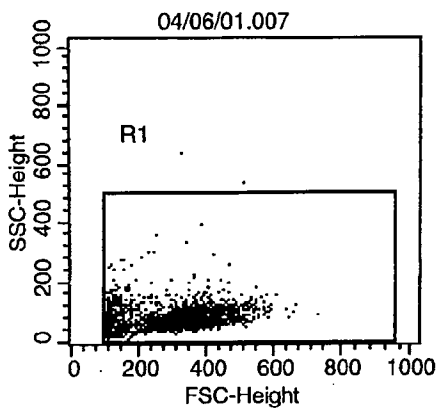


Quadrant Statistics

File: 04/06/01.006  
 Sample ID: 4  
 Tube:  
 Acquisition Date: 06-Apr-01  
 Gated Events: 9997  
 X Parameter: FL1-H FL1-Height (Log)  
 Quad Location: 37, 13

Log Data Units: Linear Values  
 Patient ID:  
 Panel:  
 Gate: G1  
 Total Events: 10000  
 Y Parameter: FL5-H FL5-Height (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	4753	47.54	47.53	9.44	7.48	2.78	2.46
LR	5244	52.46	52.44	624.27	442.71	2.36	2.08

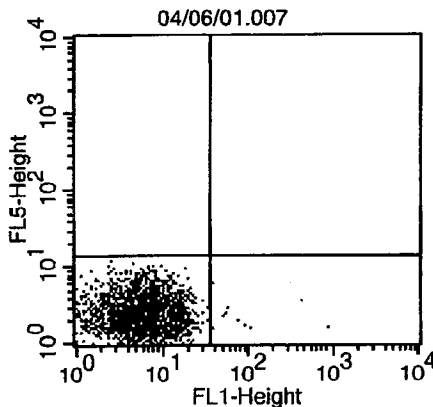


Histogram Statistics

File: 04/06/01.007  
 Sample ID: negative 4  
 Tube:  
 Acquisition Date: 06-Apr-01  
 Gated Events: 5951  
 X Parameter: FL1-H FL1-Height (Log)

Log Data Units: Linear Values  
 Patient ID:  
 Panel:  
 Gate: G1  
 Total Events: 5955

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	5951	100.00	99.93	8.65	6.12	258.86	6.21	6
M1	38, 9910	40	0.67	0.67	176.99	115.50	117.56	105.07	39



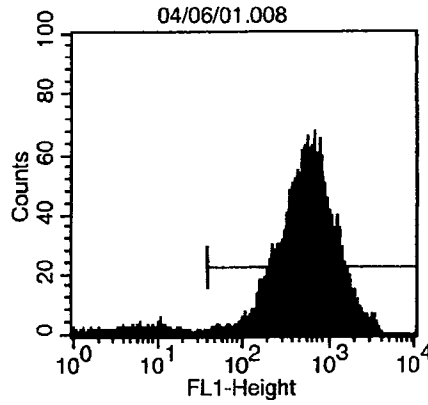
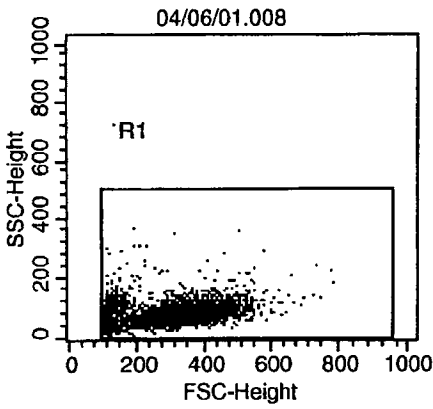
Quadrant Statistics

File: 04/06/01.007  
 Sample ID: negative 4  
 Tube:  
 Acquisition Date: 06-Apr-01  
 Gated Events: 5951  
 X Parameter: FL1-H FL1-Height (Log)  
 Quad Location: 37, 13

Log Data Units: Linear Values  
 Patient ID:  
 Panel:  
 Gate: G1  
 Total Events: 5955  
 Y Parameter: FL5-H FL5-Height (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	5909	99.29	99.23	7.51	5.99	2.75	2.43
LR	42	0.71	0.71	170.33	109.41	2.70	2.43

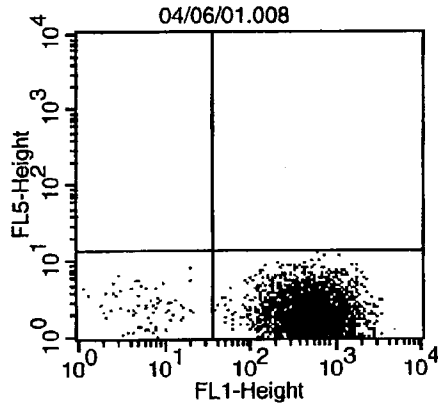




Histogram Statistics

File: 04/06/01.008                      Log Data Units: Linear Values  
 Sample ID: positive 4                    Patient ID:  
 Tube:    Panel:  
 Acquisition Date: 06-Apr-01            Gate: G1  
 Gated Events: 9312                        Total Events: 9315  
 X Parameter: FL1-H FL1-Height (Log)

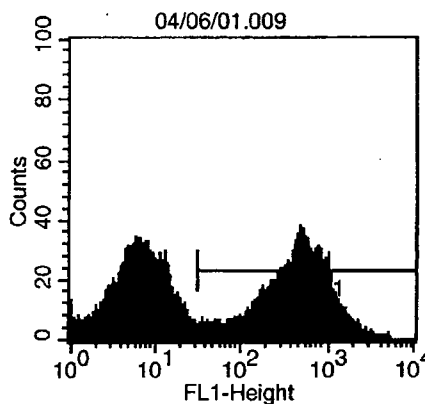
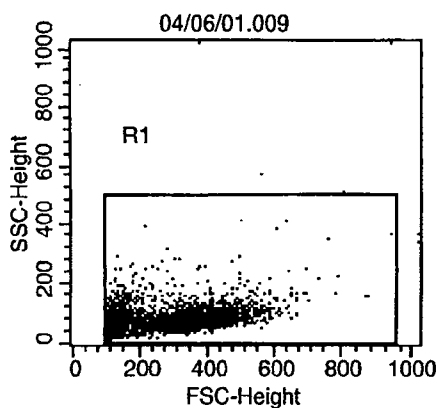
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9312	100.00	99.97	629.80	467.87	72.67	518.61	609
M1	38, 9910	9104	97.77	97.73	644.01	516.59	70.34	528.03	609



Quadrant Statistics

File: 04/06/01.008                      Log Data Units: Linear Values  
 Sample ID: positive 4                    Patient ID:  
 Tube:    Panel:  
 Acquisition Date: 06-Apr-01            Gate: G1  
 Gated Events: 9312                        Total Events: 9315  
 X Parameter: FL1-H FL1-Height (Log)    Y Parameter: FL5-H FL5-Height (Log)  
 Quad Location: 37, 13

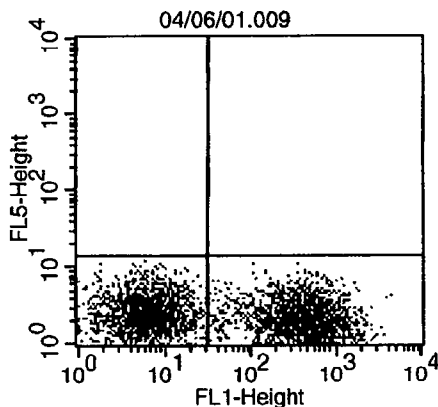
Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	0	0.00	0.00	***	***	***	***
LL	208	2.23	2.23	8.06	6.13	2.80	2.47
LR	9104	97.77	97.73	644.01	516.59	2.34	2.07



Histogram Statistics

File: 04/06/01.009                      Log Data Units: Linear Values  
 Sample ID: 5                              Patient ID:  
 Tube:                                      Panel:  
 Acquisition Date: 06-Apr-01              Gate: G1  
 Gated Events: 9988                      Total Events: 10000  
 X Parameter: FL1-H FL1-Height (Log)

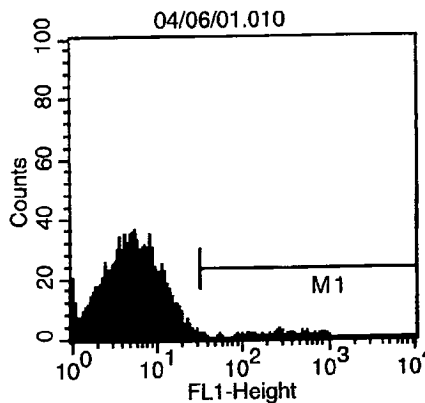
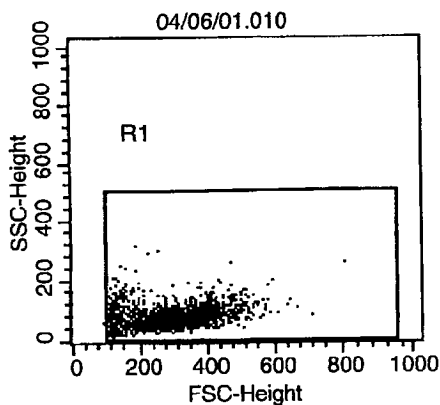
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CY	Median	Peak Ch
All	1, 9910	9988	100.00	99.88	278.61	53.41	147.15	63.21	457
M1	31, 9910	5233	52.39	52.33	524.72	377.14	83.84	425.51	457



Quadrant Statistics

File: 04/06/01.009                      Log Data Units: Linear Values  
 Sample ID: 5                              Patient ID:  
 Tube:                                      Panel:  
 Acquisition Date: 06-Apr-01              Gate: G1  
 Gated Events: 9988                      Total Events: 10000  
 X Parameter: FL1-H FL1-Height (Log)      Y Parameter: FL5-H FL5-Height (Log)  
 Quad Location: 32, 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	4770	47.76	47.70	7.83	6.25	2.82	2.49
LR	5218	52.24	52.18	526.13	379.85	2.42	2.13

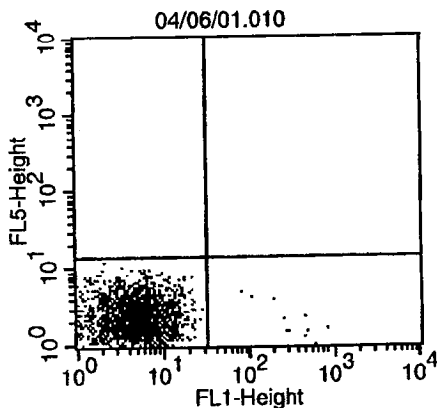


Histogram Statistics

File: 04/06/01.010  
 Sample ID: negative 5  
 Tube:  
 Acquisition Date: 06-Apr-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	9.04	5.11	422.85	5.00	5
M1	31, 9910	45	0.89	0.89	337.35	254.27	69.54	261.80	250

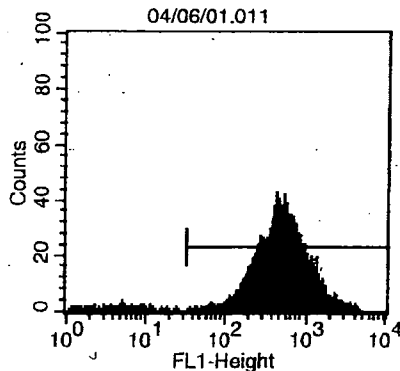
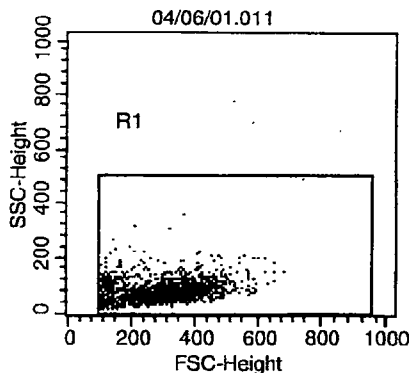


Quadrant Statistics

File: 04/06/01.010  
 Sample ID: negative 5  
 Tube:  
 Acquisition Date: 06-Apr-01  
 Gated Events: 5068  
 X Parameter: FL1-H FL1-Height (Log)  
 Quad Location: 32, 13

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

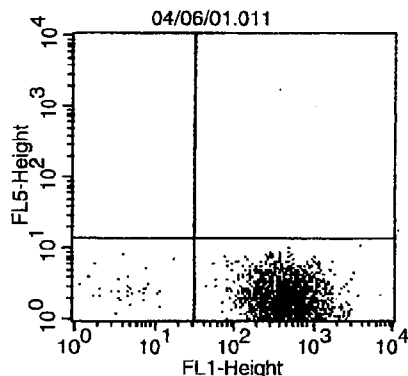
Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	0	0.00	0.00	***	***	***	***
LL	5024	99.13	99.09	6.10	4.94	2.73	2.42
LR	44	0.87	0.87	344.31	266.72	2.75	2.30



Histogram Statistics

File: 04/06/01.011 Log Data Units: Linear Values  
 Sample ID: positive 5 Patient ID:  
 Tube: Panel:  
 Acquisition Date: 06-Apr-01 Gate: G1  
 Gated Events: 5099 Total Events: 5100  
 X Parameter: FL1-H FL1-Height (Log)

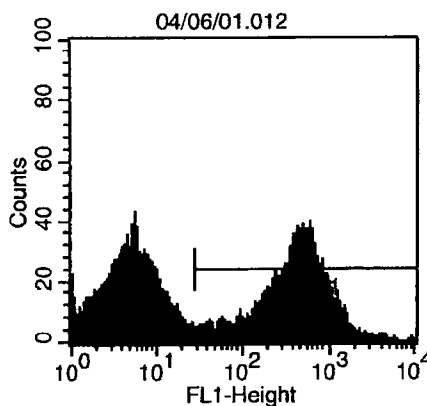
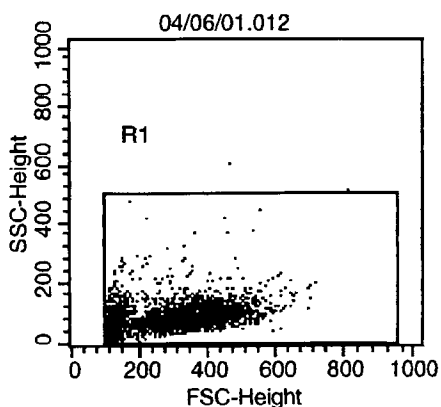
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak	Ch
All	1, 9910	5099	100.00	99.98	548.51	415.58	76.23	445.08	378	
M1	31, 9910	5006	98.18	98.16	558.60	452.31	74.36	453.16	378	



Quadrant Statistics

File: 04/06/01.011 Log Data Units: Linear Values  
 Sample ID: positive 5 Patient ID:  
 Tube: Panel:  
 Acquisition Date: 06-Apr-01 Gate: G1  
 Gated Events: 5099 Total Events: 5100  
 X Parameter: FL1-H FL1-Height (Log) Y Parameter: FL5-H FL5-Height (Log)  
 Quad Location: 32, 13

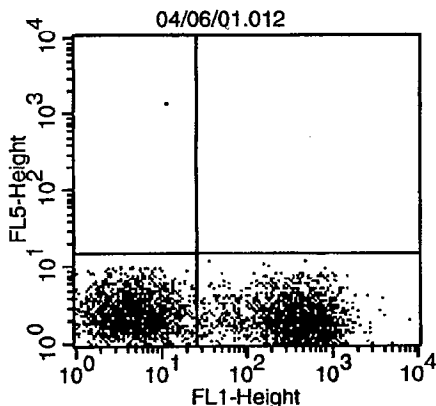
Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	0	0.00	0.00	***	***	***	***
LL	93	1.82	1.82	5.43	4.35	2.69	2.43
LR	5006	98.18	98.16	558.60	452.31	2.33	2.06



Histogram Statistics

File: 04/06/01.012                      Log Data Units: Linear Values  
 Sample ID: 6                              Patient ID:  
 Tube:                                      Panel:  
 Acquisition Date: 06-Apr-01              Gate: G1  
 Gated Events: 9995                      Total Events: 10000  
 X Parameter: FL1-H FL1-Height (Log)

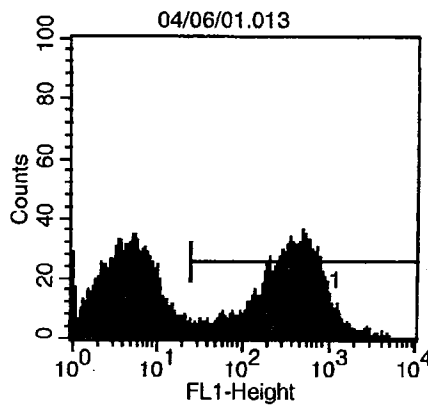
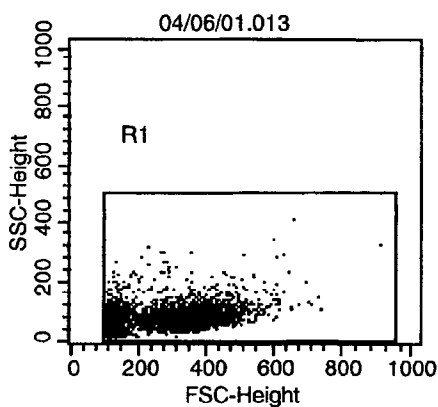
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak	Ch
All	1, 9910	9995	100.00	99.95	258.73	46.28	148.81	57.25	5	
M1	27, 9910	5245	52.48	52.45	487.46	356.82	85.18	403.15	528	



Quadrant Statistics

File: 04/06/01.012                      Log Data Units: Linear Values  
 Sample ID: 6                              Patient ID:  
 Tube:                                      Panel:  
 Acquisition Date: 06-Apr-01              Gate: G1  
 Gated Events: 9995                      Total Events: 10000  
 X Parameter: FL1-H FL1-Height (Log)      Y Parameter: FL5-H FL5-Height (Log)  
 Quad Location: 26, 14

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	4745	47.47	47.45	6.15	4.84	2.79	2.45
LR	5250	52.53	52.50	487.02	355.94	2.38	2.10

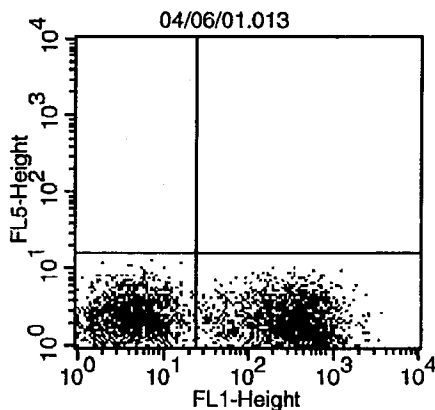


Histogram Statistics

File: 04/06/01.013  
 Sample ID: 7  
 Tube:  
 Acquisition Date: 06-Apr-01  
 Gated Events: 9994  
 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	9994	100.00	99.94	231.00	43.48	143.65	64.94	453
M1	24, 9910	5355	53.58	53.55	426.30	311.23	82.38	352.27	453

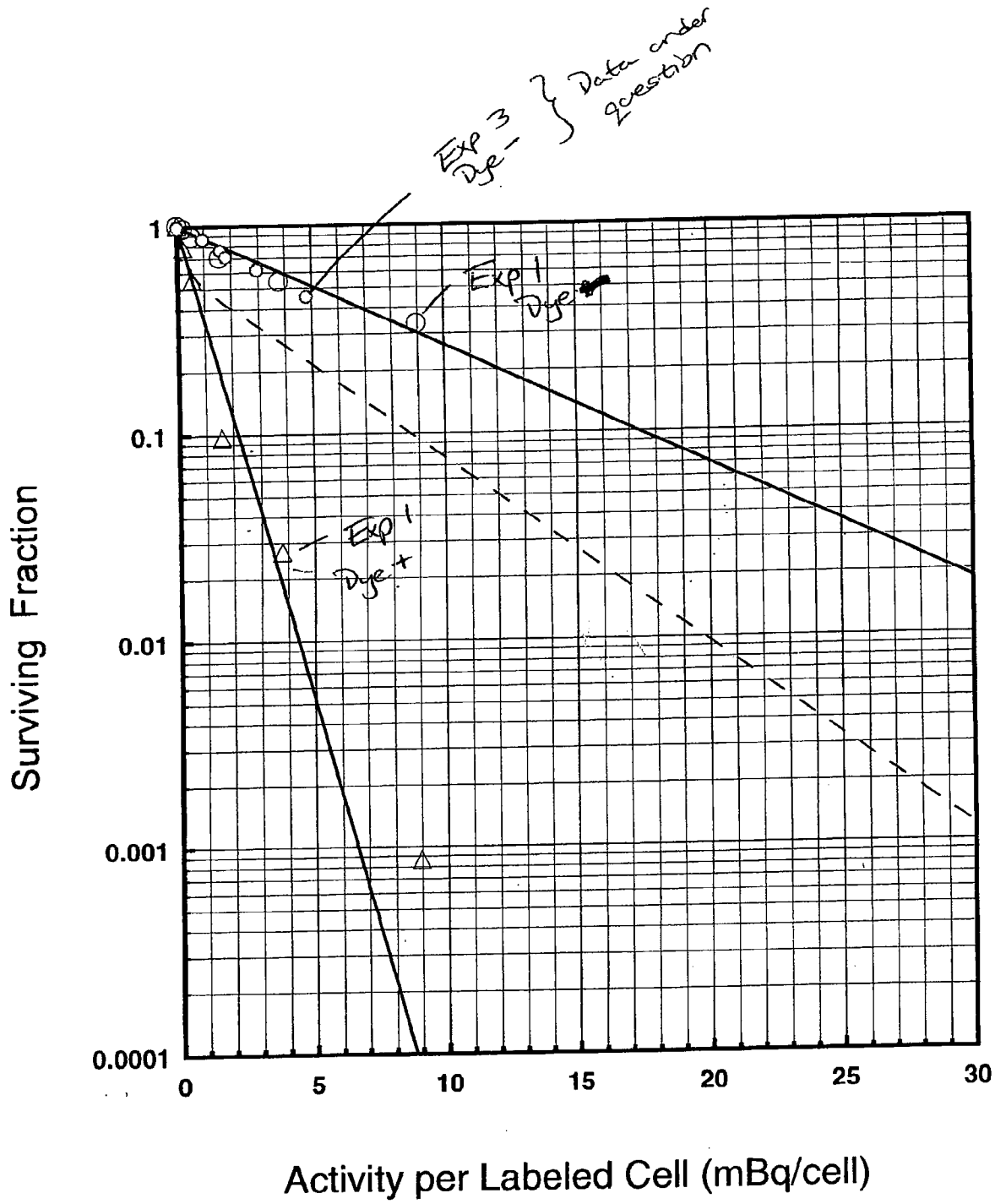


Quadrant Statistics

File: 04/06/01.013  
 Sample ID: 7  
 Tube:  
 Acquisition Date: 06-Apr-01  
 Gated Events: 9994  
 X Parameter: FL1-H FL1-Height (Log)  
 Quad Location: 25, 16

Log Data Units: Linear Values  
 Patient ID:  
 Panel:  
 Gate: G1  
 Total Events: 10000  
 Y Parameter: FL5-H FL5-Height (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	4651	46.54	46.51	5.62	4.50	2.75	2.44
LR	5343	53.46	53.43	427.20	313.03	2.41	2.12



CoulterSurvival

Sheet with colony count entered!

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!				

only counting to determine uptake

Tube #	Predicted # Cells Seeded	Actual # Cells Seeded	Colony count			Average	PE (%)	SF	SF
			1st	2nd	3rd			Uncorrected	Corrected
1	200	251	167	178	190	186	73.988	1.00	1.0000
2	200	253	193	204	169	162	62.564	0.8709	0.8456
3	200	259	166	152	148	143	56.878	0.7690	0.7688
4	200	252	150	132	136	133	56.243	0.7153	0.7602
5	200	237	129	135	119	115	46.158	0.6187	0.6239
6	200	250	100	127	100	85	36.097	0.4578	0.4879
7	200	236	81	75					
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!

Plating efficiency reasonable