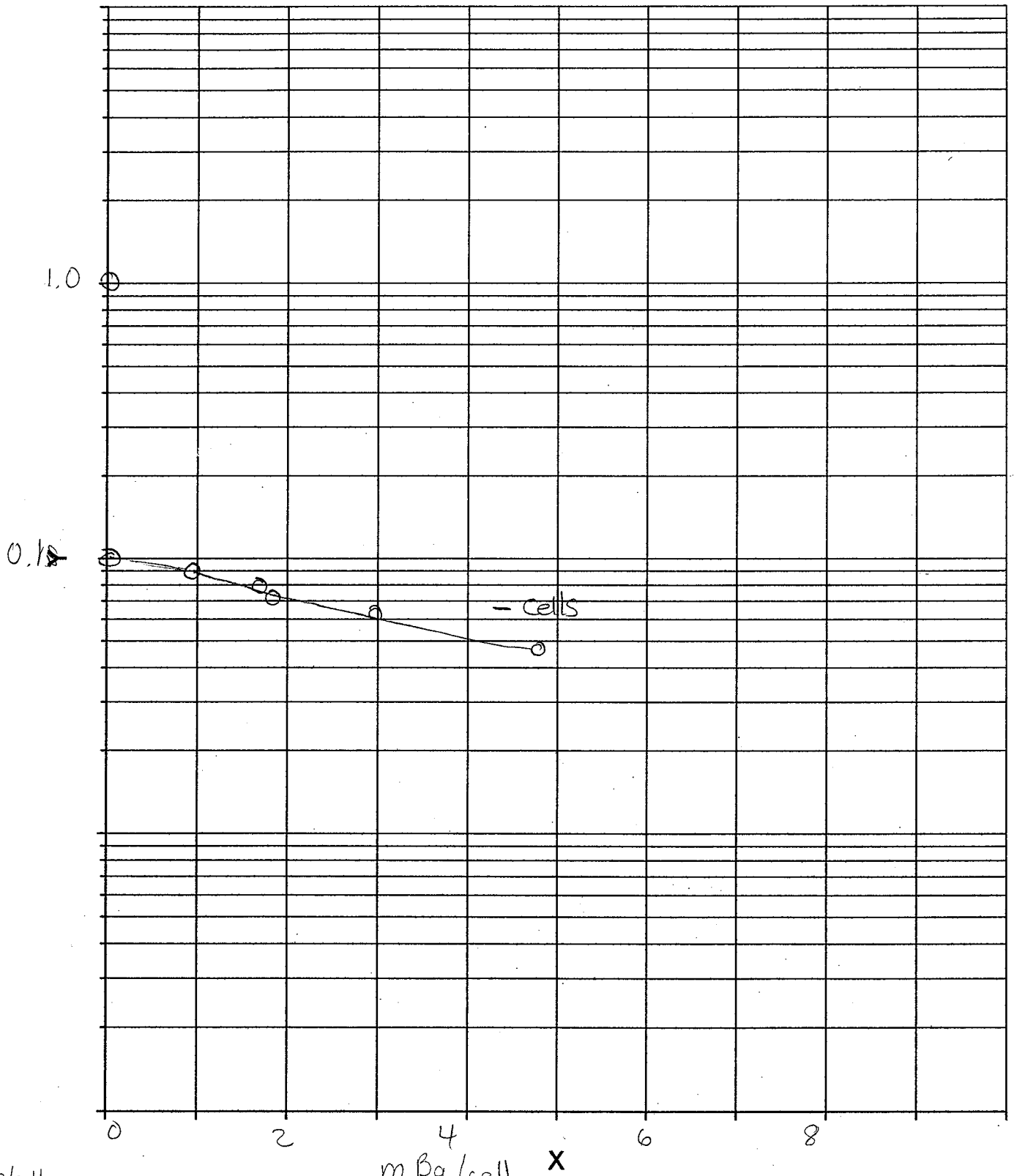
~~HEH~~ Graph

3/26/01

Sort is v. clean



mBq/cell			Av		S/S₀
0	1,2	167, 178 –	172.5	184.25	1.0
	2,2	193, 204, 191	196		
0.95	3,2	166, 152, 169			0.88
1.61	4,2	150, 132, 148			0.78
1.80	5,2	129, 135, 136			0.72
2.93	6,2	100, 127, 119			0.63
4.78	7,2	81, 75, 100			0.46

V79 COLONY FORMING ASSAY FOLLOWING FACS

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

Exp. # 3; **Investigator:** A. Bishayee **Date:** 03/26/01

1. Set the rocker-roller at 37°C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 80-90% confluent 175 cm^2 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 transfering 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% CO_2 **Date/Time:** 03/26/01; 4-00 pm
5. Prepare MEMB containing radioactivity in hood

   36 µl ^{3}HTdR (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; 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

Notes

- ~~Experiment~~ under question
- H-3 thymidine not contaminated as per subsequent test

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)

Indicates purpose of experiment was indeed to focus on labeled cells. Those were the ones which had contaminated colonies.

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 uM CFDA in prewarmed PBS as per the Table and PBS in the remaining tubes.
22. Incubate all tubes at 37°C for ~~15~~ 30 min.
23. Centrifug 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 ul) using 200 ul pipet tips
31. Again add 200 ul MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
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 ul 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 ul PBS in microcentrifuge tubes, resuspend and transfer the cell suspensions in 15 ml tubes

ERROR: timeout
OFFENDING COMM out

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.

RMA

4/6/2001 7:00 am initiate colony staining and counting

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

problem with fixing & staining 1st plate

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

50 µl 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 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
HALF LIFE(DAYS):N

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

* Sample was not added
** Sample was added twice

MediumActivity

We know that significant uptake occured because extracellular concentration at ~~reboot~~ t = 14 h is about 2/3 of what is was at t = 0.

Experiment: H-3/50%/FACS3
Date: 3/26/2001

Tube #	Medium count (CPM) 1st	2nd	3rd	CPM Average	CPM corrected for control	DPM CPM/(y e)	At µCi/ml on counting	Ao µCi/ml at addition [At/e-0.693t/T]	Ao kBq/ml at addition
1	*59*	*9*	*12*	*17*	*0*	*0*	*0*	*0*	*0*
2	*4*	*8*	*9*		*0*	*0*	*0*	*0*	*0*
3	30037	32445	32445	31642	31626	48655	0.7305	0.7306	27.0329
4	56870	54540	56435	55948	55932	86048	1.2920	1.2921	47.8091
5	80786	84560	87933	84426	84410	129861	1.9499	1.9500	72.1516
6	101253	113006	121893	112051	112034	172360	2.5880	2.5882	95.7643
7	172990	166430	166500	168640	168623	259420	3.8952	3.8956	144.1358
8				*#DIV/0!*	*0*	*0*	*0*	*0*	*0*
9					*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									

Some cells

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:0 1.00000
H[redacted] LIFE(DAYS):N

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

Experiment: H-3/50%/FACS3
Date: 03/26/01

Tube #	Suspension count (CPM) 1st	2nd	3rd	CPM Average	CPM corrected for control	DPM CPM/(y e)	A_t µCi/ml on counting	A_o µCi/ml after uptake	A_o kBq/ml after uptake
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						*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 1st	2nd	3rd	Average	Cells/ml	Hemocytometer Count in Grid 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!				

OK

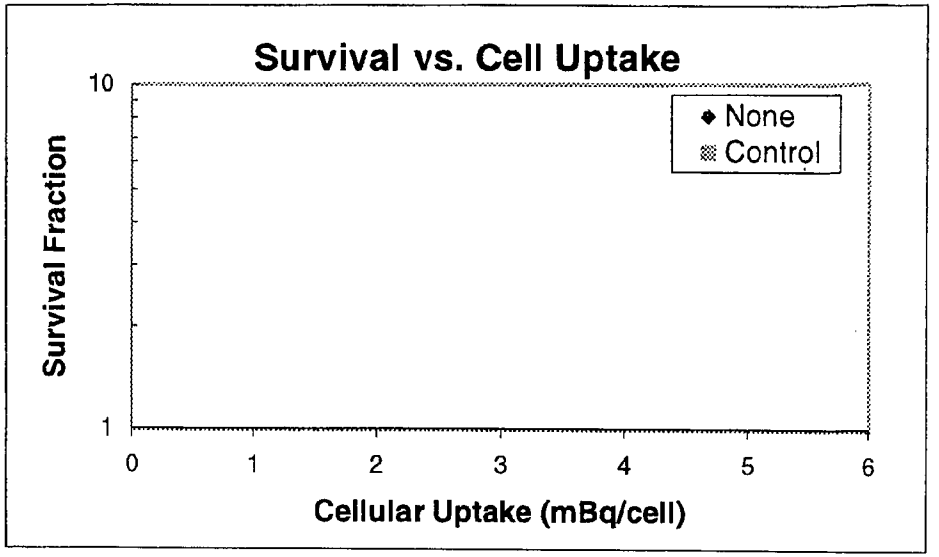
didn't Coulter count these because no radioactivity in these.

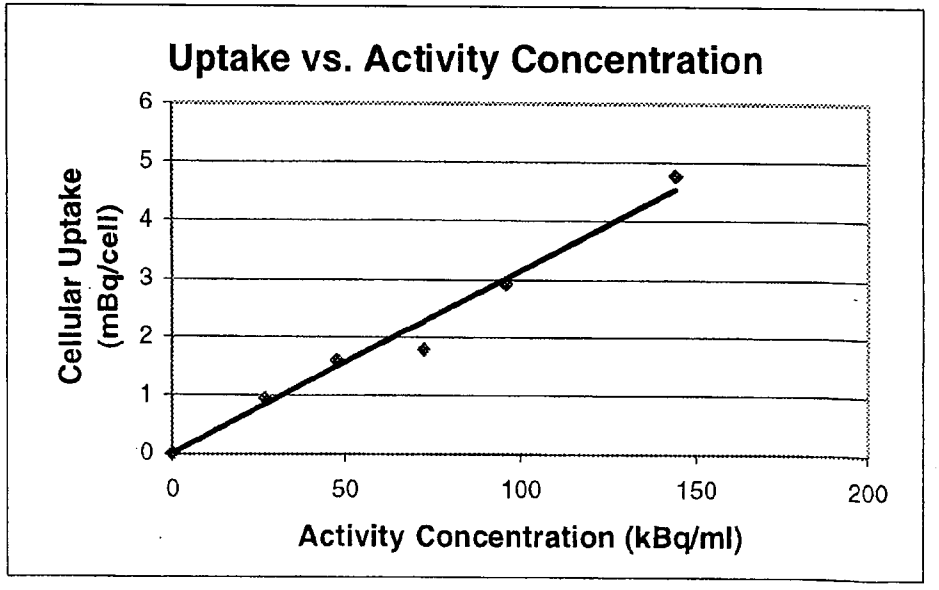
Tube #	Predicted # Cells Seeded	Actual # Cells Seeded	Colony count 1st	2nd	3rd	Average	PE (%)	SF Uncorrected	SF Corrected
1	200	*251*				*#DIV/0!*	*#DIV/0!*	*#DIV/0!*	*#DIV/0!*
2	200	*253*							
3	200	259				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
4	200	252				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
5	200	237				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
6	200	250				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
7	200	236				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
8		*#DIV/0!*				*#DIV/0!*	*#DIV/0!*	*#DIV/0!*	*#DIV/0!*
9		*#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!

Summary

Experiment:
Date/Time: 3/26/01

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*		
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!*		
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!





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				

Cell count after sorting

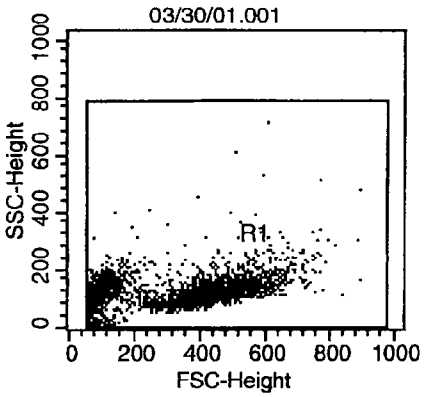
MS = 500 ul ; background = 21

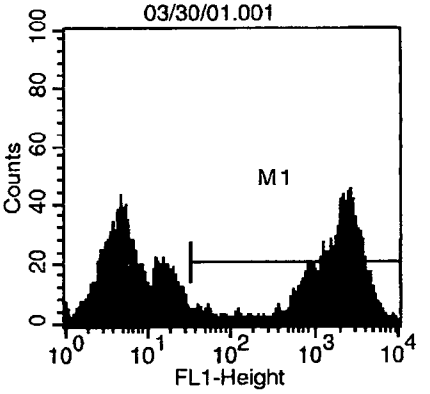
Dye(+)ve cells

Tube #	Coulter Counts	Avg.	Cell Conc. (#/ml)	# of cells plated (dil)	Vol. Plated (ul)
1	385, 395, 361	380	152133	200 (1:1000)	1315
2	1211, 1235, 1224	1223	489333	200 (1:1000)	410
3	1192, 1185, 1197	1191	476533	200 (1:1000)	420
4	1209, 1215, 1222	1215	486133	200 (1:1000)	411
5	1175, 1187, 1195	1185	474266	200 (1:1000)	420
				2000 (1:100)	420
6	1190, 1181, 1192	1187	473066	200 (1:1000)	420
				2000 (1:100)	420
7	1165, 1180, 1172	1172	468933	2000 (1:100)	425
				20,000 (1:10)	425

Dye(-)ve cells

1	365, 380, 377	374	149600	200 (1:1000)	1335
2	1127, 1135, 1145	1135	454266	200 (1:1000)	440
3	1207, 1229, 1217	1217	487066	200 (1:1000)	410
4	1177, 1189, 1162	1176	470400	200 (1:1000)	425
5	1150, 1169, 1141	1153	461333	200 (1:1000)	433
6	1135, 1169, 1147	1150	460133	200 (1:1000)	435
7	1081, 1065, 1047	1064	425733	200 (1:1000)	470



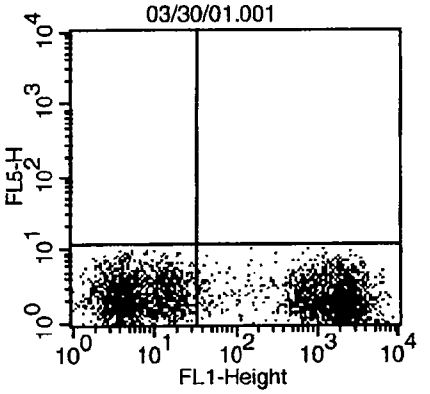


Histogram Statistics

File: 03/30/01.001
Sample ID: control 1
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	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



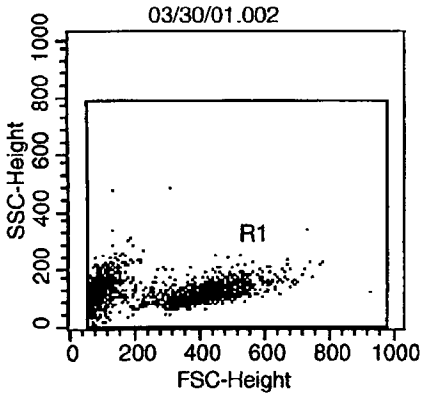
Quadrant Statistics

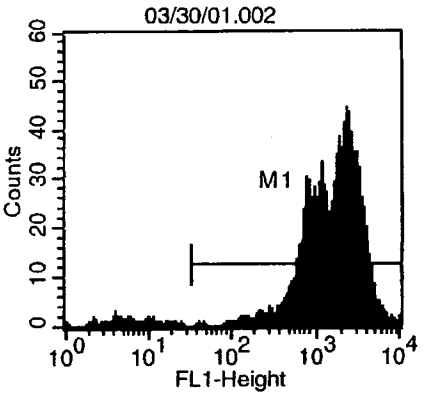
File: 03/30/01.001
Sample ID: control 1
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 9995
X Parameter: FL1-H FL1-Height (Log)
Quad Location: 33, 12

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	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 sorting



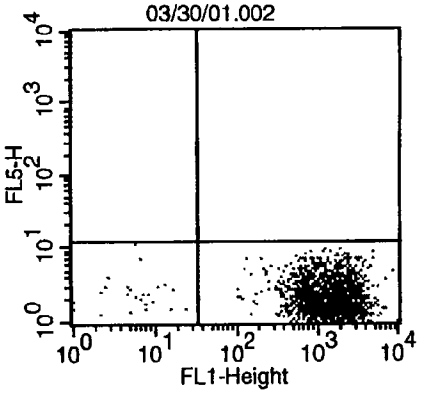


Histogram Statistics

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



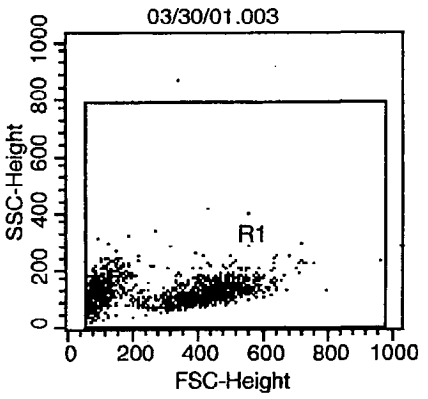
Quadrant Statistics

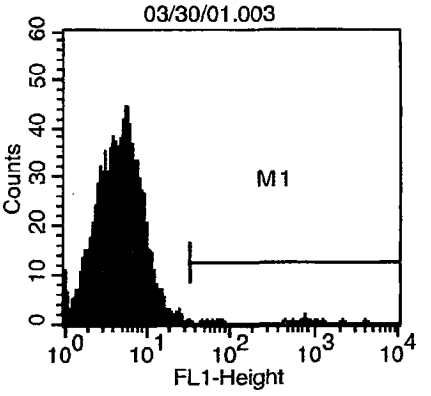
File: 03/30/01.002
Sample ID: left +
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 5067
X Parameter: FL1-H FL1-Height (Log)
Quad Location: 33, 12

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 sorting



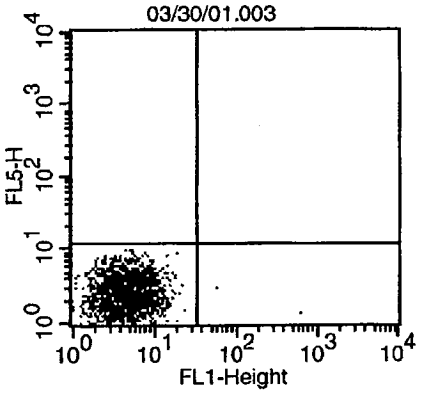


Histogram Statistics

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

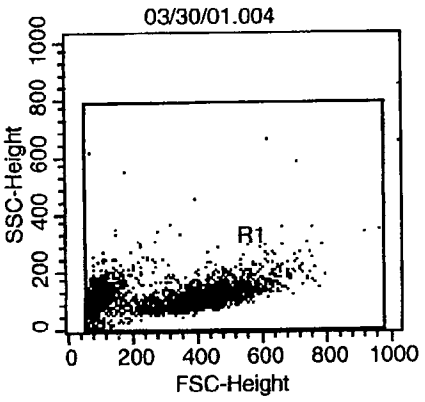


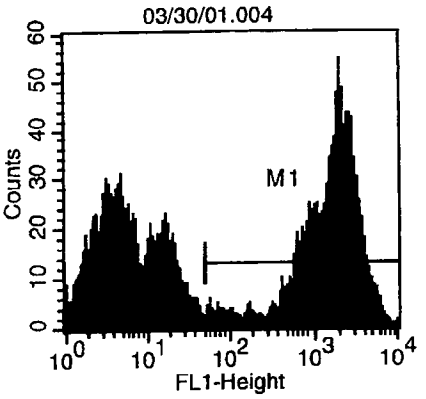
Quadrant Statistics

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



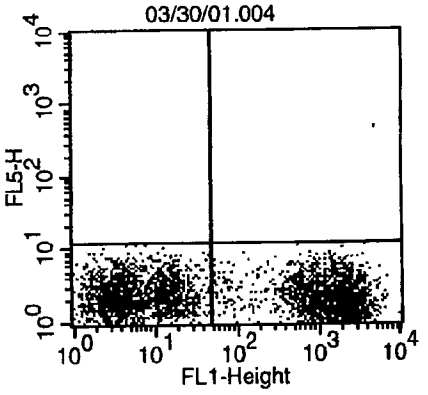


Histogram Statistics

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

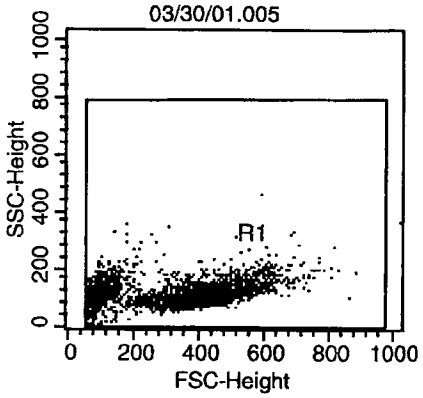


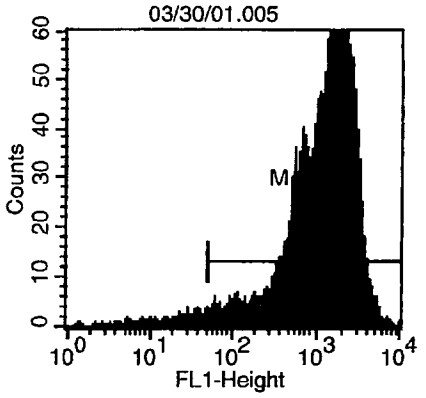
Quadrant Statistics

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



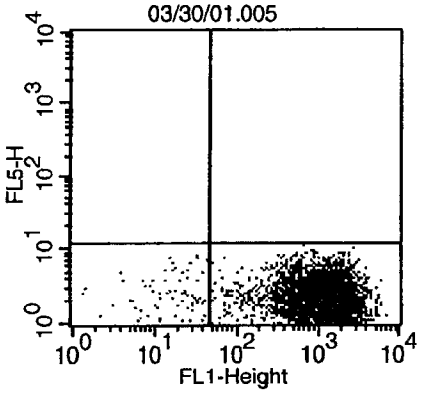


Histogram Statistics

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

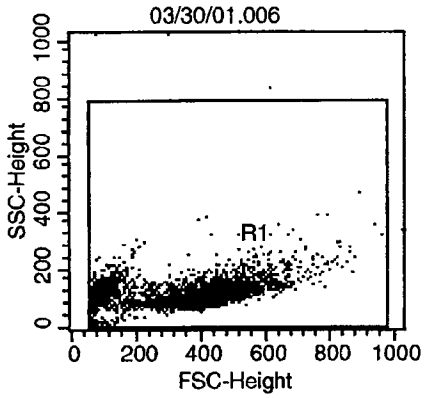


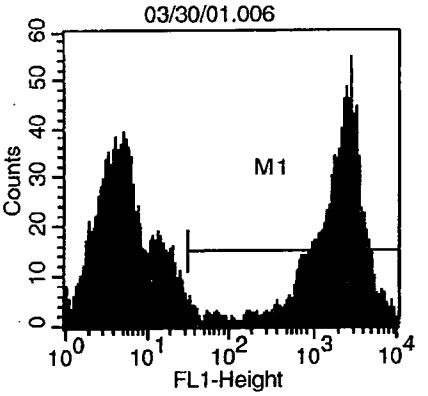
Quadrant Statistics

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



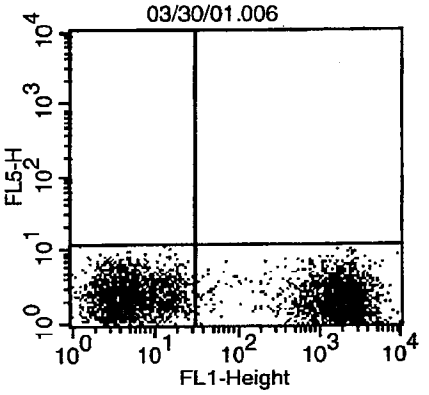


Histogram Statistics

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

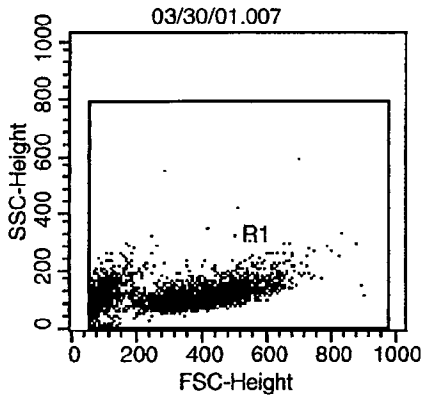


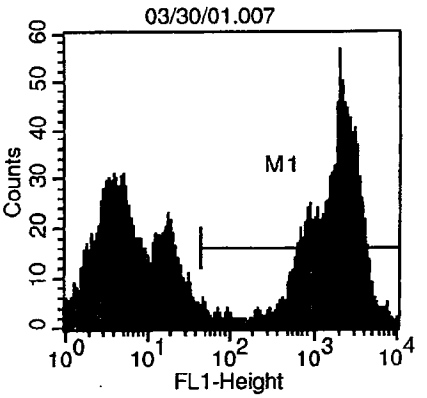
Quadrant Statistics

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



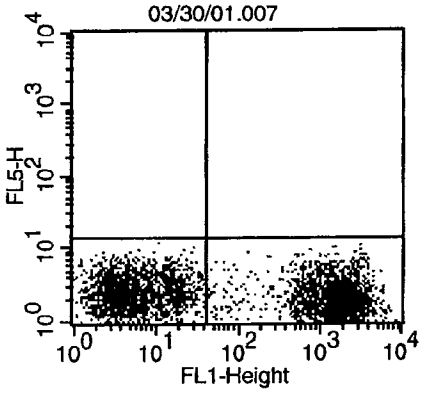


Histogram Statistics

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



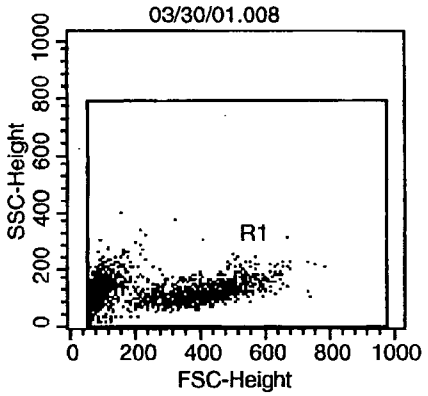
Quadrant Statistics

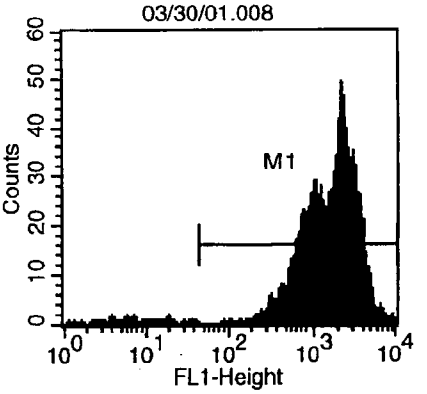
File: 03/30/01.007
Sample ID: 4
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 9993
X Parameter: FL1-H FL1-Height (Log)
Quad Location: 41, 14

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



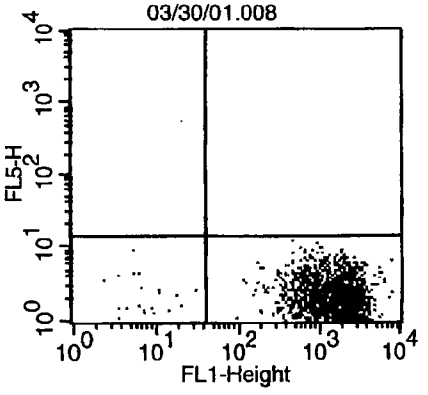


Histogram Statistics

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	CV	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



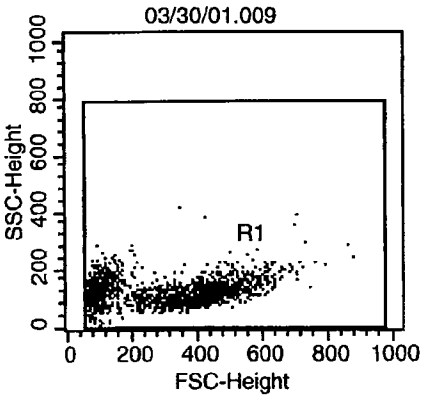
Quadrant Statistics

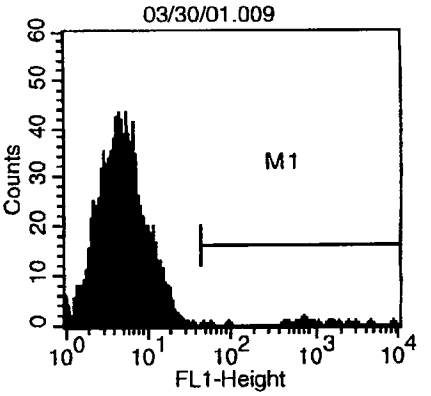
File: 03/30/01.008
Sample ID: sort 4 +
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 5190
X Parameter: FL1-H FL1-Height (Log)
Quad Location: 41, 14

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 sorting



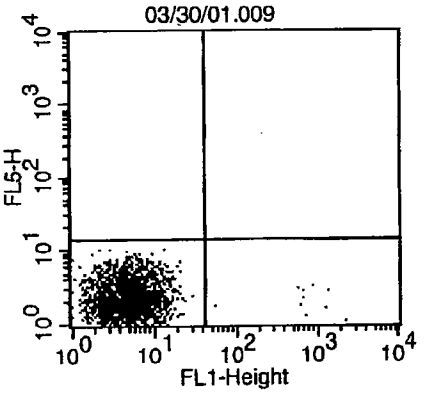


Histogram Statistics

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

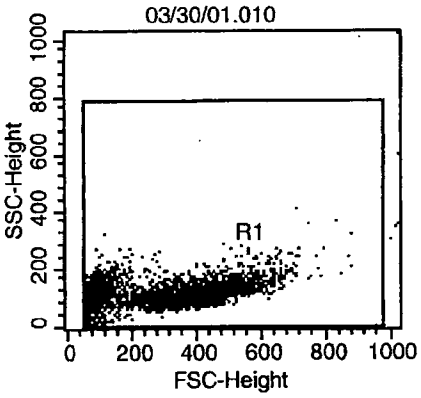


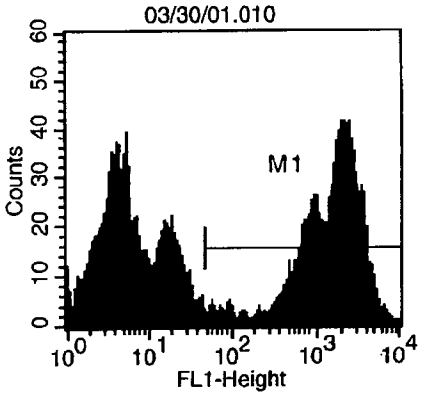
Quadrant Statistics

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



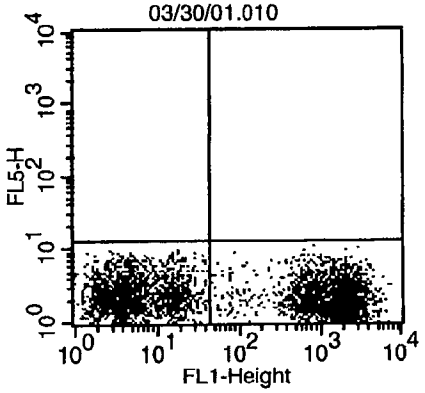


Histogram Statistics

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

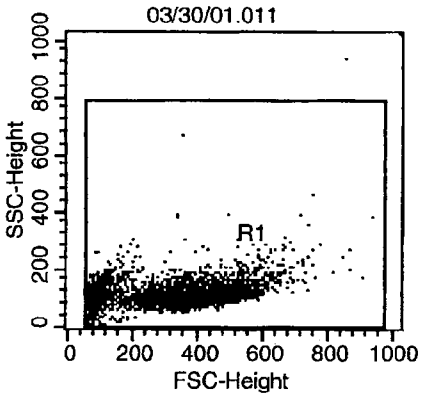


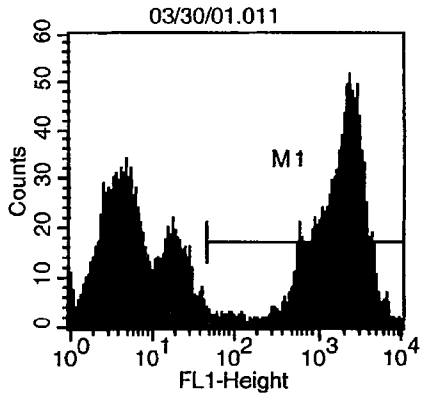
Quadrant Statistics

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



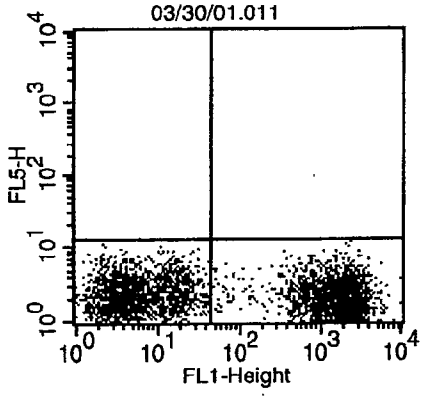


Histogram Statistics

File: 03/30/01.011
Sample ID: 6
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	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

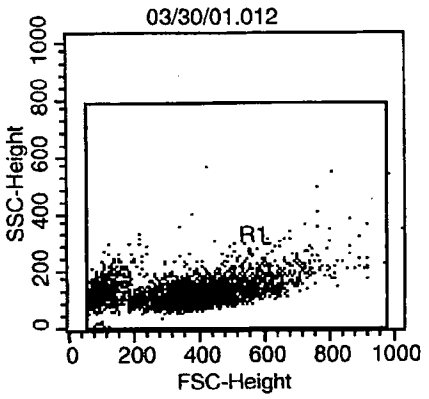


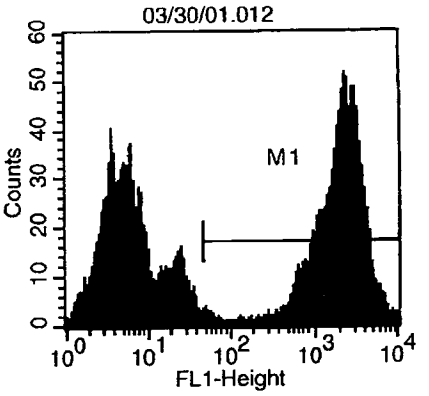
Quadrant Statistics

File: 03/30/01.011
Sample ID: 6
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 9995
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	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



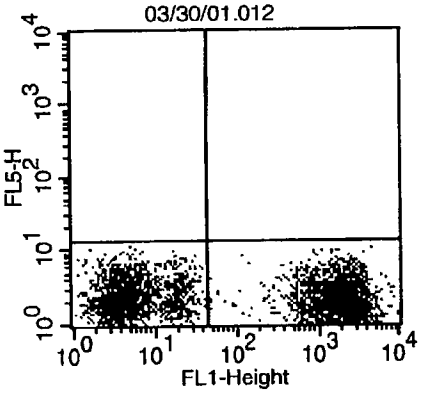


Histogram Statistics

File: 03/30/01.012
Sample ID: 7
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 9992
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	9992	100.00	99.92	1169.46	128.43	116.83	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
Sample ID: 7
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 9992
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	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

PAGE: 1

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 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	1	9.00	66.67	1.00	1.47	73.0	
2	**- 2	1	14.00	53.45	1.00	3.11	79.0	
3	**- 3	1	13.00	55.47	1.00	4.73	73.0	
4	**- 4	1	12.00	57.74	1.00	6.36	73.0	
5	**- 5	1	6.00	81.65	1.00	7.92	74.0	
6	**- 6	1	8.00	70.71	1.00	9.58	80.0	
7	**- 7	1	14.00	53.45	1.00	11.21	75.0	
8	**- 8	1	9.00	66.67	1.00	12.83	77.0	
9	**- 9	1	7.00	75.59	1.00	14.46	76.0	
10	**-10	1	7.00	75.59	1.00	16.03	70.0	
11	**-11	1	11.00	60.30	1.00	17.61	71.0	

Background