

V79 COLONY FORMING ASSAY

Experiment Name : Cell separation by FACS and SF (³HTdR cluster, 50% labeling, three ³HTdR conc.) **Exp. # :** 1; **Investigator:** A. Bishayee **Date:** 09/09/99

1. Set the rocker-roller at 37°C incubator with 5% CO₂, set the Coulter Counter, wash cells (from two 150 cm² flusk, subcultured 1:2, 24h before) with PBS, trypsinize cells, each resuspend in 9 ml MEMB, pool, pass five times through 3 cc syringe with 21 gauge needle, perform cell count by transferring 100 ul in Coulter cup containing 20 ml isotone (Coulter balanced electrolyte solution) OR 6,000,000
2. Dilute to 3,000,000 cells/ml in MEMB [Actual count : cells/ml]
3. Transfer 1 ml of cell suspension into 12 ml tubes (Falcon plastic test tube, 17x100 mm) labeled 1-8 both on cap and wall
4. Keep the tubes in the roller for 3-4 h at 37°C, 5% CO₂ **Date/Time:** 09/09/99; 3-00pm
5. Prepare MEMB containing radioactivity in hood
60 μl ³HTdR (Stock : 1 μCi/μl on 6/17/99) + ml MEMB
6. After 3-4 h, remove tubes from roller and add MEMB with or without radioactivity according to Table below. **Date/Time:** 09/09/99; 7-15 pm.

Tube # <i>Pf.</i>	³ HTdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ³ HTdR (ml) [20uCi/ml 1]	CFDA in PBS (0.05 uM) (ml)	Treatment <i>* See details</i>
1	0	1.0 1.0	1.0	0	0	None
2	0	1.0	1.0	0	2	100% dyed
3	0	1.0	1.0	0	2	50% dyed
4	0	1.0	1.0	0	2	50% dyed
5	1	1.0	0.9	0.1	2	50% dyed, whole
6	1	1.0	0.9	0.1	2	50% " "
7	3	1.0	0.7	0.3	2	50% dyed, "
8	3	1.0	0.7	0.3	2	50% dyed, "
9	6	1.0	0.4	0.6	2	50% dyed "
10	6	1.0	0.4	0.6	2	50% dyed "

- 09/09/99; 7-30 P.M.
 Date/Time: ~~09/10/99; 9-30 a.m.~~
7. Return test tubes to roller for 12 h .
 8. Next day, while test tubes are in roller label 10 gamma-tubes (13 X 100 mm VWR glass test tube)
 9. After ~12 h incubation period, remove tubes and centrifuge at 2000 rpm at 4°C for 10 min (precooled centrifuge). Date/Time: ~~09/10/99; 9-00 a.m.~~
 10. Remove buckets from centrifuge and carefully remove 150 µl of supernatant and place in prelabeled gamma-tube.
 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, resuspend in ~~2 ml of PBS, syringe and perform cell-count as well as radioactivity count by transferring aliquots.~~
 18. Add 8 ml of PBS in each tube, vortex and transfer the content to 15-ml plastic centrifuge tube
 18. Centrifuge tubes for 10 min at 2000 rpm, 4°C
 19. Decant supernatant, click tubes, vortex
 20. Add 2 ml of 0.05 µM CFDA in prewarmed PBS as per the Table and PBS in the remaining tubes.
 21. Incubate all tubes at 37°C for 15 min.
 21. Centrifuge tubes for 10 min at 2000 rpm, 4°C
 22. Decant supernatant, click tubes, vortex, add 2 ml prewarmed MEMA
 23. Incubate all tubes at 37°C for 30 min.
 24. Centrifuge and decant the supernatant, suspend in 5 ml MEMA
 25. Transfer the content of one tube to the corresponding tube
 26. Centrifuge, decant the supernatant
 27. Transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 µl) using 200 µl pipet tips
 28. Again add 200 µl ice cold MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 µl)
 29. Centrifuge tubes for 5 min at 1000 rpm, 4°C
 30. Transfer tubes at 10°C for 72 h. Date/Time: 11-00 a.m / 09/10/99
 31. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 µl wash MEMA and transfer the content to ~~eight~~¹⁰ 15 ml tubes containing 10 ml PBS by using pasteur pipet Date/Time: 09/10/99; 1-30 P.M.

32. Again add 200 ul PBS in microcentrifuge tubes, resuspend and transfer the cell suspensions in 15 ml tubes
33. Centrifuge the tubes for 10 min at 2000 rpm, 4°C (*precooled centrifuge*)
32. Decant supernatant, click tubes, vortex, pooled cells from corresponding tubes, centrifuge, decant the supernatant, resuspend in 2 ml PBS with 0.005 mM EDTA, syringe and transfer aliquots (100 ul) for cell count and radioactivity count
33. Centrifuge, decant, resuspend in 1 ml PBS with 0.005 mM EDTA to have ~10,000,000 cells/ml 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.

Tube # p
 (cluster of 1 million cells)

Treatment

1	1	100% None	
2		None	
3	2	100% dyed	
4		100% dyed	
5	3	50% dyed	
6	4	50% dyed	
7	5	50% dyed, labeled with ^3H (conc. 1)	1 $\mu\text{Ci}/\text{ml}$
8	6	50% " " " " "	" " " " "
9	7	50% dyed; labeled with ^3H (conc. 2)	3 $\mu\text{Ci}/\text{ml}$
10	8	50% " " " " "	" " " " "
	9	50% dyed, labeled with ^3H (conc. 3)	6 $\mu\text{Ci}/\text{ml}$
	10	50% dyed, labeled with ^3H (conc. 3)	

Tube #	Treatment
1	None
2	100% dyed
3	50% dyed, labeled
4	50% dyed
5	50% dyed

Tube #	# of cells (million)	CFDA
1	6	0
2	6	2
3	3	2
4	3	0
5	3	2
6	3	0
7	3	2
8	3	0
9	3	2
10	3	0
11	3	2
12	3	0
13	3	2
14	3	0
15	3	2
16	3	0
17	3	2
18	3	0

228 01 11 1971 Nov. 20-0 24 08 210011

Take 60 μ l 3H + 3ml MEMB = 20 μ ci/ml

	MEMB	MEMB + 3H (20 μ ci/ml)
	2 μ l	
5A 6A	10 μl 0.9	0.7
7A 8A	3 μ l 0.7	0.3
9A 10A	6 μ l 0.4	0.6

$$20 \mu\text{ci} = 1 \text{ ml}$$

$$2 - \frac{1}{10}$$

Prepare 20ml 0.05 μ M LPDA in 1X PBS

30 µe medium

Operator: JH-HS HOWELL PRESET TIME: 1.00 PRN 10 SEP 1999 13:42

SAMPLE REPEAT: 1 CYCLE REPEAT: 1 CORR:W RS232C/A

1 ADDIN B. F. S. ADMIN

2.00 251394: 2.00 R.C. SUR: 0.00 BRK 2513: 0.00 100%

DATA CALG: DPK, UNKNOWN REPLICATES: 1 NORM FACTOR: 1.00000

IN - LIFE (DAYS): N

RAN	POS	CH	CPM	25134	TIME	EL TIME	AVG HK	ERR
1	00-1	1	20473.33	1.93	0.52	1.47	82.0	
2	00-2	1SM	15359.60	1.99	0.62	3.37	73.0	
3	00-3	1	15337.14	1.93	0.70	5.25	81.0	
4	00-4	1GM	13600.00	1.96	0.67	6.73	80.0	
5	00-5	1	13351.11	1.77	0.23	8.23	82.0	
6	00-6	1	*7.00	75.39	1.00	10.30	80.0	
7	00-7	1	42175.00	1.99	0.24	11.66	83.0	
8	00-8	1	37407.37	1.89	0.21	12.94	85.0	
		1GM	3162.56	1.65	0.17	14.19	82.0	
		1GM	3138.22	1.76	0.14	15.22	83.0	
11	00-11	1	30913.95	1.97		17.17	85.0	
12	00-12	110M	30626.66	1.73	0.15	18.60	83.0	
13	00-13					19.03		101
14	00-14					19.45		101

* Sample was not added by mistake!

Cells suspended in 2ml

09/13/99 : 50µl

1.	712, 711, 702	2.8 M/ml	Take 10µl
2	808, 795, 789, 741, 753, 720	2.9 M/ml	for radio
3	1503, 1524, 1511	6.0 M/ml	activity
4	1547, 1518, 1539	6.1 M/ml	count
5	1509, 1556, 1519	6.1 M/ml	
6	1401, 1426, 1435	5.7 M/ml	

45, 866 cells - 1

After working

Cells suspended in 1ml : MS² 500µl

MEMA

+

1.	110, 115, 119	45, 866 cells/ml	1.3 ml
2	122, 107, 112	45, 466 cells/ml	+1.2 ml
3	121, 145, 135	54, 533 cells/ml	1.8 ml
4	95, 105, 111	41, 466 cells/ml	1.2 ml

Take 0.1 ml for radioactivity count

- i) Dilutions were made to have 20,000 cells/ml
- ii) Take 0.5 ml + 4.5 ml MEMA = 5 ml (2,000 cells/ml)
- iii) Take 0.5 ml from ii + 4.5 ml MEMA = 5 ml (200 cells/ml)

1) Plate ~~200 cells~~ 2 cells/ml 1ml (200 cells) for tube 1 X

2) Plate 1ml (200 or 2000) for tube 2-4 X

2007: 11/11/90 2nd in Collection 1960

Initial count = 1010, 1020, 1030, 1040
 Avg count = 1020
 Cell conc = 1020
 22 ml of 3,000,000 cells/ml = 66,000,000 cells

Vol. required = 22 ml

Take 22 ml cells + 22 ml MEMB = 22 ml
 1010
 1020
 1030
 1040
 Avg count = 1020
 Cell conc = 1020

2007: 11/11/90 2nd in Collection 1960
 2000 cells/ml
 2000 cells/ml
 2000 cells/ml
 2000 cells/ml

TABLE-1

Expt. # : {

Date/Time : 09/10/99; 1-45 p.m.

Tube #	Medium count for 30 ul (cpm)	Avg. cpm	dpm [cpm/0.65]	μ Ci/ml (A_t) on counting [dpm/66600]	μ Ci/ml (A_0) on addition [$A_t/e^{-\lambda t}$]
1					
2					
3					
4					
5		18407	28319	0.425	}
6		15468	23797	0.357	
7		56551	87001	1.31	}
8		42291	65063	0.98	
9		89123	137113	2.06	}
10		87770	135031	2.03	

100µl unsorted cells

USER: A D.H. HOWELL PRESET TIME: 1.00 TUE 14 SEP 1999 11:28
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
 H# ACC:N DCF:N RCM:N
 CHANNEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKS SUB: 0.00 BKS 2SIB: 0.00 LSR: 0
 DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000
 HALF LIFE (DAYS): N

SAN	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	12.00	57.74	1.00	1.89	81.0	
2	**	2	8.00	70.71	1.00	3.96	81.0	
3	**	3	888.00	6.71	1.00	6.08	82.0	
4	**	4	523.00	6.38	1.00	8.33	81.7	
5	**	5	2657.00	3.68	1.00	10.33	82.0	
6	**	6	669.00	3.87	1.00	12.93	81.0	
7	**	7	5759.00	2.64	1.00	14.96	82.0	
8	**	8	5426.00	2.72	1.00	16.98	80.0	
9	**	9	123.00	18.03	1.00	19.05	84.0	
10	**	10	126.00	17.82	1.00	21.02	82.0	
11	**	11	78.00	22.65	1.00	23.03	84.0	
12	**	12	63.00	21.95	1.00	25.06	84.0	
13	**	13	22.00	42.64	1.00	27.03	82.0	
14	**	14	14.00	53.43	1.00	29.01	84.0	
15	**	15	10.00	63.25	1.00	31.19	83.0	
16	**	16	11.00	60.30	1.00	33.42	84.0	

10
100 µl cells
before cell sorting
(50% labelled, dyed cells)

100 µl cells
following sorting
(unlabelled + undyed cells)

* S. Vials were touched with gloves.

$$\mu\text{Ci/ml} = \frac{905}{0.65 \times 60 \times 37000}$$

=>

TABLE-2

Expt. # : |

Date/Time : 09/14/99; 11-30 a.m.

Tube #	Radioactivity for 200 ul cell suspension (cpm) ¹⁰⁰	Avg. cpm ^X (0)	dpm [cpm/0.65]	μ Ci/ml (A_t) on counting ²²²⁰⁰⁰ [dpm/444000]	μ Ci/ml (A_0) after 12 h incubation [$A_t/e^{-\lambda t}$]
1					
2					
3 } 4 }	0	425			
5 } 6 }		9050	1392		0.0063
7 } 8 }		26630	2663		0.012
9 } 10 }		55920	8603		0.039

TABLE-3

Expt. # :

Date/Time :

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [uCi/ml x 10 ⁶ Cells/ml]	nCi/ cluster [pCi/cell x 4000]
1	712, 711, 702	708	2,833,333	-	
2	741, 753, 720	738	2,952,000	-	
3 } 4 }	1503, 1524, 1511	1512	6,050,666		
5 } 6 }	1547, 1518, 1539	1534	6,138,666	0.0010	4
7 } 8 }	1509, 1556, 1519	1528	6,112,000	0.0019	7.6
9 } 10 }	1401, 1426, 1435	1420	5,682,666	0.0069	27.6

	mCi/cell (50% label)	mCi/cell	Kbq/cluster [nCi/exo.037]
4	0.37	0.74	0.148
5	0.7	1.4	0.281
6	2.5	5.0	1.02

TABLE-4

Expt # :)

Date : 09/20/99;

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony for 3	SF
3.2	85	72	92	83	
4.2	15	21	12	16	
5.2	7	5	6	6	
6.3	3	1	4	0.26	

Sorted cells

TABLE-2

Expt. #: |

100

Date/Time :

05/25/00

Tube #	Radioactivity for 200 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.65]	$\mu\text{Ci/ml (A}_t\text{)}$ on counting [dpm/444000] 222000	$\mu\text{Ci/ml (A}_0\text{)}$ after 12 h incubation [$A_t/e^{-\lambda t}$]
1					
2	20				
3	22, 14 22, 14	18	27.7	0.00012	
4	11, 10 11, 10	10.5	16.2	0.00007	
5					
6					
7					
8					
9					
10					

Sorted cells

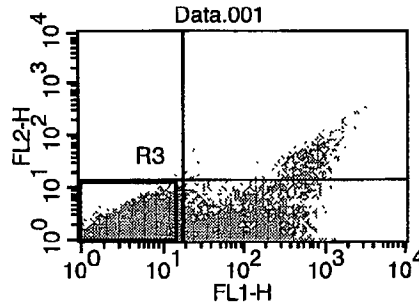
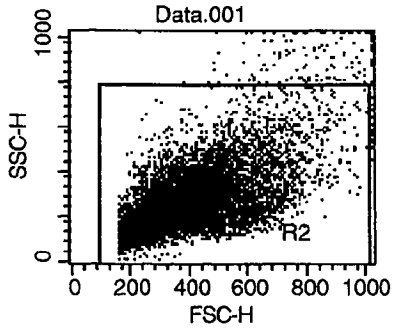
TABLE-3

Expt. #: (

Date/Time: 05/25/00

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [uCi/ml x 10 ⁶ Cells/ml]	mBq/cell
1					
2					
3	129, 145, 135	136	54,533	0.0022	0.06
4	95, 105, 111	103.6	41,466	0.0017	0.06
5					
6					
7					
8					
9					
10					

Amupass



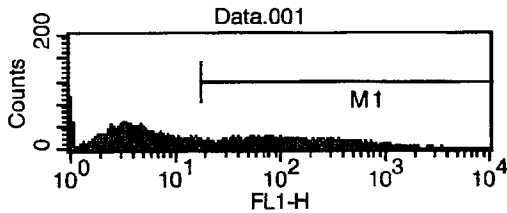
Quadrant Statistics

File: Data.001
 Sample ID:
 Tube:
 Acquisition Date: 13-Sep-99
 Gated Events: 9783
 X Parameter: FL1-H (Log)
 Quad Location: 18, 14

Log Data Units: Linear Values
 Patient ID:
 Panel:
 Gate: G2
 Total Events: 10000
 Y Parameter: FL2-H (Log)

unsat

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	1	0.01	0.01	14.33	14.33	18.27	18.27
UR	357	3.65	3.57	734.63	621.18	43.41	34.63
LL	5739	58.66	57.39	5.19	4.17	1.95	1.67
LR	3686	37.68	36.86	157.36	99.92	2.24	1.66



Histogram Statistics

File: Data.001
 Sample ID:
 Tube:
 Acquisition Date: 13-Sep-99
 Gated Events: 9783
 X Parameter: FL1-H (Log)

Log Data Units: Linear Values
 Patient ID:
 Panel:
 Gate: G2
 Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9783	100.00	97.83	89.14	16.57	215.33	8.98	1
M1	17, 9910	4099	41.90	40.99	205.73	114.43	123.48	103.66	52

① Threshold
2,281,677 - Total

09/13/99

● (633918) sorted #1

②

Threshold Total +
306115
295463 sorted #2

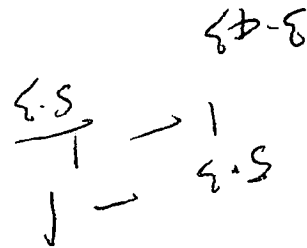
● ③

2832454 Threshold Total
(616260) - sorted #3

④

116,000? sorted
● 300152
(416152) #4

200,000
 ↓
 20,000
 ↓
 2,000
 ↓
 1
 ↓
 200



$10,000 \times 10^1 = 10^1 \times 10^4$
 $10,000 \times 10^1 = 10^1 \times 10^4$
 $5.3 \times 10^1 = 5.3 \times 10^1$

$1 \text{ mL } 10 \text{ mM} = 0.7 \text{ mg}$

$96.9 \text{ mg} =$

$10 \text{ mL } 10 \text{ mM} = 0.0969$

~~$1000 \text{ mL } 10 \text{ mM} = 9.69$~~

~~$1000 \text{ mL } 1 \text{ mM} = 0.969$~~

~~$1000 \text{ mL } 1 \text{ M} = 9609$~~

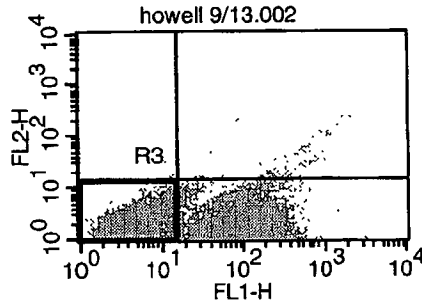
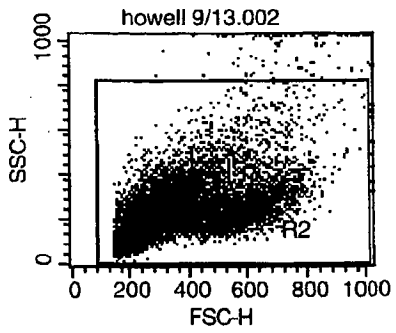
~~5 mL = 9.6 mg~~

~~$1 \text{ mL } 10 \text{ mM} = 5.3 \text{ mg}$~~

~~$1 \text{ mL } 1 \text{ mM} = 0.530 \text{ mg} = 530 \mu\text{g}$~~

~~$1000 \text{ mL } 1 \text{ mM} = 0.530 \text{ g} = 530 \text{ mg}$~~

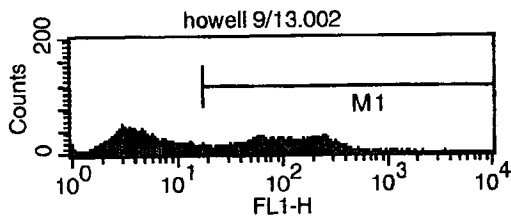
~~$1 \text{ M} = 529.29 \text{ g}$~~



Quadrant Statistics

File: howell 9/13.002 Log Data Units: Linear Values
 Sample ID: Patient ID:
 Tube: Panel: *unsorted*
 Acquisition Date: 13-Sep-99 Gate: G2
 Gated Events: 9877 Total Events: 10000
 X Parameter: FL1-H (Log) Y Parameter: FL2-H (Log)
 Quad Location: 16, 14

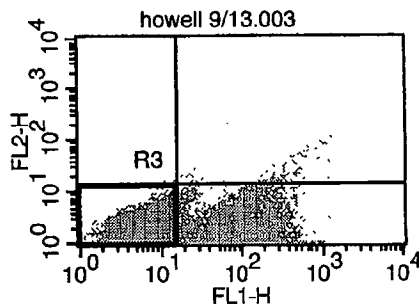
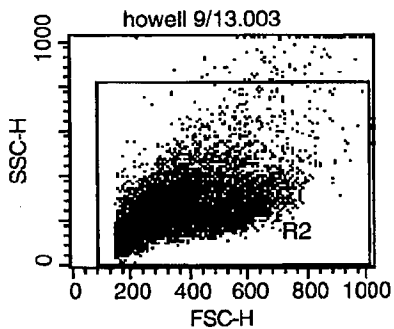
Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	4	0.04	0.04	13.28	13.19	21.56	20.86
UR	72	0.73	0.72	488.95	352.53	43.17	30.77
LL	4997	50.59	49.97	5.15	4.38	2.16	1.86
LR	4804	48.64	48.04	125.95	92.24	2.41	1.83



Histogram Statistics

File: howell 9/13.002 Log Data Units: Linear Values
 Sample ID: Patient ID:
 Tube: Panel:
 Acquisition Date: 13-Sep-99 Gate: G2
 Gated Events: 9877 Total Events: 10000
 X Parameter: FL1-H (Log)

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9877	100.00	98.77	67.43	19.92	160.98	14.46	2
M1	17, 9910	4812	48.72	48.12	132.84	96.28	94.73	97.34	55



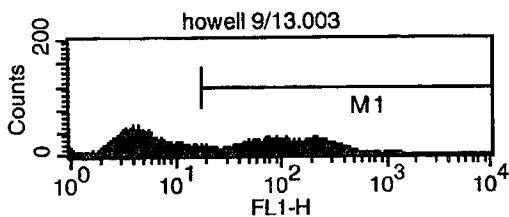
Quadrant Statistics

File: howell 9/13.003
 Sample ID:
 Tube:
 Acquisition Date: 13-Sep-99
 Gated Events: 9886
 X Parameter: FL1-H (Log)
 Quad Location: 16, 14

Log Data Units: Linear Values
 Patient ID:
 Panel:
 Gate: G2
 Total Events: 10000
 Y Parameter: FL2-H (Log)

unsorted

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	2	0.02	0.02	12.72	12.58	16.70	16.70
UR	80	0.81	0.80	390.86	270.08	29.77	25.05
LL	4897	49.53	48.97	5.48	4.73	2.04	1.75
LR	4907	49.64	49.07	131.27	95.07	2.40	1.80

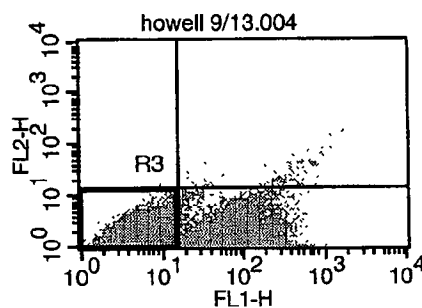
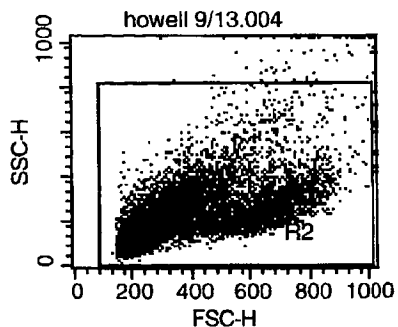


Histogram Statistics

File: howell 9/13.003
 Sample ID:
 Tube:
 Acquisition Date: 13-Sep-99
 Gated Events: 9886
 X Parameter: FL1-H (Log)

Log Data Units: Linear Values
 Patient ID:
 Panel:
 Gate: G2
 Total Events: 10000

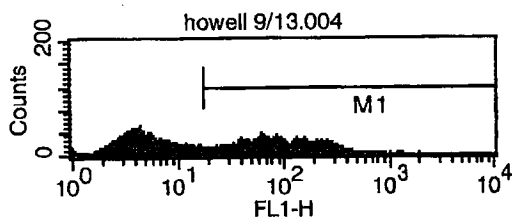
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9886	100.00	98.86	71.03	21.68	148.95	16.85	3
M1	17, 9910	4914	49.71	49.14	137.20	99.24	85.64	99.10	70



Quadrant Statistics

File: howell 9/13.004 Log Data Units: Linear Values
 Sample ID: Patient ID:
 Tube: Panel: *W. S. J. W.*
 Acquisition Date: 13-Sep-99 Gate: G2
 Gated Events: 9894 Total Events: 10000
 X Parameter: FL1-H (Log) Y Parameter: FL2-H (Log)
 Quad Location: 16, 14

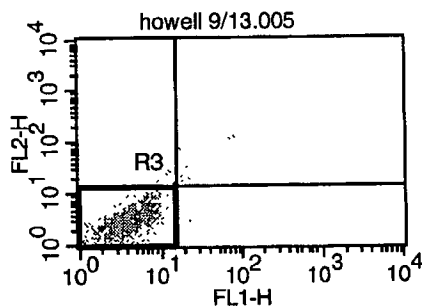
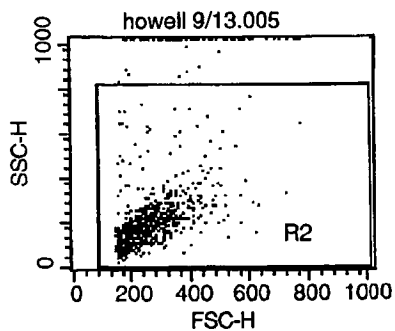
Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	1	0.01	0.01	9.73	9.73	16.25	16.25
UR	88	0.89	0.88	409.27	295.75	32.02	26.82
LL	4755	48.06	47.55	5.55	4.78	2.12	1.80
LR	5050	51.04	50.50	111.88	82.55	2.28	1.75



Histogram Statistics

File: howell 9/13.004 Log Data Units: Linear Values
 Sample ID: Patient ID:
 Tube: Panel:
 Acquisition Date: 13-Sep-99 Gate: G2
 Gated Events: 9894 Total Events: 10000
 X Parameter: FL1-H (Log)

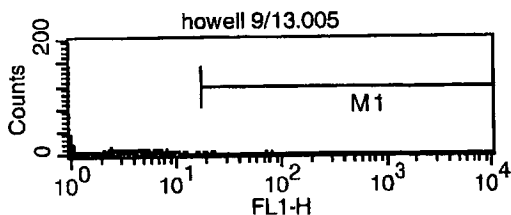
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9894	100.00	98.94	63.41	21.23	147.72	19.99	4
M1	17, 9910	5065	51.19	50.65	118.42	86.38	88.29	82.05	57



Quadrant Statistics

File: howell 9/13.005 Log Data Units: Linear Values
 Sample ID: #1 sort Patient ID:
 Tube: Panel:
 Acquisition Date: 13-Sep-99 Gate: G2
 Gated Events: 689 Total Events: 765
 X Parameter: FL1-H (Log) Y Parameter: FL2-H (Log)
 Quad Location: 16, 14

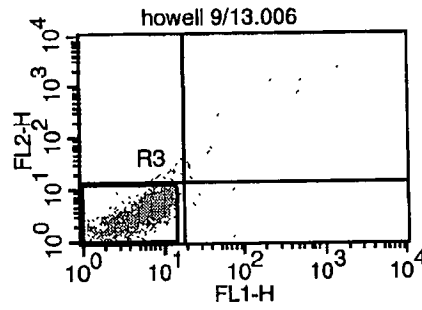
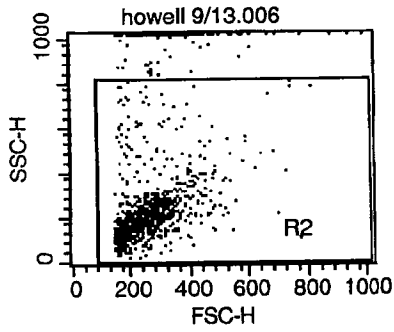
Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	4	0.58	0.52	12.06	11.86	21.07	20.44
UR	7	1.02	0.92	34.70	28.24	61.07	44.34
LL	676	98.11	88.37	4.28	3.73	3.23	2.77
LR	2	0.29	0.26	19.06	18.85	2.78	2.78



Histogram Statistics

File: howell 9/13.005 Log Data Units: Linear Values
 Sample ID: #1 sort Patient ID:
 Tube: Panel:
 Acquisition Date: 13-Sep-99 Gate: G2
 Gated Events: 689 Total Events: 765
 X Parameter: FL1-H (Log)

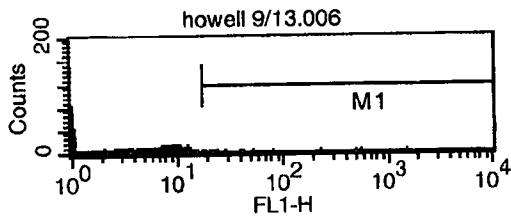
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	689	100.00	90.07	4.68	3.85	97.80	3.89	1
M1	17, 9910	8	1.16	1.05	33.10	27.35	74.74	21.38	17



Quadrant Statistics

File: howell 9/13.006 Log Data Units: Linear Values
 Sample ID: #2 sort Patient ID:
 Tube: Panel:
 Acquisition Date: 13-Sep-99 Gate: G2
 Gated Events: 1000 Total Events: 1155
 X Parameter: FL1-H (Log) Y Parameter: FL2-H (Log)
 Quad Location: 18, 14

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	14	1.40	1.21	12.71	12.27	22.33	21.52
UR	11	1.10	0.95	257.57	81.91	566.15	157.95
LL	971	97.10	84.07	5.93	4.69	4.01	3.28
LR	4	0.40	0.35	50.43	43.62	6.32	4.88



Histogram Statistics

File: howell 9/13.006 Log Data Units: Linear Values
 Sample ID: #2 sort Patient ID:
 Tube: Panel:
 Acquisition Date: 13-Sep-99 Gate: G2
 Gated Events: 1000 Total Events: 1155
 X Parameter: FL1-H (Log)

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	1000	100.00	86.58	8.98	4.95	561.56	5.78	1
M1	17, 9910	18	1.80	1.56	171.58	55.20	201.93	31.91	17