

V79 COLONY FORMING ASSAY

Experiment Name : Cell separation by FACS and SF (³HTdR cluster, 50% labeling, three ³HTdR conc.) Exp. # : 1; Investigator: A. Bishayec 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 # Pl. See attached sheet for details | ³ HTdR uCi/ml | Cells in MEMB (ml) 1.0 1.0 1.0 | MEMB (ml) | MEMB+ ³ HTdR (ml) [20uCi/ml] | CFDA in PBS (0.05 uM) (ml) | Treatment * See details |
|--|-----------------------------|--|--------------|--|--|-------------------------------|
| 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, labeled |
| 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 ¹⁰eight 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 suoematant, 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 6 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% " " " " " 1 |
| 9 | 7 | 50% dyed, labeled with ^3H (conc. 2) 3 $\mu\text{Ci}/\text{ml}$ |
| 10 | 8 | 50% " " " " " 3 |
| | 9 | 50% dyed, labeled with ^3H (conc. 3) 6 $\mu\text{Ci}/\text{ml}$ |
| | 10 | 50% dyed, labeled with ^3H (conc. 3) |

| Site | # | Treatment |
|------|---|------------------------------|
| 1 | | None |
| 2 | | 100% dyed |
| 3 | | 50% dyed, labeled |
| 4 | | 50% dyed |
| 5 | | 50% dyed |

Tube # # of cells
 (million) CFDA

| 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 |

200 01 11 4073 400 20-0 2000 2000

Take 60 μ l 3H + 3 μ l 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 0.2 | 0.3 |
| 9A 10A | 6 μ l 0.4 | 0.6 |

$$20 \mu\text{ci} = 1 \mu\text{l} \times \frac{1}{10}$$

Prepare 20 μ l 0.05 μ M LPDA in 1X PBS

30 µl medium

SCR: 6 ID:H3 HOWELL PRESET TIME: 1.00 FRI 10 SEP 1999 13:42
CYCLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
1 ADD:N ODF:N RCM:N
INLET I-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
ATA CALD: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000
ALF LIFE(DAYS):N

| QM | POS | UH | CPM | 2SIG% | TIME | EL TIME | AVG H# | ERR |
|----|-----|----|----------|-------|------|---------|--------|-----|
| 1 | ** | 1 | 20453.33 | 1.93 | 0.52 | 1.47 | 82.0 | |
| 2 | ** | 2 | 15362.60 | 1.99 | 0.62 | 3.37 | 79.0 | |
| 3 | ** | 3 | 15337.14 | 1.93 | 0.70 | 5.25 | 81.0 | |
| 4 | ** | 4 | 15600.00 | 1.96 | 0.67 | 6.93 | 80.0 | |
| 5 | ** | 5 | 15651.11 | 1.77 | 0.23 | 8.23 | 82.0 | |
| 6 | ** | 6 | *7.00 | 75.59 | 1.00 | 10.30 | 80.0 | |
| 7 | ** | 7 | 42175.00 | 1.99 | 0.24 | 11.66 | 83.0 | |
| 8 | ** | 8 | 42407.55 | 1.89 | 0.26 | 12.94 | 83.0 | |
| 9 | ** | 9 | 84182.86 | 1.65 | 0.17 | 14.19 | 82.0 | |
| 10 | ** | 10 | 84065.22 | 1.76 | 0.14 | 15.82 | 83.0 | |
| 11 | ** | 11 | 86915.96 | 1.97 | 0.12 | 17.27 | 83.0 | |
| 12 | ** | 12 | 88626.66 | 1.73 | 0.15 | 18.60 | 83.0 | |
| 13 | ** | 13 | | | | 19.03 | | 101 |
| 14 | ** | 14 | | | | 19.45 | | 101 |

* Sample was not added by mistake!

Cells suspended in 2ml

09/11/99 : 50µl

| | | | |
|----|------------------------------|----------|-----------|
| 1. | 712, 711, 702 | 2.8 M/ml | Take 10µl |
| 2 | 808, 795, 785, 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 cu - 1

After sorting

Cells suspended in 1ml : MS² 500µl MFMA +

| | | | |
|----|---------------|------------------|---------|
| 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

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

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

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

Report: 11/1/90 Date in Laboratory: 11/1/90

Initial count = 1010, 1020, 995, 1015
 Avg count = 1007.5
 Cell conc. = 1203333
 22 ml @ 3,000,000 cells/ml = 66,000,000 cells

Vol. required = $\frac{\text{desired conc.}}{\text{stock conc.}}$

Take ml Cells + ml MEMB = 22 ml
 1. 10.1 ml MEMB + 11.9 ml Cells
 2. 10.2 ml MEMB + 11.8 ml Cells
 3. 10.3 ml MEMB + 11.7 ml Cells
 4. 10.4 ml MEMB + 11.6 ml Cells
 Avg count = 1007.5
 Cell conc. = 1203333

1. 0.2 ml MEMB + 2 ml Cells = 2.2 ml
 2. 0.2 ml MEMB + 2 ml Cells = 2.2 ml
 3. 0.2 ml MEMB + 2 ml Cells = 2.2 ml

1. 100 cells (100 cells)
 2. 100 cells (100 cells)

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 _c) on counting [dpm/66600] | μ Ci/ml (A _o) on addition [A _c /e ^{-λt}] |
|--------|---------------------------------|----------|-------------------|---|---|
| 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 ~~media~~ cells

BE: 6 ID: H3 HOWELL PRESET TIME: 1.00 TUE 14 SEP 1999 11:25
FILE REPEAT: 1 CYCLE REPEAT: 1 SCR: N RS232: N
ACC: N BDF: N RCM: N
CHANNEL 1-LL: 0 UL: 400 ZSIGMA: 2.00 BKG SUB: 0.00 BKG ZSIG: 0.00 LSR: 0
STA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000
ALF LIFE(DAYS): N

| 9M | POS | CH | CPM | ZSIG% | 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 | 889.00 | 6.71 | 1.00 | 6.08 | 82.0 | |
| 4 | ** | 4 | 923.00 | 6.58 | 1.00 | 8.32 | 81.0 | |
| 5 | ** | 5 | 2657.00 | 3.88 | 1.00 | 10.55 | 82.0 | |
| 6 | ** | 6 | 2669.00 | 3.67 | 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 | 83.00 | 21.95 | 1.00 | 25.06 | 84.0 | |
| 13 | ** | 13 | 22.00 | 42.64 | 1.00 | 27.03 | 82.0 | |
| 14 | ** | 14 | 14.00 | 53.45 | 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.

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

=

TABLE-2

Expt. # : |

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

| Tube # | Radioactivity for 200 ¹⁰⁰ ul cell suspension (cpm) | Avg. cpm | 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 |

| | mPu/cell (50% label) | mPu/cell | Kb/cell [nCi/exo 0.37] |
|---|-------------------------|----------|---------------------------|
| 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] | $\mu\text{Ci/ml } (A_t)$ on counting [dpm/444000] 222000 | $\mu\text{Ci/ml } (A_0)$ after 12 h incubation [$A_t e^{-\lambda t}$] |
|--------|--|----------|----------------|--|---|
| 1 | | | | | |
| 2 | 22 | | | | |
| 3 | 42, 53 22, 14 | 18 | 27.7 | 0.00012 | |
| 4 | 62, 60 11, 10 | 10.5 | 16.2 | 0.00007 | |
| 5 | | | | | |
| 6 | | | | | |
| 7 | | | | | |
| 8 | | | | | |
| 9 | | | | | |
| 10 | | | | | |

Sorted cells

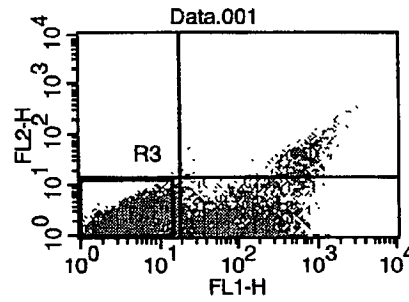
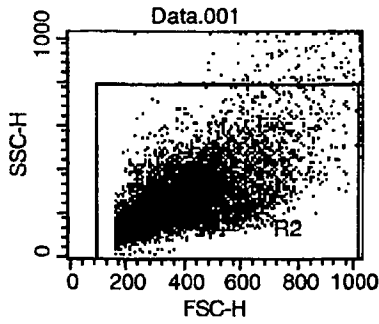
TABLE-3

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 | | | | | |

Anupam



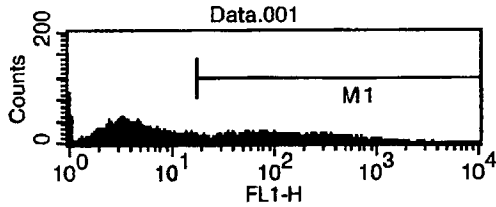
Quadrant Statistics

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)

unsold

| 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
2,281,677 - Total Threshold

09/13/99

● ● (633918) - sorted #1

②
Threshold Total +
306115
295463 ← sorted #2

● ● (3)
2832454 Threshold Total
(616260 - sorted) #3

④
116,000? sorted
300,152
● ● (416152) #4

200,000
 ↓
 20,000
 ↓
 2,000
 ↓
 200

3-48
 5.3
 ↓
 5.3
 ↓

$10,000 \times V_1 = 10,000 \times 10$
 $10,000 \times V_1 = 100,000$
 $V_1 = 10$

1 mL 10 mM = 0.7 mg

96.9 mg =

10 mL 10 mM = 0.969 g

1000 mL 10 mM = 9.69 g

1000 mL 1M = 0.969 g

1000 mL 1M = 96.9 g

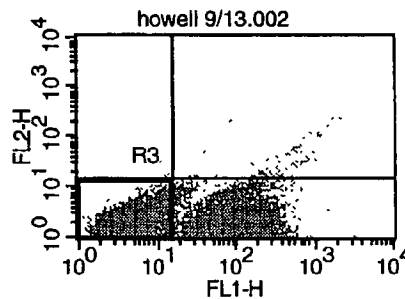
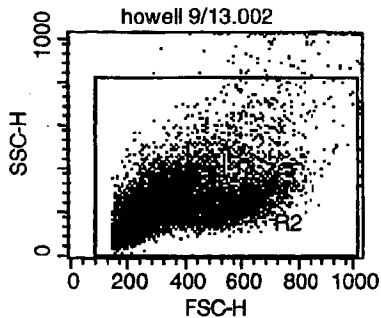
~~5 mL 9.6 mg~~

1 mL 10 mM = 5.3 mg

1 mL 1M = 0.530 mg = 530 µg

1000 mL 1M = 0.530 g = 530 mg

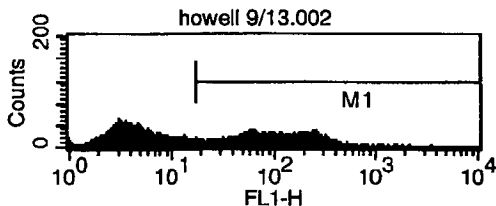
1000 mL 1M = 529.29 g



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

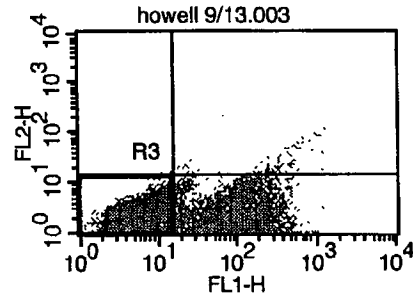
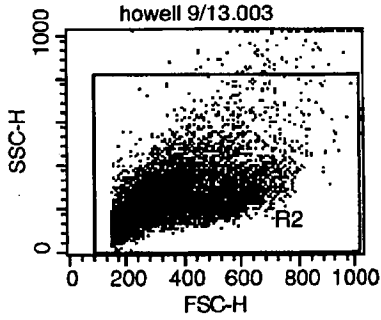
| Quad | Events | % Gated | % Total | X Mean | X Geo Mean | Y Mean | Y Geo Mean |
|------|--------|---------|---------|--------|------------|--------|------------|
| UL | 4 | 0.04 | 0.04 | 13.28 | 13.19 | 21.56 | 20.86 |
| UR | 72 | 0.73 | 0.72 | 488.95 | 352.53 | 43.17 | 30.77 |
| LL | 4997 | 50.59 | 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 |

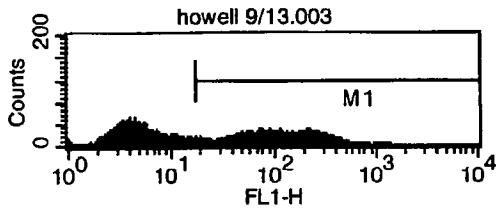


Quadrant Statistics

File: howell 9/13.003 Log Data Units: Linear Values
 Sample ID: Patient ID:
 Tube: Panel:
 Acquisition Date: 13-Sep-99 Gate: G2
 Gated Events: 9886 Total Events: 10000
 X Parameter: FL1-H (Log) Y Parameter: FL2-H (Log)
 Quad Location: 16, 14

unsorted

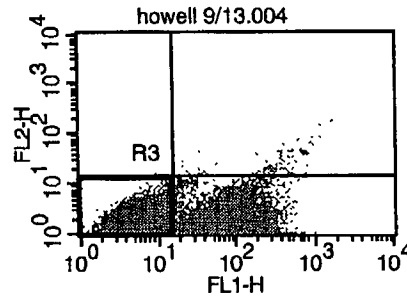
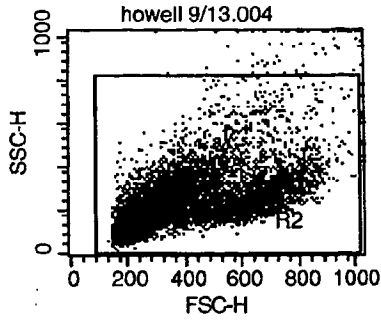
| Quad | Events | % Gated | % Total | X Mean | X Geo Mean | Y Mean | Y Geo Mean |
|------|--------|---------|---------|--------|------------|--------|------------|
| UL | 2 | 0.02 | 0.02 | 12.72 | 12.58 | 16.70 | 16.70 |
| UR | 80 | 0.81 | 0.80 | 390.86 | 270.08 | 29.77 | 25.05 |
| LL | 4897 | 49.53 | 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 Log Data Units: Linear Values
 Sample ID: Patient ID:
 Tube: Panel:
 Acquisition Date: 13-Sep-99 Gate: G2
 Gated Events: 9886 Total Events: 10000
 X Parameter: FL1-H (Log)

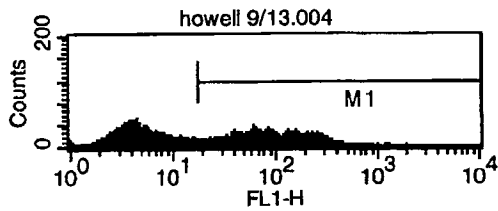
| Marker | Left, Right | Events | % Gated | % Total | Mean | Geo Mean | CV | Median | Peak Ch |
|--------|-------------|--------|---------|---------|--------|----------|--------|--------|---------|
| All | 1, 9910 | 9886 | 100.00 | 98.86 | 71.03 | 21.68 | 148.95 | 16.85 | 3 |
| M1 | 17, 9910 | 4914 | 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: *ur srfw*
 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

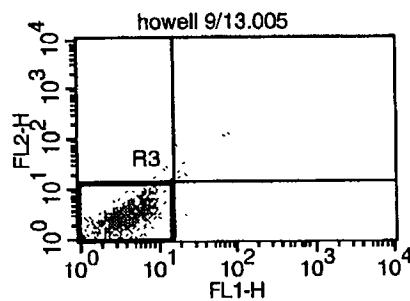
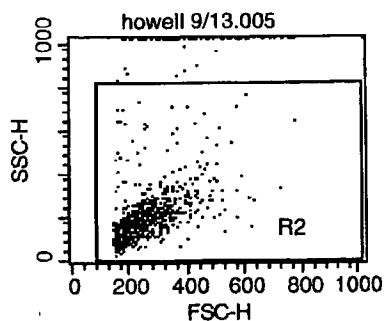
| Quad | Events | % Gated | % Total | X Mean | X Geo Mean | Y Mean | Y Geo Mean |
|------|--------|---------|---------|--------|------------|--------|------------|
| UL | 1 | 0.01 | 0.01 | 9.73 | 9.73 | 16.25 | 16.25 |
| UR | 88 | 0.89 | 0.88 | 409.27 | 295.75 | 32.02 | 26.82 |
| LL | 4755 | 48.06 | 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)

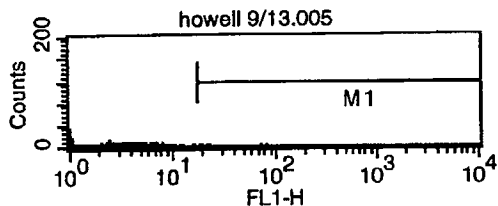
| Marker | Left, Right | Events | % Gated | % Total | Mean | Geo Mean | CV | Median | Peak Ch |
|--------|-------------|--------|---------|---------|--------|----------|--------|--------|---------|
| All | 1, 9910 | 9894 | 100.00 | 98.94 | 63.41 | 21.23 | 147.72 | 19.99 | 4 |
| M1 | 17, 9910 | 5065 | 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

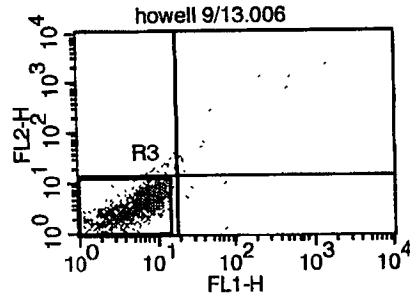
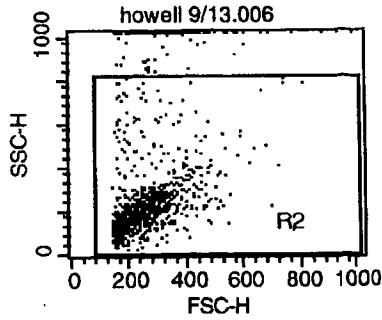
| Quad | Events | % Gated | % Total | X Mean | X Geo Mean | Y Mean | Y Geo Mean |
|------|--------|---------|---------|--------|------------|--------|------------|
| UL | 4 | 0.58 | 0.52 | 12.06 | 11.86 | 21.07 | 20.44 |
| UR | 7 | 1.02 | 0.92 | 34.70 | 28.24 | 61.07 | 44.34 |
| LL | 676 | 98.11 | 88.37 | 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)

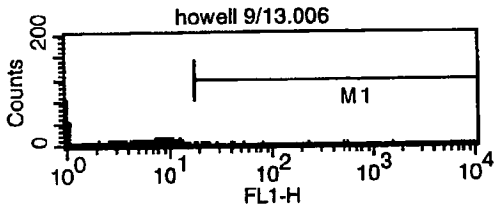
| Marker | Left, Right | Events | % Gated | % Total | Mean | Geo Mean | CV | Median | Peak Ch |
|--------|-------------|--------|---------|---------|-------|----------|-------|--------|---------|
| All | 1, 9910 | 689 | 100.00 | 90.07 | 4.68 | 3.85 | 97.80 | 3.89 | 1 |
| M1 | 17, 9910 | 8 | 1.16 | 1.05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