# PROTOCOLS

100% by A



## V79 COLONY FORMING ASSAY

**Experiment Name :** $^3$HTdR toxicity (cluster, 100% labeling); **Exp. # :** 2;
**Investigator:** [redacted] **Date:** 11/30/98

1. Set the rocker-roller at 37°C incubator with 5% $CO_2$, set the Coulter Counter, wash cells (from two 150 $cm^2$ 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 transfering 100 ul in Coulter cup containing 20 ml isotone (Coulter balanced electrolyte solution)
2. Dilute to ~4,000,000 cells/ml in MEMB [Actual count : 3,728,333 cells/ml]
3. Transfer 1 ml of cell suspension into ten 12 ml tubes (Falcon plastic test tube, 17x100 mm) labeled 1-10 both on cap and wall
4. Keep the tubes in the roller for 3-4 h at 37°C, 5% $CO_2$
   **Date/Time:** 11/30/98; 4-00 p.m.
5. Prepare MEMB containing radioactivity in hood
   14 µl $^3$HTdR (Stock : 1 µCi/µl on 11/12/98) + 3 ml MEMB
6. After 3-4 h, remove test tubes from roller and add MEMB with or without radioactivity according to Table below. **Date/Time:** 11/30/98; 7-00 p.m

| Tube # | $^3$HTdR uCi/ml | Cells in MEMB (ml) | MEMB (ml) | MEMB+ $^3$HTdR (ml) [4uCi/ml] |
|---|---|---|---|---|
| 1 | 0 | 1.0 | 1.0 | 0 |
| 2 | 0 | 1.0 | 1.0 | 0 |
| 3 | 0.01 | 1.0 | 0.995 | 0.005 |
| 4 | 0.05 | 1.0 | 0.975 | 0.025 |
| 5 | 0.1 | 1.0 | 0.950 | 0.050 |
| 6 | 0.2 | 1.0 | 0.900 | 0.100 |
| 7 | 0.5 | 1.0 | 0.750 | 0.250 |
| 8 | 0.75 | 1.0 | 0.625 | 0.375 |
| 9 | 1 | 1.0 | 0.500 | 0.500 |
| 10 | 2 | 1.0 | 0 | 1 |

1000 µl → 4 µCi





7. Return test tubes to roller for 12 h. **Date/Time:** 11/30/98; 7-20 p.m
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:** 12/1/98; 9-30 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 7 ml of MEMA
18. Centrifuge tubes for 10 min at 2000 rpm, 4°C
19. Decant supernatant, click tubes, vortex, transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips
20. Again add 200 ul ice cold MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
21. Centrifuge tubes for 5 min at 1000 rpm, 4°C
22. Transfer tubes at 10°C for 72 h. **Date/Time:** 12/1/98; 11-30 a.m.
23. Transfer 30 ul supernatant in three sets of 20 ml scintillation vials containing 6 ml liquid scintillation cocktail (Aquasol) from 150 ul supernatant removed earlier (Step 10) and count them for radioactivity **Date/Time:** 12/1/98; 2-20 p.m
24. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the content to ten 12 ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA by using pasteur pipet
    **Date/Time:** 12/04/98; 10-00 a.m
25. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes
26. Centrifuge the tubes for 10 min at 2000 rpm, 4°C (*precooled centrifuge*)
27. Labeling and preparation of dilution tubes and colony dishes
    - load 66, 60 mm petri dishes with 4 ml MEMA
    - load 40 sterile tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2, X.3, X.4, X.5 etc.
28. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
29. Centrifuge tubes for 10 min at 2000 rpm, 4°C

30. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
31. Centrifuge tubes for 10 min at 2000 rpm, 4°C
32. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
33. Determine cell concentration by transfering 100 µl to Coulter cup
34. Vortex tube, transfer 0.5 ml into dilusion tube X.5, vortex tube X.5, transfer 0.5 ml into dilution tube X.4, vortex tube X.4 and transfer 0.5 ml to tube X.3, vortex tube X.3 and transfer 0.5 ml to tube X.2 and vortex. Keep tubes on ice.
35. Transfer 1 ml from dilution tubes into dishes labeled X.2, X.3, X.4 (in triplicate). Only X.2 should be seeded for control T-tubes.
36. Transfer 100 µl of cell suspension (in triplicate) to 20 ml scintillation vial containing 6 ml cocktail (Aquasol)
37. Incubate petridishes for 1 week
38. Count vials for radioactivity **Date/Time :** 12/04/98; 12-00 Noon
39. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
40. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.

100% by B

3 V79/100



## V79 COLONY FORMING & ASSAY

**Experiment Name : 100 %** cluster, 3HTdR); **Exp.**
**Investigator:** **Date:**
**May 3, 2001**

1. Set the rocker-roller at 37°C incubator with 5% $CO_2$, set the Coulter Counter, wash cells (from two 150 $cm^2$ flasks, sub-cultured 20x10(6) cells/flask 24h before) with PBS, trypsinize cells, each re-suspend in 9 ml MEMB, pass 5x through 10 cc syringe with 21 gauge needle, count the cells using Couter counter (100 ul cell suspension / 20 ml Isotone
2. Dilute to ~4,000,000 cells/ml in MEMB [Actual count : 4 200 000 cells/ml)
   [404 + 1051 + 1053 / 3] - 2 ] x 100
3. Transfer 1 ml of cell suspension into ten 12 ml tubes (Falcon plastic test tube, 17x100 mm) labeled 1-10 both on cap and wall
4. Keep the tubes in the roller for 3-4 h at 37°C, 5% $CO_2$ **Date/Time: May 3, 2001/ 18:20**





5. Prepare MEMB containing radioactivity in hood
   60 µl $^3$HTdR (Stock : µCi/µl on ) + 5 ml MEMB

6. After 3-4 h, remove test tubes from roller and add MEMB with or without radioactivity according to Table below.
   **Date/Time: May 3, 2001 /** 20 : 15

| Tube # | $^3$HTdR uCi/ml | Cells in MEMB (ml) | MEMB (ml) | MEMB+ $^3$HTdR (ml) [12 uCi/ml] |
|---|---|---|---|---|
| 1 | 0 | 1.0 | 1.0 | 0 |
| 2 | 0 | 1.0 | 1.0 | 0 |
| 3 | 1 | 1.0 | 0.830 | 0.170 |
| 4 | 1.5 | 1.0 | 0.750 | 0.250 |
| 5 | 2.0 | 1.0 | 0.670 | 0.330 |
| 6 | 2.5 | 1.0 | 0.680 | 0.420 |
| 7 | 3.0 | 1.0 | 0.500 | 0.500 |
| 8 | 4.0 | 1.0 | 0.330 | 0.670 |
| 9 | 5.0 | 1.0 | 0.170 | 0.830 |
| 10 | 6.0 | 1.0 | 0 | 1 |

7. Return test tubes to roller for 12 h
   **Date/Time: May 3, 2001 /** 20 : 20

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: May 4, 2001** / 10:10 am
10. Remove buckets from centrifuge and carefully remove 150 µl of supernatant and place in pre-labeled gamma-tube.
11. Decant supernatant, click tubes, vortex, re-suspend in 10 ml wash MEMA
12. Centrifuge tubes for 10 min at 2000 rpm, 4°C
13. Decant supernatant, click tubes, vortex, re-suspend in 10 ml wash MEMA
14. Centrifuge tubes for 10 min at 2000 rpm, 4°C
15. Decant supernatant, click tubes, vortex, re-suspend in 10 ml wash MEMA
16. Centrifuge tubes for 10 min at 2000 rpm, 4°C
17. Decant supernatant, click tubes, vortex, re-suspend in 7 ml of MEMA
18. Centrifuge tubes for 10 min at 2000 rpm, 4°C
19. Decant supernatant, click tubes, vortex, transfer the cell suspension in polypropylene micro-centrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips
20. Again add 200 ul ice cold MEMA, re-suspend and transfer the cell suspensions in the same polypropylene micro-centrifuge tubes (Total volume ~400 ul)
21. Centrifuge tubes for 5 min at 1000 rpm, 4°C
22. Transfer tubes at 10°C for 72 h.
    **Date/Time:** MAY 4, 2001 / 11:55 am
23. Transfer 30 ul supernatant in three sets of 20 ml scintillation vials containing 6 ml liquid scintillation cocktail (EcoLume) from 150 ul supernatant removed earlier (Step 10) and count them for radioactivity **Date/Time:** MAY 4, 2001/
24. After 72 h, carefully remove the supernatant from the top, re-suspend pellet in 200 ul wash MEMA and transfer the content to ten 12 ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA by using pasteur pipet
    **Date/Time:** MAY 7, 2001 / 13:00
25. Again add 200 ul wash MEMA in micro-centrifuge tubes, re-suspend and transfer the cell suspensions in 12 ml tubes
26. Centrifuge the tubes for 10 min at 2000 rpm, 4°C (***precooled centrifuge***)
27. Labeling and preparation of dilution tubes and colony dishes
    - load 66, 60 mm petri dishes with 4 ml MEMA
    - load 40 sterile tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2, X.3, X.4, X.5 etc.





28. Decant supernatant, click tubes, vortex, re-suspend in 10 ml wash MEMA
29. Centrifuge tubes for 10 min at 2000 rpm, 4°C
30. Decant supernatant, click tubes, vortex, re-suspend in 10 ml wash MEMA
31. Centrifuge tubes for 10 min at 2000 rpm, 4°C
32. Decant supernatant, click tubes, vortex, re-suspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
33. Determine cell concentration by Coulter counting (100 µl cell suspension + 20 ml Isotone II)
34. Vortex tube, transfer 0.5 ml into dilusion tube X.5, vortex tube X.5, transfer 0.5 ml into dilution tube X.4, vortex tube X.4 and transfer 0.5 ml to tube X.3, vortex tube X.3 and transfer 0.5 ml to tube X.2 and vortex. Keep tubes on ice.
35. Transfer 1 ml from dilution tubes into dishes labeled X.2, X.3, X.4 (in triplicate). Only X.2 should be seeded for control T-tubes.
36. Transfer 200 µl of cell suspension (in triplicate) to 20 ml scintillation vial containing 6 ml cocktail (EcoLume)
37. Incubate P60's for 1 week
38. Count vials for radioactivity **Date/Time :**
39. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
40. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.

41. For HGPRT$^-$ – plate $2\times10^6$ cells / T75ml + 15ml MEMA
    0 day – May 7, 2001
    3 day May 10, 2001
    * On May 11 all the T75 flasks were contaminated. Why?

100 % by C

## V79 COLONY FORMING ASSAY

**Experiment Name :** $^3$HTdR toxicity (cluster, 100% labeling) **Exp. # :** 5

**Investigator:** **Date:** 7/16/01

**Serum/Lot #s:** Gibco Lot# 1023285 FCS

1. Set the rocker-roller at 37°C incubator with 5% $CO_2$, set the Coulter Counter, wash cells (from one 225 $cm^2$ flasks, subcultured 1:2 the day before) with PBS-PS, trypsinize cells with 2 ml trypsin 3 min at 37°C, resuspend in 10 ml MEMB, pool, pass five times through 10 cc syringe with 21 gauge needle, perform cell count by transferring 100 ul in Coulter cup containing 20 ml Isotone II (Coulter balanced electrolyte solution).
2. Dilute to ~4,000,000 cells/ml in MEMB [Actual count : $4.05 \times 10^6$ cells/ml)
   10135, 10089, 10152 } 10125 x 400 = $4.05 \times 10^6$ cells/ml
3. Transfer 1 ml of cell suspension into 10 14 ml tubes (Falcon polypropylene test tube, 17x100 mm) labeled 1-10 both on cap and wall.
4. Keep the tubes in the roller for 3-4 h at 37°C, 5% $CO_2$ **Date/Time:** 2:15 pm 7/16/01
5. Prepare MEMB containing radioactivity in hood
   160 µl $^3$HTdR (Stock : 1 µCi/µl on 7/5/01 ) + ml MEMB
   Manufacturer: NEN NET-027E Lot #: Calibration: 7/5/01
6. After 3-4 h, remove tubes from roller and add MEMB with radioactivity according to Table below.





| Tube # | $^3$HTdR µCi/ml | Cells in MEMB (ml) | MEMB (ml) | MEMB+ $^3$HTdR [40 µCi/ml] (ml) |
|---|---|---|---|---|
| 1 | 0 | 1.0 | 1.0 | 0 |
| 2 | 0 | 1.0 | 1.0 | 0 |
| 3 | 1 | 1.0 | 0.95 | 0.05 |
| 4 | 2 | 1.0 | 0.90 | 0.10 |
| 5 | 3 | 1.0 | 0.85 | 0.15 |
| 6 | 4 | 1.0 | 0.8 | 0.20 |
| 7 | 5 | 1.0 | 0.75 | 0.25 |
| 8 | 10 | 1.0 | 0.5 | 0.5 |
| 9 | 15 | 1.0 | 0.25 | 0.75 |
| 10 | 20 | 1.0 | 0 | 1.0 |
| | | | 3ml | 3ml |

Make 8 ml
160 µl H-3
+ 3.84 ml MEMB

7. Return test tubes to roller for 12-14 h. **Date/Time:** 07/16/01; 6-30 pm
8. Next day, while tubes are in roller label 10 gamma-tubes (13 X 100 mm VWR glass tube)
9. After ~12-14 h incubation period, remove all tubes and centrifuge at 2000 rpm at ~~4°C~~ for 10 min (~~precooled centrifuge~~). **Date/Time:**
10. Remove buckets from centrifuge and carefully remove 150 µl of supernatant from tubes containing radioactivity and place in pre-labeled gamma-tubes.
11. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
12. Centrifuge tubes for 10 min at 2000 rpm
13. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
14. Centrifuge tubes for 10 min at 2000 rpm
17. Decant supernatant, click tubes, vortex, resuspend in 10 ml MEMA.
18. Centrifuge tubes for 10 min at 2000 rpm.
22. Decant supernatant completely, click tubes, vortex.
23. Transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 µl) using 200 µl pipette tip.
24. Again add 200 µl MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 µl)
25. Centrifuge tubes for 5 min at 1000 rpm, 4°C
26. Transfer tubes at 10.5°C for 72 h. **Date/Time:** 10:30am 7/17/01
27. Transfer 30 µl supernatant in three sets of 7 ml scintillation vials and add 6 ml liquid scintillation cocktail (Ecoscint) from 150 ul supernatant removed earlier and count them for radioactivity **Date/Time:** 10:30am 7/17/01

28. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the content to ten 12 ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA by using Pasteur pipette

**Date/Time:** 7/22/01

29. Again add 200 µl wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes
30. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***
31. Labeling and preparation of dilution tubes and colony dishes
    - load 60 mm tissue culture dishes with 4 ml MEMA
    - load 40 sterile tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2, X.3, X.4, X.5 etc.
32. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
33. Centrifuge tubes for 10 min at 2000 rpm, 4°C
34. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
35. Centrifuge tubes for 10 min at 2000 rpm, 4°C
36. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 5 cc syringe with 21 gauge needle
37. Determine cell concentration by transferring 100 µl to Coulter cup
38. Vortex tube, transfer 0.5 ml into dilution tube X.5, vortex tube X.5, transfer 0.5 ml into dilution tube X.4, vortex tube X.4 and transfer 0.5 ml to tube X.3, vortex tube X.3 and transfer 0.5 ml to tube X.2 and vortex. Keep tubes on ice.
39. Transer $2x10^6$ cells to T75 flask for mutant expression.
39. Transfer 1 ml from dilution tubes into dishes labeled X.2, X.3, X.4 (in triplicate). Only X.2 should be seeded for control T-tubes.
40. Transfer 200 µl of cell suspension (in triplicate) to 7 ml scintillation vial containing 6 ml cocktail (Ecolume)
41. Incubate tissue culture dishes for 1 week
42. Count vials for radioactivity **Date/Time:**
43. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
44. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.

50% by A



## V79 COLONY FORMING ASSAY

**Experiment Name :** $^3$HTdR toxicity (cluster, 50% labeling); **Exp. # :** 1;
**Investigator:** **Date:** 12/28/98

1. Set the rocker-roller at 37°C incubator with 5% $CO_2$, set the Coulter Counter, wash cells (from two 150 $cm^2$ 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 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 : 2,172,000cells/ml)
3. Transfer 1 ml of cell suspension into 20 12 ml tubes (Falcon plastic test tube, 17x100 mm) labeled 1-10 both on cap and wall
4. Keep the tubes in the roller for 3-4 h at 37°C, 5% $CO_2$ **Date/Time:** 12/28/98; 3-00 pm
5. Prepare MEMB containing radioactivity in hood
   30 µl $^3$HTdR (Stock : 1 µCi/µl on 11/12/98 ) + 3 ml MEMB
6. After 3-4 h, remove first set of 10 test tubes from roller and add MEMB with or without radioactivity according to Table below. **Date/Time:** 12/28/98; 7-15 pm.

| Tube # | $^3$HTdR uCi/ml | Cells in MEMB (ml) | MEMB (ml) | MEMB+ $^3$HTdR (ml) [8uCi/ml] |
|---|---|---|---|---|
| 1 | 0 | 1.0 | 1.0 | 0 |
| 2 | 0 | 1.0 | 1.0 | 0 |
| 3 | 0.02 | 1.0 | 0.995 | 0.005 |
| 4 | 0.1 | 1.0 | 0.975 | 0.025 |
| 5 | 0.2 | 1.0 | 0.950 | 0.050 |
| 6 | 0.4 | 1.0 | 0.900 | 0.100 |
| 7 | 1 | 1.0 | 0.750 | 0.250 |
| 8 | 1.5 | 1.0 | 0.625 | 0.375 |
| 9 | 2 | 1.0 | 0.500 | 0.500 |
| 10 | 4 | 1.0 | 0 | 1 |

7. Return test tubes to roller for 12 h. **Date/Time:** 12/28/98; 7-30 pm.





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:** 12/29/98; 9-30 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 7 ml of MEMA
18. Centrifuge tubes for 10 min at 2000 rpm, 4°C
19. Decant supernatant, click tubes,vortex
20. Add 10 ml of wash MEMA to second set of tubes containing 2,000,000 cells, and follow steps 11-17, suspend in 7 ml of MEMA and transfer cells to the corresponding tubes containing 2,000,000 cells in step 19
21. Centrifug tubes for 10 min at 2000 rpm, 4°C
22. Decant supernatant, click tubes, vortex
23. Transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips
24. Again add 200 ul ice cold MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
25. Centrifuge tubes for 5 min at 1000 rpm, 4°C
26. Transfer tubes at 10°C for 72 h. **Date/Time:** 12/29/98; 11-15 a.m.
27. Transfer 30 ul supernatant in three sets of 20 ml scintillation vials containing 6 ml liquid scintillation cocktail (Aquasol) from 150 ul supernatant removed earlier (Step 10) and count them for radioactivity **Date/Time:**
28. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the content to ten 12 ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA by using pasteur pipet **Date/Time:**
29. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes
30. Centrifuge the tubes for 10 min at 2000 rpm, 4°C (*precooled centrifuge*)

31. Labeling and preparation of dilution tubes and colony dishes
    - load 66, 60 mm petri dishes with 4 ml MEMA
    - load 40 sterile tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2, X.3, X.4, X.5 etc.
32. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
33. Centrifuge tubes for 10 min at 2000 rpm, 4°C
34. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
35. Centrifuge tubes for 10 min at 2000 rpm, 4°C
36. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
37. Determine cell concentration by transfering 100 µl to Coulter cup
38. Vortex tube, transfer 0.5 ml into dilusion tube X.5, vortex tube X.5, transfer 0.5 ml into dilution tube X.4, vortex tube X.4 and transfer 0.5 ml to tube X.3, vortex tube X.3 and transfer 0.5 ml to tube X.2 and vortex. Keep tubes on ice.
39. Transfer 1 ml from dilution tubes into dishes labeled X.2, X.3, X.4 (in triplicate). Only X.2 should be seeded for control T-tubes.
40. Transfer 200 µl of cell suspension (in triplicate) to 20 ml scintillation vial containing 6 ml cocktail (Aquasol)
41. Incubate petridishes for 1 week
42. Count vials for radioactivity **Date/Time :** 01/04/87, 3-00 P.M.
43. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
44. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.

50% by B

8



**COLONY FORMING ASSAY – V79 cells / 50 % cluster/ 3HTdR**

**Experiment Name :** $^3$HTdR toxicity (cluster, 50% labeling); **Exp. Dec., 26, 2000**

**Experiment performed by:** [redacted] **Date:** 12/26/00

1. Set the rocker-roller at 37°C incubator with 5% $CO_2$, set the Coulter Counter, wascells (from 4x 175 $cm^2$ flask, sub-cultured 1:2, 24h before) with DPBS, trypsinize cells (2 ml/flask), re-suspend cells in 15 ml MEMB/flask, pool 2 flask in 50mm conical tube,, spin the cells down (4 o C, 2000 rpm, 10"), aspirate supernatant, and wash the cells in 15 ml DPBS, aspirate supernatant, pass five times through 10 cc syringe with 21 gauge needle, perform cell count by transfer 100 µl in Coulter cup containing 20 ml EcoLume
2. Dilute to ~2,000,000 cells/ml in MEMB [Actual count :1 985 600 cells/ml)
3. Transfer 1 ml of cell suspension into 20 12 ml tubes (Falcon plastic test tube, 17x100 mm) labeled 1-10 both on cap and wall
4. Keep the tubes in the roller for 3-4 h at 37°C, 5% $CO_2$ **Date/Time: Dec., 26, 2000 / 16:30**
5. Prepare MEMB containing radioactivity in hood

   720 µl $^3$HTdR (Stock :1 µCi/µl on Nov., 7, 2000 ) + 3.8 ml MEMB
6. After 3-4 h, remove first set of 10 test tubes from roller and add MEMB with or without radioactivity according to Table below. **Date/Time: Dec.,26, 2000 / 20:30**

| Tube # | $^3$HTdR µCi/ml | Cells in MEMB (ml) | MEMB (ml) | MEMB+$^3$HTdR Working sol. Activity [160µCi/ml] (ml) |
|---|---|---|---|---|
| 1 | 0 | 1.0 | 1.0 | 0 |
| 2 | 0 | 1.0 | 1.0 | 0 |
| 3 | 5 | 1.0 | 0.94 | 0.06 |
| 4 | 10 | 1.0 | 0.875 | 0.125 |
| 5 | 20 | 1.0 | 0.750 | 0.250 |
| 6 | 30 | 1.0 | 0.625 | 0.375 |
| 7 | 40 | 1.0 | 0.500 | 0.500 |
| 8 | 50 | 1.0 | 0.375 | 0.625 |
| 9 | 50 | 1.0 | 0.250 | 0.750 |
| 10 | 80 | 1.0 | 0 | 1 |

7. Return test tubes to roller for 12 h . **Date/Time: Dec.,26, 2000 / 20:45**





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: Dec., 27, 2000 /** ~~1345~~ 11:25 ( ~~13:00~~ 15hrs )
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 5 ml of MEMA
18. Centrifuge tubes for 10 min at 2000 rpm, 4°C
19. Decant supernatant, click tubes, vortex
20. Add 10 ml of wash MEMA to second set of tubes containing 2,000,000 cells, and follow steps 11-17, suspend in 5 ml of MEMA and transfer cells to the coresponding tubes containing 2,000,000 cells in step 19
21. Centrifug tubes for 10 min at 2000 rpm, 4°C
22. Decant supernatant, click tubes, vortex, resuspend in 2 ml MEMA nonlabeled samples
23. Pass five times through 3 cc syringe with 21 gauge needle
24. Using 200 µl pipet tips transfer the cell suspension into Helena tubes (400 µl) with attached caps,
25. Again add 200 µl ice cold MEMA, re-suspend and transfer the cell suspensions in the same Helena tubes (400 µl) (Total volume ~400 µl)
26. Centrifuge tubes for 5 min at 1000 rpm, 4°C
28. Transfer tubes at 10°C for 72 h. **Date/Time: Dec., 27, 2000 /** 1500
29. Transfer 30 µl supernatant in three sets of 20 ml scintillation vials containing 6 ml liquid scintillation cocktail (EcoLume) from 150 µl supernatant removed earlier (Step 10) and count them for radioactivity **Date/Time:**
30. After 72 h, carefully remove the supernatant from the top, re-suspend pellet in 200 µl wash MEM-A and transfer the content into ten 12 ml tubes , (Falcon polypropylene tube), labeled 1-10 both on cap and wall) containing 10 ml wash MEM-A by using Pasteur pipet **Date/Time: Dec.,30, 2000 /**
31. Again add 200 µl wash MEMA in micro-centrifuge tubes, re-suspend and transfer the cell suspensions into 12 ml tubes

32. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***
33. Labeling and preparation of dilution tubes and colony dishes
    - load 60 mm petri dishes with 4 ml MEMA
    - load 40 sterile tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2, X.3, X.4, X.5 etc.
34. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
35 Centrifuge tubes for 10 min at 2000 rpm, 4°C
36. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
37. Centrifuge tubes for 10 min at 2000 rpm, 4°C
38. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
39. Determine cell concentration by transfering 100 µl to Coulter cup
40. Vortex tube, transfer 0.5 ml into dilusion tube X.5, vortex tube X.5, transfer 0.5 ml into dilution tube X.4, vortex tube X.4 and transfer 0.5 ml to tube X.3, vortex tube X.3 and transfer 0.5 ml to tube X.2 and vortex. Keep tubes on ice.
41. Transfer 1 ml from dilution tubes into dishes labeled X.2, X.3, X.4 (in triplicate). Only X.2 should be seeded for control T-tubes.
42. Transfer 200 µl of cell suspension (in triplicate) to 20 ml scintillation vial containing 6 ml cocktail (Aquasol)
43. Incubate Petri dishes for 1 week
44. Count vials for radioactivity **Date/Time :**
45. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
46. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.

50% by C



## V79 COLONY FORMING ASSAY

**Experiment Name :** $^3$HTdR toxicity (cluster, 50% labeling); **Exp. # : ;** 2
**Investigator:** **Date:** 4/19/2001

1. Set the rocker-roller at 37°C incubator with 5% $CO_2$, set the Coulter Counter, wash cells (from two 175 $cm^2$ flasks, sub-cultured 1:2, 2 days 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)
2. Dilute to ~2,000,000 cells/ml in MEMB [Actual count : 2.0 x $10^6$ cells/ml) Flasks 95% confluent
3. Transfer 1 ml of cell suspension into 20 12 ml tubes (Falcon plastic test tube, 17x100 mm) labeled 1-10 both on cap and wall
4. Keep the tubes in the roller for 3-4 h at 37°C, 5% $CO_2$ **Date/Time:** 3:15 pm
5. Prepare MEMB containing radioactivity in hood
   60 µl $^3$HTdR (Stock : 1 µCi/µl on ) + 2.94 ml MEMB
6. After 3-4 h, remove first set of 10 test tubes from roller and add MEMB with or without radioactivity according to Table below. **Date/Time:** 6:00 pm

Add 1 ml of MEMB to the second set of tubes.

| Tube # | $^3$HTdR uCi/ml | Cells in MEMB (ml) | MEMB (ml) | MEMB+ $^3$HTdR (ml) [20uCi/ml] |
|---|---|---|---|---|
| 1 | 0 | 1.0 | 1.0 | 0 |
| 2 | 0 | 1.0 | 1.0 | 0 |
| 3 | 0.1 | 1.0 | 0.99 | 0.01 |
| 4 | 0.5 | 1.0 | 0.95 | 0.05 |
| 5 | 0.75 | 1.0 | 0.925 | 0.075 |
| 6 | 1 | 1.0 | 0.9 | 0.1 |
| 7 | 2 | 1.0 | 0.8 | 0.2 |
| 8 | 4 | 1.0 | 0.6 | 0.4 |
| 9 | 5 | 1.0 | 0.5 | 0.5 |
| 10 | 10 | 1.0 | 0 | 1 |

Prepare 3 ml ($\frac{20\,\mu Ci}{ml}$) = 60 µCi

9 ml + 10 ml for 2nd set





7. Return test tubes to roller for 12 h . **Date/Time:** 6:17pm
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:** 7:35 am 4/20/2001
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 5 ml of MEMA
18. ~~Centrifuge tubes for 10 min at 2000 rpm, 4°C~~
19. ~~Decant supernatant, click tubes, vortex~~
20. Add 10 ml of wash MEMA to second set of tubes containing 2,000,000 cells, and follow steps 11-17, suspend in 5 ml of MEMA and transfer cells to the corresponding tubes containing 2,000,000 cells in step 19 ← wash second set with 2 ml MEMA and transfer that too to 1st set
21. Centrifuge tubes for 10 min at 2000 rpm, 4°C
22. Decant supernatant, click tubes, vortex
23. Transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips
24. Again add 200 ul ice cold MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul) had difficulty with transfer due to inconsistent decanting. Leftover volumes different.
25. Centrifuge tubes for 5 min at 1000 rpm, 4°C 4/20/2001
26. Transfer tubes at 10°C for 72 h. **Date/Time:** 10:40am Recommend correcting cell only counts as per Mueller's idea. programmed into spreadsheet.
27. Transfer 30 ul supernatant in three sets of 20 ml scintillation vials containing 6 ml liquid scintillation cocktail (Aquasol) from 150 ul supernatant removed earlier (Step 10) and count them for radioactivity **Date/Time:** Ecolume
28. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the content to ten 12 ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA by using pasteur pipet
    **Date/Time:** 11:30 am 4/23/2001
29. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell

suspensions in 12 ml tubes

30. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***
31. Labeling and preparation of dilution tubes and colony dishes
    - load 66, 60 mm petri dishes with 4 ml MEMA
    - load 40 sterile tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2, X.3, X.4, X.5 etc.
32. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
33. Centrifuge tubes for 10 min at 2000 rpm, 4°C
34. ~~Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA~~
35. ~~Centrifuge tubes for 10 min at 2000 rpm, 4°C.~~
36. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
37. Determine cell concentration by transfering 100 µl to Coulter cup
38. Vortex tube, transfer 0.5 ml into dilusion tube X.5, vortex tube X.5, transfer 0.5 ml into dilution tube X.4, vortex tube X.4 and transfer 0.5 ml to tube X.3, vortex tube X.3 and transfer 0.5 ml to tube X.2 and vortex. Keep tubes on ice.
39. Transfer 1 ml from dilution tubes into dishes labeled X.2, X.3, X.4 (in triplicate). Only X.2 should be seeded for control T-tubes.
40. Transfer 200 µl of cell suspension (in triplicate) to 20 ml scintillation vial containing 6 ml cocktail (Aquasol)
41. Incubate petridishes for 1 week
42. Count vials for radioactivity **Date/Time :**
43. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
44. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.

| | |
|---|---|
| 1.2 | 92, 111, 119 |
| 2.2 | 78, 85, 74 |
| 3.2 | 142, 126, 120 |
| 4.2 | 120, 129, 121 |
| 5.2 | 64, 68, 79 |
| 6.2 | 92, 101, 78 |
| 7.2 | 74, 62, 94 |
| 8.2 | 89, 69, 67 |
| 9.2 | 85, 87, 97 |
| 10.2 | 71, 58, 55 |

RAW DATA

1999 Paper Figure 3 100% △

TABLE-3

Expt. # : 2 Date/Time : 12/21/98; 4-00 P.M

| 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] |
|---|---|---|---|---|
| 1 | 770, 793, 789 | 784 | 3136000 | – |
| 2 | 721, 756, 724 | 732 | 2930666 | – |
| 3 | 751, 777, 757 | 761 | 3046666 | 0·00037 |
| 4 | 721, 694, 690 | 701 | 2806666 | 0·00161 |
| 5 | 743, 754, 768 | 755 | 3020000 | 0·00294 |
| 6 | 759, 766, 724 | 749 | 2998666 | 0·00636 |
| 7 | 753, 740, 713 | 735 | 2941333 | 0·0186 |
| 8 | 750, 754, 775 | 759 | 3038666 | 0·0247 |
| 9 | 707, 687, 674 | 689 | 2757333 | 0·036 |
| 10 | 620, 625, 620 | 621 | 2486666 | 0·079 |

**TABLE-4**

**Expt # :** 3 **Date :**

| Tube.dilution | Colony 1 | Colony 2 | Colony 3 | Avg Colony | SF |
|---|---|---|---|---|---|
| 1·2 | 143 | 159 | 138 | 139·3 | |
| 2·2 | 126 | 129 | 141 | | |
| 3·2 | 140 | 135 | 129 | 134·6 | 0·9665 |
| 4·2 | 132 | 129 | 124 | 128·3 | 0·9210 |
| 5·2 | 106 | 113 | 119 | 112·6 | 0·8086 |
| 6·2 | 100 | 92 | 85 | 92 | 0·6602 |
| 7·2 | 50 | 43 | 36 | 43 | 0·3086 |
| 8·2 | 20 | 17 | 19 | 18·66 | 0·1339 |
| 9·3 | 79 | 70 | 62 | 7·0 | 0·0504 |
| 10·3 | 49 | 38 | 30 | 0·39 | 0·0028 |

Figure 5 Expt #2

**TABLE-3**

**Expt. # :** 2 ✓ **Date/Time :** 12/04/98 ; 1000 am

| 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] |
|---|---|---|---|---|
| 1 | 252, 262, 270 | 261 | | |
| 2 | 245, 221, 259 | 241 | | |
| 3 | 276, 236, 249 | 253.6 | 1014666 | 0.0007 |
| 4 | 302, 279, 271 | 284 | 1136000 | 0.0023 |
| 5 | 284, 294, 291 | 289 | 1158666 | 0.0059 |
| 6 | 404, 416, 426 | 415 | 1661333 | 0.008 |
| 7 | 299, 295, 336 | 310 | 1240000 | 0.029 |
| 8 | 244, 244, 237 | 239 | 958666 | 0.0471 |
| 9 | 231, 225, 228 | 228 | 912000 | 0.0711 |
| 10 | 279, 288, 300 | 289 | 1156000 | 0.1205 |

**TABLE-4**

Expt # : 2 Date : 12/11/98

| Tube.dilution | Colony 1 | Colony 2 | Colony 3 | Avg Colony | SF |
|---|---|---|---|---|---|
| 1·2 | 120 | 111 | 99 | 116·5 | |
| 2·2 | 131 | 121 | 117 | | |
| 3·2 | 104 | 114 | 96 | 104·6 | 0·8984 |
| 4·2 | 93 | 100 | 86 | 93 | 0·7982 |
| 5·2 | 70 | 78 | 63 | 70·3 | 0·6037 |
| 6·2 | 58 | 68 | 49 | 58·3 | 0·5007 |
| 7·2 | 29 | 22 | 19 | 23·3 | 0·2002 |
| 8·3 | 104 | 115 | 94 | 10·4 | 0·0895 |
| 9·4 | 116 | 126 | 107 | 1·16 | 0·0099 |
| 10·4 | 23 | 29 | 18 | 0·23 | 0·009 |

RAW DATA

1999 Paper Fig 3 O Fig 6 ▽ 50%

**TABLE-3**

**Expt. # :** 2 **Date/Time :** 01/15/99 ; 11-00 a.m.

| 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] | Kbq/cell |
|---|---|---|---|---|---|
| 1 | ~~488~~, 482, 478 | 482 | 1930666 | – | |
| 2 | 510, 472, 463 | 481 | 1926666 | – | |
| 3 | 517, 505, 506 | 509 | 2037333 | 0.00075 | 0.111 |
| 4 | 468, 458, 455 | 460 | 1841333 | 0.00397 | 0.587 |
| 5 | 528, 527, 524 | 526 | 2105333 | 0.00691 | 1.022 |
| 6 | 622, 648, 609 | 626 | 2505333 | 0.01373 | 2.032 |
| 7 | ~~345~~, 442, 422, 432 | 432 | 1728000 | 0.03347 | 4.953 |
| 8 | 467, 438, 438 | 447 | 1790666 | 0.05941 | 8.792 |
| 9 | 481, 452, 522 | 485 | 1940000 | 0.08051 | 11.91 |
| 10 | 487, 516, 482 | 495 | 1980000 | 0.18071 | 26.74 |

**TABLE-4**

**Expt # :** 2 **Date :** 11/22/99

| Tube.dilution | Colony 1 | Colony 2 | Colony 3 | Avg Colony | SF |
|---|---|---|---|---|---|
| 1·2 | 145 | 139 | 121 | 131·66 | - |
| 2·2 | 129 | 119 | 137 | | - |
| 3·2 | 130 | 135 | 124 | 129·66 | 0·9848 |
| 4·2 | 125 | 115 | 109 | 116·33 | 0·8835 |
| 5·2 | 95 | 101 | 107 | 101 | 0·7671 |
| 6·2 | 85 | 92 | 78 | 85 | 0·6456 |
| 7·2 | 59 | 69 | 78 | 68·6 | 0·5215 |
| 8·2 | 49 | 58 | 67 | 58 | 0·4405 |
| 9·2 | 38 | 44 | 33 | 38 | 0·2911 |
| 10·3 | 100 | 95 | 106 | 10·33 | 0·0784 |

**TABLE-3**

**Expt. # :** (

**Date/Time :** 01/01/99; 12-00 noon

| 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/labeled cell [pci/cell x 74] |
|---|---|---|---|---|---|
| 1 | 518, 550, 569 | 545 | 2182666 | – | |
| 2 | 482, 500, 501 | 494 | 1977333 | – | |
| 3 | 458, 485, 510 | 484 | 1937333 | 0.00136 | 0.10064 |
| 4 | 541, 483, 488 | 504 | 2016000 | 0.00634 | 0.4692 |
| 5 | 531, 525, 523 | 526 | 2105333 | 0.01196 | |
| 6 | 609, 657, 632 | 639 | 2557333 | 0.02158 | |
| 7 | 506, 524, 485 | 505 | 2020000 | 0.06792 | |
| 8 | 432, 440, 428 | 433 | 1733333 | 0.10148 | |
| 9 | 497, 453, 507 | 485 | 1942666 | 0.13687 | |
| 10 | 453, 475, 508 | 478 | 1914666 | 0.27043 | |

**TABLE-4**

**Expt # :** 1 **Date :** 01/08/99

| Tube.dilution | Colony 1 | Colony 2 | Colony 3 | Avg Colony | SF |
|---|---|---|---|---|---|
| 1·2 | | | | | |
| 2·2 | 99 | 89 | 91 | 93 | — |
| 3·2 | 80 | 74 | 68 | 74 | 0·7956 |
| 4·2 | 65 | 71 | 59 | 65 | 0·6989 |
| 5·2 | 40 | 48 | 57 | 48·3 | 0·5197 |
| 6·2 | 39 | 34 | 37 | 37 | 0·3942 |
| 7·2 | 28 | 26 | 23 | 26 | 0·2759 |
| 8·3 | 102 | 110 | 92 | 10·13 | 0·1089 |
| 9·3 | 33 | 47 | 40 | 4·0 | 0·043 |
| 10·4 | 90 | 84 | 79 | 0·84 | 0·009 |

RAW DATA

1999 Paper - Figure 7 100%

Data analyzed designated "only"

**TABLE-3**

**Expt. # :** 2 **Date/Time :** 03/05/99, 10-00 a.m.

| 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] |
|---|---|---|---|---|
| 1 | | | | |
| 2 | | | | |
| 3 | 480, 495, 478 | 484 | 1937333 | 0·04516 |
| 4 | 450, 477, 458 | 461 | 1846666 | 0·1073 |
| 5 | 575, 589, 567 | 577 | 2308000 | 0·2038 |
| 6 | | | | |
| 7 | | | | |
| 8 | 435, 449, 429 | 437 | 1750666 | 0·0434 |
| 9 | 445, 455, 462 | 454 | 1816000 | 0·1057 |
| 10 | 540, 559, 547 | 548 | 2194666 | 0·1875 |
| 11 | | | | |
| 12 | | | | |
| 13 | 560, 550, 542 | 550 | 2202666 | 0·0436 |
| 14. | 610, 625, 609 | 614 | 2458666 | 0·1208 |
| 15 | 675, 685, 687 | 682 | 2729333 | 0·2266 |

only

160

**TABLE-4**

**Expt # :** 2 **Date :** 03/12/99

| Tube.dilution | Colony 1 | Colony 2 | Colony 3 | Avg Colony | SF |
|---|---|---|---|---|---|
| 1.2 | 160 | 155 | 168 | 158.83 | – |
| 2.2 | 152 | 157 | 161 | | – |
| 3.2 | 17 | 22 | 27 | 22 | 0.138 |
| 4.4 | 23 | 30 | 38 | 0.33 | 0.0019 |
| 5.4 | 3 | 2 | 4 | 0.03 | 0.00018 |
| 6.2 | 156 | 142 | 150 | 147.5 | |
| 7.2 | 143 | 149 | 145 | | |
| 8.2 | 18 | 25 | 30 | 24.33 | 0.165 |
| 9.4 | 14 | 19 | 25 | 019 – | 0.0013 |
| 10.4 | 3 | 4 | 5 | 0.04 | 0.00027 |
| 11.2 | 160 | 167 | 172 | 160.66 | |
| 12.2 | 151 | 149 | 165 | | |
| 13.2 | 20 | 24 | 28 | 24 0.147 | 0.147 |
| 14.4 | 23 | 27 | 35 | 0.28 | 0.0017 |
| 15.4 | 3 | 3 | 2 | 0.023 | 0.00016 |

only

**TABLE-3**

**Expt. # :** 1 **Date/Time :** 03/01/99

| 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] |
|---|---|---|---|---|
| 1 | 640, 666, 685 | | | |
| 2 | 701, 722, 735 | | | |
| 3 | 735, 742, 722 | 733 | 2932000 | 0.0465 |
| 4 | 760, 750, 742 | 750 | 3002666 | 0.1205 |
| 5 | 775, 790, 769 | 778 | 3112000 | 0.2301 |
| 6 | 711, 729, 731 | | | |
| 7 | 699, 719, 698 | | | |
| 8 | 765, 777, 759 | 767 | 3068000 | 0.0402 |
| 9 | 745, 755, 761 | 753 | 3014666 | 0.1259 |
| 10 | 762, 772, 760 | 764 | 3058666 | 0.2389 |
| 11 | 685, 672, 669 | | | |
| 12 | 701, 735, 723 | | | |
| 13 | 795, 780, 776 | 783 | 3134666 | 0.0404 |
| 14 | 740, 722, 729 | 730 | 2921333 | 0.1138 |
| 15 | 811, 788, 792 | 797 | 3188000 | 0.23 |

only

**TABLE-4**

**Expt # :** 1 **Date :** 03/06/99

| Tube.dilution | Colony 1 | Colony 2 | Colony 3 | Avg Colony | SF |
|---|---|---|---|---|---|
| 1·2 | 155 | 160 | 149 | 150 | |
| 2·2 | 145 | 139 | 152 | | |
| 3 2 | 21 | 20 | 17 | 19·33 | 0·1288 |
| 4·4 | 23 | 26 | 21 | 0·23 | 0·0015 |
| 5·4 | 2 | 2 | 1 | 0·016 | 0·00012 |
| 6·2 | 174 | 185 | 170 | 174·5 | |
| 7·2 | 169 | 172 | 177 | | |
| 8·2 | 25 | 27 | 29 | 27 | 0·1547 |
| 9·4 | 26 | 30 | 35 | 0·3 | 0·0017 |
| 10·4 | 2, | 3, | 3 | 0·026 | 0·00016 |
| 11·2 | 182 | 169 | 178 | 171·66 | |
| 12·2 | 177 | 161 | 163 | | |
| 13·2 | 26 | 31 | 37 | 31·33 | 0·1925 |
| 14·4 | 22 | 26 | 29 | 0·25 | 0·0015 |
| 15·4 | 2 | 2 | 2 | 0·02 | 0·00011 |

only

**TABLE-3**

**Expt. # :** 1 **Date/Time :** 01/29/99

| 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] |
|---|---|---|---|---|
| 1 | 664, 644, 696 | | | |
| 2 | 716, 726, 721 | | | |
| 3 | 651, 675, 664 | | | |
| 4 | 643, 681, 636 | | | |
| 5 | 700, 690, 717 | 702 | 2809333 | 0.0228 |
| 6 | 618, 618, 657 | 631 | 2524000 | 0.0706 |
| 7 | 664, 691, 653 | 669 | 2677333 | 0.1315 |
| 8 | 536, 505, 557 | 532 | 2130666 | 0.0299 |
| 9 | 642, 626, 633 | 633 | 2534666 | 0.0761 |
| 10 | 541, 558, 569 | 556 | 2224000 | 0.1697 |

only

**TABLE-4** (1)

**Expt # :** 2/5/99 **Date :** 2/5/99

| Tube.dilution | Colony 1 | Colony 2 | Colony 3 | Avg Colony | SF |
|---|---|---|---|---|---|
| 1·2 | 150 | 135 | 139 | 137·33 | |
| 2·2 | 129 | 140 | 131 | | |
| 3·2 | 107 | 100 | 98 | 105·33 | 0·767 |
| 4·2 | 14 | 109 | 107 | | |
| 5·2 | 35 | 40 | 29 | 34·66 | 0·2524 |
| 6·3 | 18 | 20 | 16 | 1·8 | 0·0131 |
| 7·4 | 13 | 13 | 14 | 0·13 | 0·00097 |
| 8·2 | 40 | 47 | 32 | 39·6 | 0·3765 |
| 9·4 | 146 | 157 | 167 | 156 | 0·0149 |
| 10·4 | 14 | 16 | 15 | 0·15 | 0·00094 |

only

# RAW DATA

2001 Paper Fig 1 100%

Data analyzed designated "only"

**TABLE-3**

MS = 500 µl

**Expt. # :** 2

**Date/Time :** 12/06/99

| 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] | Ag/cell [pCi/cell x 37] |
|---|---|---|---|---|---|
| 1 | 801, 784, 832 | | | | |
| 2 | 829, 836, 827 | | | | |
| 3 | 759, 747, 732 | 746 | 298400 | 0.0087 | 0.3224 |
| 4 | 745, 713, 696 | 718 | 287200 | 0.0373 | 1.3785 |
| 5 | 824, 782, 807 | 804 | 321733 | 0.0914 | 3.382 |
| 6 | 808, 792, 771 | 790 | 316133 | 0.2065 | 7.64 |
| 7 | 728, 732, 702 | 727 | 290933 | 0.3743 | 13.85 |
| 8 | 648, 648, 651 | 649 | 259600 | 0.7399 | 27.38 |
| 9 | 725, 723, 713 | 720 | 288000 | 1.11 | 41.16 |
| 10 | 656, 647, 634 | 645 | 258266 | 1.76 | 65.01 |

12/13/99

| | | | | | |
|---|---|---|---|---|---|
| 1-2 | 170, | 181, | 185 | 178.66 | |
| 2-2 | 169, | 179, | 178 | | |
| 3-2 | 108 | 99 | 117 | 108 | 0.6045 |
| 4-2 | 45 | 54 | 64 | 54.33 | 0.3041 |
| 5-3 | 86 | 90 | 95 | 9.03 | 0.0506 |
| 6-4 | 31 | 36 | 42 | 0.36 | 0.002 |
| 7-4 | 3 | 4 | 5 | 0.04 | 0.00022 |
| 8-4 | 1 | 1 | 0 | 0.0066 | 0.000037 |

a  $S = \mathrm{Exp}(-a/a_0)$;  $a_0 =$

0.624
0.827

D013434

**TABLE-3**

MS = 500µl

**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] | µCi/cell [pCi/cell ÷ 37] |
|---|---|---|---|---|---|
| 1 | 546, 523, 543 | | | | |
| 2 | 606, 619, 627 | | | | |
| 3 | 521, 533, 529 | 527 | 210800 | 0.00664 | 0.246 |
| 4 | 611, 601, 622 | 611 | 244,533 | 0.013 | 0.484 |
| 5 | | | | | |
| 6 | 575, 585, 595 | 585 | 234000 | 0.059 | 2.21 |
| 7 | 535, 540, 549 | 541 | 216533 | 0.106 | 3.93 |
| 8 | 621, 603, 613 | 612 | 244933 | 0.249 | 9.21 |
| 9 | 550, 560, 569 | 559 | 223866 | 0.366 | 13.55 |
| 10 | | | | | |

11/9/99

| | | | |
|---|---|---|---|
| 1.2 | 134, 144, 146 | 125.33 | |
| 2.2 | 112, 100, 116 | | |
| 3.2 | 95, 99, 102 | 98.66 | 0.7872 |
| 4.2 | 70, 72, 76 | 72.66 | 0.5798 |
| 5.2 | 34, 40, 25 | 33 | 0.2633 |
| 7.3 | 91, 90, 82 | 8.76 | 0.0699 |
| 8.4 | 43, 45, 46 | 0.44 | 0.0035 |
| 9.4 | 16, 15, 23 | 0.18 | 0.0014 |

B013445

Cell count

MS = 50 µl

| | | Avg. count | Vol. contains $10^6$ cells (ml) |
|---|---|---|---|
| 1 | ~~521~~, 545, 567, 551 | 554.3 | 0.450 |
| 2 | 552, 543, 541 | 545.3 | 0.458 |
| 3 | 468, 455, 449 | 457.3 | 0.547 |
| 4 | 649, 683, 666 | 666 | 0.375 |
| 5 | 561, 523, 550 | 544.6 | 0.458 |
| 6 | 605, 590, 603 | 599.3 | 0.417 |
| 7. | 588, 574, 614 | 592 | 0.422 |
| 8 | 750, 767, 739 | 752 | 0.332 |
| 9. | 789, 772, 769 | 776 | 0.322 |
| 10. | 721, 719, 710 | 716 | 0.348 |
| 11 | 630, 666, 639 | 645 | 0.387 |
| 12. | 612, 631, 645 | 629 | 0.397 |
| 13 | 565, 574, 591 | 576 | 0.434 |
| 14 | 638, 610, 580 | 609 | 0.418 |

only 569 ± 51

672 ± 76

12/20/99

B001724

12/27/99

| | | | |
|---|---|---|---|
| 1.2 | 155, 165, 142 | 145.16 | |
| 2.2 | 135, 129, 145 | | |
| 3.2 | 49, 58, 68 | 58.33 | 0.4018 |
| only 4.3 | 89, 101, 110 | 10.0 | 0.0688 |
| 5.4 | 58, 62, 75 | 0.68 | 0.0047 |
| 6.4 | 12, 15, 9 | 0.12 | 0.0008 |
| 7.4 | 5, 7, 9 | 0.07 | 0.00048 |
| 8.2 | 175, 185, 181 | 173.83 | |
| 9.2 | 169, 173, 160 | | |
| 10.2 | 51, 56, 62 | 56.33 | 0.3241 |
| 11.3 | 112, 104, 119, | 11.16 | 0.0642 |
| 12.3 | 75, 87, 64 | 7.53 | 0.0433 |
| 13.4 | 27, 35, 44 | 0.35 | 0.002 |
| 14.4 | 9, 14, 20 | 0.14 | 0.00082 |

B0017ZG

| | Coulter Count | Avg. Cell Count | Vol. containing $10^6$ cells (ml) 01/07/00 |
|---|---|---|---|
| 1 | 525, 540, 523 | 529 | 0·472 |
| 2 | 560, 574, 550 | 561 | 0·445 |
| 3 | 251, 249, 248 | 249 | 1·00 (~0·8 plated) |
| 4 | ~~408~~, ~~395~~, 446, 424, 430 \| 433 | | 0·577 |
| 5. | 309, 282, 312 | 359 | 0·695 |
| 6. | 396, 373, 396 | 388 | 0·644 |
| 7. | 452, 432, 429 | 437 | 0·571 |
| 8. | 423, 443, 437 | 434 | 0·576 |
| 9 | 383, 367, 385 | 378 | 0·661 |
| 10. | 375, 379, 408 | 387 | 0·645 |
| 11. | 401, 398, 399 | 399 | 0·626 |
| 12 | 345, 365, 340 | 350 | 0·714 |
| 13 | 395, 367, 385 | 382 | 0·654 |
| 14 | 436, 449, 458 | 447 | 0·358 |

only

973-972-6474 (fax)
bishayan@umdnj.edu



**TABLE-4**

**Expt # :** **Date :**

| Tube.dilution | Colony 1 | Colony 2 | Colony 3 | Avg Colony for x.2 | SF | SE (%) |
|---|---|---|---|---|---|---|
| 1.2 | 157 | 162 | 149 | 161.16 | | |
| 2.2 | 168 | 172 | 159 | | | |
| 3.2 | 106 | 99 | 93 | 99.3 | 0.6163 | 4 |
| 4.2 | 98 | 87 | 77 | 87.3 | 0.5419 | 7 |
| 5.3 | 79 | 80 | 92 | 8.36 | 0.0519 | 5 |
| 6.3 | 37 | 32 | 28 | 3.23 | 0.0200 | |
| 7.4 | 39 | 48 | 57 | 0.48 | 0.0029 | 10 |
| 8.2 | 142 | 138 | 132 | 131.83 | | |
| 9.2 | 131 | 129 | 179 | | | |
| 10.2 | 36 | 44 | 51 | 43.6 | 0.3312 | |
| 11.2 | 18 | 26 | 37 | 27 | 0.2048 | |
| 12.3 | 40 | 47 | 34 | 4.03 | 0.0306 | |
| 13.4 | 132 | 139 | 118 | 1.29 | 0.0098 | |
| 14.4 | 20 | 16 | 13 | 0.16 | 0.0012 | |

only

**TABLE-3**

**Expt. # :** 1 **Date/Time :** 02/28/00

| 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] |
|---|---|---|---|---|
| 1 | 511, 526, 532 | | | |
| 2 | 546, 530, 519 | | | |
| 3 | 520, 540, 543 | 534 | 2137333 | 0.0027 |
| 4 | 475, 495, 482 | 484 | 1936000 | 0.0042 |
| 5 | 531, 521, 547 | 533 | 2132000 | 0.0041 |
| 6 | 480, 499, 501 | 493 | 1973333 | 0.0067 |
| 7 | 507, 519, 489 | 505 | 2020000 | 0.0083 |
| 8 | 465, 472, 482 | 473 | 1892000 | 0.0102 |
| 9 | 555, 532, 521 | 536 | 2144000 | 0.0092 |
| 10 | 411, 408, 429 | 416 | 1664000 | 0.0123 |
| 11 | 460, 469, 472 | 467 | 1868000 | 0.0159 |
| 12 | 422, 410, 404 | 412 | 1648000 | 0.0240 |

KBq/dm Cpci/cm x 148

0.4088 ~~0.4080~~ ~~0.548~~ ~~0.1385~~

0.6269
0.6101
0.99
1.23
1.52
1.37
1.82
2.36
3.65

**TABLE-4**

**Expt # :** 1 **Date :** 03/06/00

| Tube.dilution | Colony 1 | Colony 2 | Colony 3 | Avg Colony | SF |
|---|---|---|---|---|---|
| 1·2 | 126 | 139 | 119 | 121·33 | |
| 2·2 | 109 | 120 | 115 | | |
| 3·2 | 104 | 115 | 124 | 114·33 | 0·9423 |
| 4·2 | 98 | 107 | 117 | 107·33 | 0·8846 |
| 5·2 | 100 | 109 | 92 | 100·33 | 0·8269 |
| 6·2 | 95 | 88 | 102 | 95 | 0·7829 |
| 7·2 | 80 | 89 | 72 | 80·33 | 0·6621 |
| 8·2 | 67 | 87 | 76 | 76·66 | 0·6319 |
| 9·2 | 76 | 83 | 90 | 83 | 0·6841 |
| 10·2 | 68 | 79 | 59 | 68·66 | 0·5659 |
| 11·2 | 43 | 51 | 61 | 51·66 | 0·4258 |
| 12·2 | 37 | 44 | 52 | 44·33 | 0·3654 |

**TABLE-3**

**Expt. # :** 2 **Date/Time :** 01/14/00

| 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] | Kbq/clm [pCi/cell × 148] |
|---|---|---|---|---|---|
| 1 | 598, 607, 588 | | | | |
| 2 | 575, 566, 559 | | | | |
| 3 | 457, 435, 461 | 451 | 1804000 | 0.0097 | 1.44 |
| 4 | 522, 535, 542 | 533 | 2132000 | 0.0339 | 5.01 |
| 5 | 545, 519, 527 | 530 | 2121333 | 0.0788 | 11.66 |
| 6 | 488, 475, 462 | 475 | 1900000 | 0.1596 | 23.63 |
| 7 | 562, 575, 569 | 568 | 2274666 | 0.2072 | 30.67 |
| 8 | 513, 522, 530 | | | | |
| 9 | 501, 511, 507 | | | | |
| 10 | 475, 465, 470 | 470 | 1880000 | 0.0099 | 1.46 |
| 11 | 495, 489, 512 | 498 | 1994666 | 0.0323 | 4.79 |
| 12 | 445, 439, 452 | 445 | 1781333 | 0.0739 | 10.93 |
| 13 | 432, 417, 420 | 423 | 1692000 | 0.1579 | 23.37 |
| 14 | 455, 429, 439 | 441 | ~~2164000~~ 1764000 | 0.2151 | 31.84 |

only

**TABLE-4**

**Expt # :** 2

**Date :** 01/21/00

| Tube.dilution | Colony 1 | Colony 2 | Colony 3 | Avg Colony | SF |
|---|---|---|---|---|---|
| 1.2 | 158 | 148 | 152 | 149 | |
| 2.2 | 141 | 139 | 156 | | |
| 3.2 | 130 | 138 | 123 | 130.33 | 0.8747 |
| 4.2 | 49 | 60 | 54 | 54.33 | 0.3646 |
| 5.3 | 97 | 103 | 91 | 9.7 | 0.0651 |
| 6.4 | 67 | 58 | 76 | 0.67 | 0.0045 |
| 7.4 | 16 | 13 | 11 | 0.13 | 0.00089 |
| 8.2 | 132 | 142 | 129 | 133.66 | |
| 9.2 | 140 | 128 | 131 | | |
| 10.2 | 113 | 120 | 128 | 120.33 | 0.9002 |
| 11.2 | 77 | 83 | 90 | 83.33 | 0.6234 |
| 12.2 | 13 | 16 | 20 | 16.33 | 0.1221 |
| 13.3 | 43 | 51 | 60 | 5.13 | 0.0384 |
| 14.4 | 80 | 90 | 71 | 0.8033 | 0.0060 |

only

**TABLE-3**

**Expt. # :** 1

**Date/Time :** 12/10/99

| 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] | KBq/cluster [pCi/cell × 148] |
|---|---|---|---|---|---|
| 1 | 635, 649, 660 | 648 | | | |
| 2 | 711, 722, 745 | 726 | | | |
| 3 | 672, 666, 659 | 665 | 2662666 | 0·0102 | 1·51 |
| 4 | 782, 799, 786 | 789 | 3156000 | 0·0278 | 4·11 |
| 5 | 881, 862, 872 | 871 | 3486666 | 0·0669 | 9·91 |
| 6 | 835, 852, 821 | 836 | 3344000 | 0·1222 | 18·09 |
| 7 | 811, 829, 835 | 825 | 3300000 | 0·1817 | 26·89 |
| 8 | 756, 742, 769 | 755 | | | |
| 9 | 701, 695, 714 | 703 | | | |
| 10 | 649, 661, 639 | 649 | 2598666 | 0·01000 | 1·48 |
| 11. | 852, 829, 840 | 840 | 3361333 | 0·0323 | 4·79 |
| 12. | 633, 615, 609 | 619 | 2476000 | 0·0748 | 11·08 |
| 13. | 719, 709, 715 | 714 | 2857333 | 0·1154 | 17·08 |
| 14. | 695, 727, 711 | 711 | 2844000 | 0·1700 | 25·17 |

only

**TABLE-4**

**Expt # :** \ **Date :** 11/17/99

| Tube.dilution | Colony 1 | Colony 2 | Colony 3 | Avg Colony | SF |
|---|---|---|---|---|---|
| 1.2 | 148 | 159 | 162 | 160.5 | |
| 2.2 | 165 | 172 | 157 | | |
| 3.2 | 123 | 129 | 136 | 129.3 | 0.8058 |
| only 4.2 | 58 129 | 68 118 | 79 137 | 68.3 12.8 | 0.4257 0.079 |
| 5.3 6.4 | 81 | 90 | 72 | 0.81 | 0.005 |
| 7.4 | 12 | 21 | 16 | 0.16 | 0.0010 |
| 8.2 | 135 | 149 | 151 | 140.33 | |
| 9.2 | 141 | 129 | 137 | | |
| 10.2 | 123 | 117 | 112 | 117.3 | 0.8361 |
| 11.2 | 73 | 82 | 92 | 82.3 | 0.5867 |
| 12.2 | 23 | 26 | 20 | 23 | 0.1639 |
| 13.3 | 39 | 50 | 42 | 4.36 | 0.031 |
| 14.4 | 99 | 112 | 88 | 0.99 | 0.0071 |

RAW DATA

2001 Paper Figure 2A 50%

**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] | KBr/counter (pci/cell x 148) |
|---|---|---|---|---|---|
| 1 | 575, 566, 569 | | | | |
| 2 | 582, 572, 568 | | | | |
| 3 | 595, 582, 601 | 592 | 2370666 | 0.0138 | 2.05 |
| 4 | 555, 565, 549 | 556 | 2225333 | 0.0663 | 9.82 |
| 5 | 579, 599, 582 | 586 | 2346666 | 0.1905 | 28.19 |
| 6 | 611, 623, 607 | 613 | 2454666 | 0.5239 | 77.54 |
| 7 | 635, 619, 622 | 625 | 2501333 | 0.7827 | 115.85 |
| 8 | 601, 609, 611 | | | | |
| 9 | 551, 561, 571 | | | | |
| 10 | 569, 557, 547 | 557 | 2230669 | 0.0141 | 2.08 |
| 11 | 529, 535, 542 | 535 | 2141333 | 0.0665 | 9.85 |
| 12 | 565, 570, 568 | 567 | 2268000 | 0.1895 | 28.05 |
| 13 | 582, 592, 579 | 584 | 2337333 | 0.463 | 68.55 |
| 14 | 575, 562, 555 | 564 | 2256000 | 0.7571 | 112.05 |

only

**TABLE-4**

**Expt # :** **Date :**

| Tube.dilution | Colony 1 | Colony 2 | Colony 3 | Avg Colony | SF |
|---|---|---|---|---|---|
| 1.2 | 140 | 148 | 137 | 149.16 | |
| 2.2 | 161 | 152 | 157 | | |
| 3.2 | 105 | 112 | 120 | 112.33 | 0.7531 |
| 4.2 | 67 | 72 | 63 | 67.33 | 0.4514 |
| 5.2 | 13 | 20 | 16 | 16.33 | 0.1095 |
| 6.3 | 24 | 37 | 30 | 3.03 | 0.0203 |
| 7.4 | 38 | 43 | 49 | 0.43 | 0.0029 |
| 8.2 | 169 | 152 | 160 | 148.33 | |
| 9.2 | 142 | 138 | 129 | | |
| 10.2 | 95 | 104 | 114 | 104.33 | 0.7034 |
| 11.2 | 35 | 44 | 54 | 44.33 | 0.2988 |
| 12.3 | 60 | 68 | 53 | 6.03 | 0.0407 |
| 13.4 | 60 | 67 | 52 | 0.63 | 0.0042 |
| 14.4 | 11 | 7 | 5 | 0.076 | 0.00052 |

10⁻⁶ DMSO

0 DMSO

only

**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] | K Dpm/Counts (pci/cell x 148) |
|---|---|---|---|---|---|
| 1 | 575, 549, 562 | | | | |
| 2 | 492, 488, 475 | | | | |
| 3 | 511, 501, 493 | 501 | 2006666 | 0.0199 | 2.95 |
| 4 | 562, 549, 537 | 549 | 2197333 | 0.0785 | 11.62 |
| 5 | 589, 599, 582 | 590 | 2360000 | 0.2192 | 32.45 |
| 6 | 575, 565, 559 | 566 | 2265333 | 0.6334 | 93.75 |
| 7 | 670, 665, 669 | 668 | 2672000 | 0.8147 | 120.57 |
| 8 | 603, 619, 592 | | | | |
| 9 | 585, 599, 572 | | | | |
| 10 | 435, 425, 411 | 423 | 1694666 | 0.027 | 4.02 |
| 11 | 611, 623, 609 | 614 | 2457333 | 0.0818 | 12.11 |
| 12 | 607, 603, 589 | 599 | 2398666 | 0.2228 | 32.98 |
| 13 | 629, 630, 641 | 633 | 2533333 | 0.622 | 92.07 |
| 14 | 686, 698, 679 | 688 | 2753333 | 0.8786 | 130.03 |

only

**TABLE-4**

**Expt # :** **Date :**

| Tube.dilution | Colony 1 | Colony 2 | Colony 3 | Avg Colony | SF |
|---|---|---|---|---|---|
| 1·2 | 150 | 161 | 147 | 151·16 | |
| 2·2 | 139 | 151 | 159 | | |
| 3·2 | 84 | 90 | 97 | 90·33 | 0·5975 |
| 4·2 | 40 | 45 | 51 | 45·33 | 0·2999 |
| 5·2 | 12 | 15 | 13 | 13·33 | 0·0882 |
| 6·2 | 17 | 15 | 14 | 15·33 | 0·0101 |
| ~~7·2~~ 8·2 | ~~17~~ 165 | ~~23~~ 160 | ~~30~~ 179 | 0·23 160·83 | 0·00145 |
| 9·2 | 160 | 149 | 152 | | |
| 10·2 | 48 | 56 | 62 | 55·33 | 0·3440 |
| 11·2 | 27 | 33 | 40 | 33·33 | 0·2072 |
| 12·3 | 15 | 17 | 19 | 17 | 0·0105 |
| 13·4 | 19 | 26 | 32 | 0·25 | 0·00159 |
| 14·4 | 1 | 2 | 1 | 0·013 | 0·00008 |

1% DMSO

0% DMSO

only

TABLE-3

Expt. # : 1 Date/Time : 11/08/99 ; 3-00 p.m.

| Kbq/cluster [nCi/cluster x 0·037] | 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 | 411, 427, 437 | 425 | 1700000 | | |
| | 2 | 520, 545, 539 | 534 | | | |
| 0·124 | 3 | 535, 521, 552 | 536 | 2144000 | 0·00084 | 3·35 |
| 0·620 | 4 | 482, 493, 475 | 483 | 1933333 | 0·00418 | 16·75 |
| 1·083 | 5 | 436, 427, 419 | 427 | 1709333 | ~~0·585~~ 0·00732 | 29·27 |
| 1·359 | 6 | 501, 492, 485 | 492 | 1970666 | 0·00918 | 36·73 |
| 2·604 | 7 | 562, 550, 572 | 561 | 2245333 | 0·0175 | 70·36 |
| 6·07 | 8 | 449, 462, 475 | 462 | 1848000 | 0·041 | 164·0 |
| 5·55 | 9 | 555, 579, 567 | 567 | 2268000 | 0·0375 | 150·0 |
| 1465 | 10 | 409, 399, 417 | 408 | 1633333 | 0·099 | 396 |

**TABLE-4**

**Expt # :**

**Date :** 11/15/99

| Tube.dilution | Colony 1 | Colony 2 | Colony 3 | Avg Colony | SF |
|---|---|---|---|---|---|
| 1·2 | 156 | 166 | 149 | 151·83 | |
| 2·2 | 139 | 149 | 152 | | |
| 3·2 | 137 | 146 | 157 | 146·6 | 0·9659 |
| 4·2 | 145 | 131 | 138 | 138 | 0·9089 |
| 5·2 | 137 | 126 | 116 | 126·3 | 0·832 |
| 6·2 | 115 | 112 | 130 | 119 | 0·7837 |
| 7·2 | 95 | 102 | 88 | 95 | 0·6256 |
| 8·2 | 68 | 87 | 76 | 77 | 0·5071 |
| 9·2 | 68 | 77 | 72 | 72·3 | 0·4764 |
| 10·2 | 40 | 32 | 24 | 32 | 0·2107 |

SE (%)

15%

$\frac{4.8}{32} \times 100$

# RAW DATA

B's 100% Experiments

# of cells recovered from clusters after 72 hrs at 37°.5°C



**TABLE-3**

**Expt. # :** HPRT/1 **Date/Time :**

| Tube # | Coulter count for 100 ul cell suspension | Avg. count (Backg ↓) | Cells/ml [Avg. count x 4000] x10^6 | pCi/cell [uCi/ml x10^6 Cells/ml] | Total # of recovered cells | % of recovery from cluster |
|---|---|---|---|---|---|---|
| 1 | 304, 266, 288 | 858/3 - 1 | 1.14 | 2.28 | | 0.57 |
| 2 | 395, 341, 307 | 1043/3 - 1 | 1.38 | 2.76 | | 69% |
| 3 | 414, 346, 336 | 1096/3 - 1 | 1.46 | 2.96 | | 73% |
| 4 | 379, 408, 331 | 1118/3 - 1 | 1.49 | | | 75% |
| 5 | 360, 367, 316 | 1043/3 - 1 | 1.38 | 2.76 | | 69% |
| 6 | 354, 290, 269 | 913/3 - 1 | 1.21 | 2.42 | | 61% |
| 7 | 246, 233, 198 | 677/3 - 1 | 0.89 | 1.78 | | 45% |
| 8 | 319, 322, 333 | 974/3 - 1 | 1.29 | 2.58 | | 65% |
| 9 | 298, 294, 314 | 906/3 - 1 | 1.20 | 2.40 | | 60% |
| 10 | 373, 328, 335 | 1036/3 - 1 | 1.38 | 2.76 | | 69% |

Background = 1

Volume counted = 500 µl

**TABLE-4**

**Expt # :** **Date :**

| | Tube.dilution | Colony 1 | Colony 2 | Colony 3 | Avg Colony PE | SF |
|---|---|---|---|---|---|---|
| 0 | 1.3 | 37 | 48 | 35 | ~~37.5~~ | > 0.34 - 1 |
| 0 | 2.3 | 38 | 36 | 31 | ~~(144+125)/2~~ | |
| 0.01 | 3.3 | 152 | 57 | 47 | 52/146 0.35 | - 1.16 |
| 0.05 | 4.3 | 57 | 65 | 72 | 65/149 0.44 | - 1.42 |
| 0.1 | 5.3 | 29 | 22 | 22 | 24/139 0.17 | - 0.55 ? |
| 0.2 | 6.3 | 50 | 51 | 47 | 49/121 0.40 | - 1.29 |
| 0.5 | 7.3 | 38 | 50 | 43 | 44/90 0.49 | - 1.58 |
| 0.75 | 8.3 | 39 | 60 | 48 | 49/129 0.38 | - 1.23 |
| 1 | 9.3 | 41 | 34 | 38 | 38/120 0.32 | - 1.00 |
| 2 | 10.3 | 19 | 19 | 20 | 19/138 0.14 | - 0.45 |

**TABLE-3**

**Expt. # :** Dec, 14, 2000 **Date/Time :** Dec, 18, 2000 / 15:05

| Tube # | Coulter count for 100 ul cell suspension | Avg. count | Cells/ml [Avg. count x 400] | pCi/cell [uCi/ml x $10^6$ Cells/ml] | Recovery |
|---|---|---|---|---|---|
| 1 | 1232, 1209, 1265 | 1230 | 492133 | | ~ 25% |
| 2 | 2714, ~~1511~~ 2620, 2702 | 2678 | 1069467 | | ~ 50% |
| 3 | 4688, 4336, 4734 | 4586 | 1832400 | | |
| 4 | 3389, 3356, 3387 | 3377 | 1,348.933 | | |
| 5 | 3674, 3592, 3655 | 3633 | 1,451,200 | | |
| 6 | 4846, ~~3534~~ 4827, 4888 | 4853 | 1.938.800 | | |
| 7 | 4414, 4549, 4327 | 4430 | 1,772,000 | | |
| 8 | 4116, 4010, 4114 | 4080 | 1,632,000 | | |
| 9 | 5151 5125 5068 | 5148 | 2,057 200 | | |
| 10 | 4263 4058 ~~3877~~ 4013 | 4111 | 1642533 | | |

X-3 ← for survival

X3, X4

4.2 × 10^6

Background - 5 counts

Coulter Mode - 500µl

① ② ③ samples processed by me

**TABLE-4**

**Expt # :** V79, H-3, 100% **Date :** Dec., 14, 2000

| Tube.dilution | Colony 1 | Colony 2 | Colony 3 | Avg Colony | SF |
|---|---|---|---|---|---|
| 1.2 | 197 | 200 | 202 | | |
| 2.2 | 73 | 69 | 82. | | |
| 3.2 | 62 | 63 | 62 | | |
| 4.2 | 61 | 60 | 55 | | |
| 5.2 | 38 | 47 | 41 | | |
| 6.2. | 49 | 42 | 37 | | |
| 7.2. | 32 | 37 | 36 | | |
| 8.2.3 | 220 | 222 | 211 | | |
| 9.2.3 | 182 | 175 | 170 | | |
| 10.2.3 | 168 | 185 | 174 | | |

For tube:

| | | | |
|---|---|---|---|
| 3.3 | 401 | 411 | 410 |
| 4.3 | 323 | 347 | 326 |
| 5.3 | 297 | 283 | 272 |
| 6.3 | 296 | 320 | 283 |
| 7.3 | 207 | 217 | 201 |

7.4, 8.4, 9.4, 10.4 - too many colonies.

**TABLE-3**

**Expt. # :** V79, 100%, H3 **Date/Time :** MAY 3 /2001 - start

| Tube # | Coulter count for 100 ul cell suspension | Avg. count | Cells/ml [Avg. count x 4000] | pCi/cell [μCi/ml x10$^6$ Cells/ml] |
|---|---|---|---|---|
| 1 | 6479, 6522, 6564 | | 2605467 | 0,39 |
| 2 | 7117, 6864, 6859 | | 2775467 | 0,37 |
| 3 | 6336, 6410, 6373 | | 2546000 | 0,40 |
| 4 | 7404, 7377, 7432 | | 2958533 | 0,34 |
| 5 | 7595, 7546, 7263 | | 2984000 | 0,34 |
| 6 | 6399, 6607, 6325 | | 2574267 | 0,39 |
| 7 | 5950, 6039, 5761 | | 2363467 | 0,41 |
| 8 | 6655, 6821, 6607 | | 2674533 | 0,38 |
| 9 | 5587 5416 5598 | | 2210267 | 0,45 |
| 10 | 7052 7258 7144 | | 2857333 | 0,36 |

For Mn ↓ plate 0,15ml of cells per P60'

Bolger - 8-11
Hoechst - 500 μl

↑ Volume for $10^6$ cells

CoulterSurvival

riment: 37014
e/Time: 5/3/01

| Tube # | Coulter count 1st | 2nd | 3rd | Average | Cells/ml | Hemocytometer Count in Grid 1st | 2nd | 3rd | 4th |
|---|---|---|---|---|---|---|---|---|---|
| *1* | *6479* | *6522* | *6564* | *6522* | *2605467* | | | | |
| *2* | *7117* | *6864* | *6859* | *6947* | *2775467* | | | | |
| 3 | 6336 | 6410 | 6373 | 6373 | 2546000 | | | | |
| 4 | 7404 | 7377 | 7432 | 7404 | 2958533 | | | | |
| 5 | 7595 | 7546 | 7263 | 7468 | 2984000 | | | | |
| 6 | 6399 | 6607 | 6325 | 6444 | 2574267 | | | | |
| 7 | 5950 | 6039 | 5761 | 5917 | 2363467 | | | | |
| 8 | 6655 | 6821 | 6607 | 6694 | 2674533 | | | | |
| 9 | 5587 | 5416 | 5598 | 5534 | 2210267 | | | | |
| 10 | 7052 | 7258 | 7144 | 7151 | 2857333 | | | | |

| Tube # | Predicted # Cells Seeded | Actual # Cells Seeded | Colony count 1st | 2nd | 3rd | Average | PE (%) | SF Uncorrected | SF Corrected |
|---|---|---|---|---|---|---|---|---|---|
| *1* | 200 | *261* | *80* | *78* | *95* | *85* | *31.531* | *1.00* | *1.0000* |
| *2* | 200 | *278* | *87* | *86* | *83* | | | | |
| 3 | 200 | 255 | 59 | 62 | 53 | 58 | 22.781 | 0.6837 | 0.7225 |
| 4 | 200 | 296 | 61 | 52 | 52 | 55 | 18.590 | 0.6483 | 0.5896 |
| 5 | 200 | 298 | 51 | 47 | 44 | 47 | 15.862 | 0.5580 | 0.5031 |
| 6 | 200 | 257 | 64 | 54 | 51 | 56 | 21.883 | 0.6640 | 0.6940 |
| 7 | 200 | 236 | 41 | 33 | 45 | 40 | 16.783 | 0.4676 | 0.5323 |
| 8 | 200 | 267 | 59 | 59 | 61 | 60 | 22.309 | 0.7033 | 0.7075 |
| 9 | 200 | 221 | 42 | 48 | 33 | 41 | 18.550 | 0.4833 | 0.5883 |
| 10 | 200 | 286 | 69 | 76 | 71 | 72 | 25.198 | 0.8487 | 0.7992 |

Plate 200,000 for MN
1,000,000 for 6TGRT

MS = 500 µl; Background = 14

DAY 0 of mutant expression

**TABLE-3**

**Expt. #:** V79, 100%, HTdR **Date/Time:** 05/25/01; 3-00 pm

| 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] |
|---|---|---|---|---|
| 1 | 6134, 6219, 6161 | 6171 | 2,468,533 | |
| 2 | ~~6789, 6628, 6961 7301, 7004, 7688~~ | 6792 | 2,717,066 | |
| 3 | 6934, 6950, 6751 | 6878 | 2,751,333 | |
| 4 | 7188, 7166, 7101 | 7151 | 2,860,666 | |
| 5 | 7464, 7568, 7366 | 7466 | 2,986,400 | |
| 6 | ~~7036~~, 6720, 6864, 6800 | 6794 | 2,717,866 | |
| 7 | 6437, 6516, 6442 | 6465 | 2,586,000 | |
| 8 | 6548, 6789, 6661 | 6666 | 2,666,400 | |
| 9 | 6044, 6415, 6176 | 6211 | 2,484,666 | |
| 10 | 6515, 6607, 6469 | 6530 | 2,612,133 | |

4/6 → 4/6

Mode - 500µl
Bckgr - See above

For survival:

1,2
2,2
3,2
4,2
5,2 5,3
6,2 6,3
7,2 7,3
8,2 8,2
9,2 9,3
10,2 10,3

**TABLE-4**

**Expt #** V79, 100%, HICR **Date :**

~~38. Count vials for radioactivity~~ **Date/Time :**

| Tube.dilution | Colony 1 | Colony 2 | Colony 3 | Avg Colony | SF |
|---|---|---|---|---|---|
| 1.2 | 86 | 78 | 72 | | |
| 2.2 | 50 | 76 | 63 | | |
| 3.2 | 71 | 81 | 68 | | |
| 4.2 | 68 | 64 | 66 | | |
| 5.2 | 62 | 55 | 55 | | |
| 6.2 | 81 | 66 | 67 | | |
| 7.2 | 29 (?) | 38 | 46 | | |
| 8.2 | 54 | 53 | 51 | | |
| 9.2 | 38 | 46 | 38 | | |
| 10.2 | 40 | 36 | 36 | | |

**TABLE-3** V79, Hilak, 100% June 25, 2001

**Expt. # :** [redacted] **Date/Time :** $2 \times 10^5$ cells

| Background in Coulter | Tube # | Coulter count for 100 ul cell suspension | Avg. count | Cells/ml [Avg. count x 400 – Background] | pCi/cell [uCi/ml x $10^6$ / Cells/ml] |
|---|---|---|---|---|---|
| 8 | 1 | 6005 20% | | [illegible] | [illegible] |
| 12 | 2 | 5067, 5096, 4980 | | 2 012 533 | 11,00 |
| 12 | 3 | 6270, 6373, 6307 | | 2 526 133 | 0,79 |
| 8 | 4 | 5934, 5676, 5619 | | 2 296 400 | 0.87 |
| 8 | 5 | 5904, 5828, 5708 | | 2 359 200 | [illegible] |
| 8 | 6 | 5903, 5821, 5843 | | 2 339 067 | [illegible] |
| 8 | 7 | 5432, 5614, 5667 | | 2 221 733 | 0.90 |
| 8 | 8 | 5456, 5370, 5454 | | 2 167 467 | 0.92 |
| 12 | 9 | 5410, | | 2 159 200 | 0,93 |
| 8 | 10 | 5637, 5539, 5701 | | 2 219 867 | 0.91 |

Mode - 500µl

Plot for SE

1, 2
[illegible]

CoulterSurvival

iiment: 37063
e/Time: ######

| Tube # | Coulter count 1st | 2nd | 3rd | Average | Cells/ml | Hemocytometer Count in Grid 1st | 2nd | 3rd | 4th |
|---|---|---|---|---|---|---|---|---|---|
| *1* | *6003* | *5803* | | *5903* | *2358000* | | | | |
| *2* | *5067* | *5096* | *4980* | *5048* | *2015867* | | | | |
| 3 | 6270 | 6373 | 6309 | 6317 | 2523733 | | | | |
| 4 | 5934 | 5676 | 5619 | 5743 | 2294000 | | | | |
| 5 | 5904 | 5828 | 5686 | 5806 | 2319200 | | | | |
| 6 | 5903 | 5821 | 5843 | 5856 | 2339067 | | | | |
| 7 | 5438 | 5627 | 5667 | 5577 | 2227733 | | | | |
| 8 | 5456 | 5370 | 5454 | 5427 | 2167467 | | | | |
| 9 | 5410 | | | 5410 | 2160800 | | | | |
| 10 | 5637 | 5539 | 5497 | 5558 | 2219867 | | | | |

| Tube # | Predicted # Cells Seeded | Actual # Cells Seeded | Colony count 1st | 2nd | 3rd | Average | PE (%) | SF Uncorrected | SF Corrected |
|---|---|---|---|---|---|---|---|---|---|
| *1* | 200 | *236* | *100* | *90* | | *85* | *38.867* | *1.00* | *1.0000* |
| *2* | 200 | *202* | *78* | *72* | | | | | |
| 3 | 200 | 252 | 97 | 107 | 93 | 99 | 39.228 | 1.1647 | 1.0093 |
| 4 | 200 | 229 | 58 | 61 | 57 | 59 | 25.574 | 0.6902 | 0.6580 |
| 5 | 200 | 232 | 68 | 51 | 63 | 61 | 26.158 | 0.7137 | 0.6730 |
| 6 | 200 | 234 | 61 | 39 | 49 | 50 | 21.234 | 0.5843 | 0.5463 |
| 7 | 200 | 223 | 57 | 42 | 39 | 46 | 20.649 | 0.5412 | 0.5313 |
| 8 | 200 | 217 | 43 | 47 | 34 | 41 | 19.070 | 0.4863 | 0.4906 |
| 9 | 200 | 216 | 47 | 55 | 49 | 50 | 23.294 | 0.5922 | 0.5993 |
| 10 | 200 | 222 | 61 | 61 | 52 | 58 | 26.128 | 0.6824 | 0.6722 |

# RAW DATA

C's 100% Experiments

Only part

## Coulter Counts After Resuspending Pellets (500ml recon.)

| | | | $2\times10^6$ cells |
|---|---|---|---|
| 1) | 3642 3420 3681 | $3581\times400 = 1.43\times10^6$/ml | 1.40 ml |
| 2) | 3584 3611 3603 | $3599\times400 = 1.44\times10^6$/ml | 1.40 ml |
| 3) | 3942 3986 3899 | $3942\times400 = 1.58\times10^6$/ml | 1.27 ml (1.2) |
| 4) | 3691 3715 3671 | $3692\times400 = 1.48\times10^6$/ml | 1.35 ml (1.2) |
| 5) | 3879 3764 4033 | $3892\times400 = 1.56\times10^6$/ml | 1.28 ml (1.2) |
| 6) | 3381 3457 3429 | $3422\times400 = 1.37\times10^6$/ml | 1.46ml (1.2) |
| 7) | 3177 3184 3164 | $3175\times400 = 1.27\times10^6$/ml | 1.57 (1.2) |
| 8) | 2683 2560 2568 | $2604\times400 = 1.04\times10^6$/ml | 1.92 (1.2) |
| 9) | 2595 2538 2661 | $2598\times400 = 1.04\times10^6$/ml | 1.92 (1.2) |
| 10) | 2687 2770 2835 | $2764\times400 = 1.45\times10^6$/ml | 1.92 (1.2) |

Backgrd. 12

~~BACK~~ V79 Exp. 6 Survival
~~Fixed~~ 10/1/00
Stain 10/8/00

Colony Counts

1.2) 105 104 98
2.2) 97 78 87
3.2) 55 66 61
4.2) 56 48 41
5.2) 47 44
6.2) 31 39 42
7.2) 34 28 24
8.2) 16 26 14
9.2) 26 36 21

9.3) 164 175 158

10.3) 139 170 156

For hprt expression

**TABLE-3** 100% cluster, H7dR, V79 cells

**Expt. # :** 5 July 20, 2001 **Date/Time :** ~12:00 am

| Tube # | Coulter count for 100 ul cell suspension | Avg. count | Cells/ml [Avg. count - Bckgr x 400] - Bckgr. Total in 2ml | pCi/cell [uCi/ml x $10^6$ Cells/ml] $2\times10^6$ cells (in 1) |
|---|---|---|---|---|
| 1 | 4827 4878 4587 | 1 896 800 | 3 793 600 | 1.05 |
| 2 | 5348 5384 5312 | 2 130 400 | 4 260 800 | 0.94 |
| 3 | 4752 4858 4672 | 1 894 133 | 3 788 267 | 1.06 |
| 4 | 5120 4944 4934 | 1990 933 | 3 981 867 | 1.00 |
| 5 | 5457 5625 5562 | 2 210 400 | 4 420 800 | 0.91 |
| 6 | 5265 5084 ~~4772~~ | ~~2 061 733~~ 2 061 000 | 4 122 000 | 0.97 |
| 7 | 4929 4878 4753 | 1 932 533 | 3 865 067 | 1.04 |
| 8 | 5115 4965 5094 | 2 014 400 | 4 028 800 | 0.95 |
| 9 | 4615 4711 4583 | 1, 839 733 | 3 679 466 | 1, 08 |
| 10 | 4662 4596 4647 | 1 845 200 | 3 690 400 | 1,08 |

Bckgr - 22
Mode - 500 gm

~~Lab 101~~ Colony Counts for Survival

| ~~Tube~~ | ~~Dilution~~ | ~~Colony Counts~~ |
|---|---|---|
| 1 | ~~200~~ | ~~153~~ 114 114 |
| 2 | 200 | 85 81 |
| 3 | 200 | 63 68 83 |
| 4 | 200 | 94 84 73 |
| 5 | ~~200~~ | 66 ~~108~~ 67 |
| 6 | 200 | 61 ~~93~~ 73 |
| 7 | 200 | 56 55 52 |
| 8 | 200 | 47 ~~36~~ 37 |
| 9 | 200 | 46 ~~38~~ 54 |
| ~~10~~ | ~~200~~ | ~~40~~ 30 30 |
| | | |
| | | |
| | | |
| | | |

5000/1442

# RAW DATA

## B's 50% Experiments

CoulterSurvival

Experiment: H-3/50%/Exp4
Date/Time: ######

| Tube # | Coulter count 1st | 2nd | 3rd | Average | Cells/ml | Hemocytometer Count in Grid 1st | 2nd | 3rd | 4th |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 4873 | 4965 | 4933 | 4924 | 1964267 | | | | |
| 2 | 4226 | 4377 | 4142 | 4248 | 1694133 | | | | |
| 3 | 4918 | 4874 | 5043 | 4945 | 1972800 | | | | |
| 4 | 4777 | 4802 | 4712 | 4764 | 1900267 | | | | |
| 5 | 4429 | 4375 | 4438 | 4414 | 1760400 | | | | |
| 6 | 3883 | 3776 | 3929 | 3863 | 1539867 | | | | |
| 7 | 4333 | 4442 | 4358 | 4378 | 1745867 | | | | |
| 8 | 3505 | 3507 | 3370 | 3461 | 1379067 | | | | |
| 9 | 4404 | 4177 | 4329 | 4303 | 1716133 | | | | |
| 10 | 3908 | 3857 | | 3883 | 1547800 | | | | |

| Tube # | Predicted # Cells Seeded | Actual # Cells Seeded | Colony count 1st | 2nd | 3rd | Average | PE (%) | SF Uncorrected | SF Corrected |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 200 | 196 | 78 | 91 | 74 | 72 | 39.544 | 1.00 | 1.0000 |
| 2 | 200 | 169 | 59 | 67 | 65 | | | | |
| 3 | 200 | 197 | 62 | 60 | 66 | 63 | 31.765 | 0.8664 | 0.8033 |
| 4 | 200 | 190 | 42 | 52 | 35 | 43 | 22.628 | 0.5945 | 0.5722 |
| 5 | 200 | 176 | 72 | 68 | 72 | 71 | 40.142 | 0.9770 | 1.0151 |
| 6 | 200 | 154 | 36 | 41 | 35 | 37 | 24.245 | 0.5161 | 0.6131 |
| 7 | 200 | 175 | 35 | 36 | 27 | 33 | 18.711 | 0.4516 | 0.4732 |
| 8 | 200 | 138 | 47 | 41 | | 44 | 31.906 | 0.6083 | 0.8068 |
| 9 | 200 | 172 | 73 | 69 | 55 | 66 | 38.264 | 0.9078 | 0.9676 |
| 10 | 200 | 155 | 37 | 37 | 25 | 33 | 21.321 | 0.4562 | 0.5392 |

CoulterSurvival

Experiment: 37056
Date/Time: ######

| Tube # | Coulter count 1st | 2nd | 3rd | Average | Cells/ml | Hemocytometer Count in Grid 1st | 2nd | 3rd | 4th |
|---|---|---|---|---|---|---|---|---|---|
| *1* | *9698* | *9493* | *9590* | *9594* | *3835467* | | | | |
| *2* | *9431* | *9585* | *9579* | *9532* | *3810667* | | | | |
| 3 | 8615 | 8504 | 8509 | 8543 | 3415067 | | | | |
| 4 | 7846 | 7901 | 7781 | 7843 | 3135067 | | | | |
| 5 | 8421 | 8249 | 8343 | 8338 | 3333067 | | | | |
| 6 | 6623 | 6463 | 6314 | 6467 | 2584667 | | | | |
| 7 | 6725 | 6757 | 6703 | 6728 | 2689333 | | | | |
| 8 | 6821 | 6891 | 6741 | 6818 | 2725067 | | | | |
| 9 | 6277 | 6426 | 6108 | 6270 | 2506133 | | | | |
| 10 | 6437 | 6187 | 6240 | 6288 | 2513200 | | | | |

| Tube # | Predicted # Cells Seeded | Actual # Cells Seeded | Colony count 1st | 2nd | 3rd | Average | PE (%) | SF Uncorrected | SF Corrected |
|---|---|---|---|---|---|---|---|---|---|
| *1* | 200 | *384* | *96* | *104* | | *111* | *29.139* | *1.00* | *1.0000* |
| *2* | 200 | *381* | *125* | *116* | *116* | | | | |
| 3 | 200 | 342 | 68 | 70 | 82 | 73 | 21.473 | 0.6583 | 0.7369 |
| 4 | 200 | 314 | 53 | 58 | 51 | 54 | 17.225 | 0.4847 | 0.5911 |
| 5 | 200 | 333 | 57 | 72 | 64 | 64 | 19.302 | 0.5775 | 0.6624 |
| 6 | 200 | 258 | 60 | 59 | 51 | 57 | 21.924 | 0.5087 | 0.7524 |
| 7 | 200 | 269 | 51 | 51 | 57 | 53 | 19.707 | 0.4758 | 0.6763 |
| 8 | 200 | 273 | 62 | 60 | 68 | 63 | 23.241 | 0.5685 | 0.7976 |
| 9 | 200 | 251 | 72 | 60 | 48 | 60 | 23.941 | 0.5386 | 0.8216 |
| 10 | 200 | 251 | 52 | 59 | 57 | 56 | 22.282 | 0.5027 | 0.7647 |

# CoulterSurvival

Experiment: Feb., 05, 2001
Date/Time: Feb., 05, 2001

| Tube # | Coulter count 1st | 2nd | 3rd | Average | Cells/ml | Hemocytometer Count in Grid 1st | 2nd | 3rd | 4th |
|---|---|---|---|---|---|---|---|---|---|
| *1* | *9086* | *8946* | *9076* | *9036* | *3609200* | | | | |
| *2* | *8917* | *8917* | *8867* | *8900* | *3554933* | | | | |
| 3 | 8917 | 9114 | 9002 | 9011 | 3599200 | | | | |
| 4 | 9448 | 9392 | 9363 | 9401 | 3755200 | | | | |
| 5 | 8884 | 8632 | 8699 | 8738 | 3490133 | | | | |
| 6 | 8078 | 8171 | 8017 | 8089 | 3230267 | | | | |
| 7 | 7868 | 7929 | 7830 | 7876 | 3145067 | | | | |
| 8 | 8354 | 8632 | 8271 | 8419 | 3362400 | | | | |
| 9 | 8760 | 8515 | 8415 | 8563 | 3420133 | | | | |
| 10 | 6490 | 6666 | 6773 | 6643 | 2652000 | | | | |

| Tube # | Predicted # Cells Seeded | Actual # Cells Seeded | Colony count 1st | 2nd | 3rd | Average | PE (%) | SF Uncorrected | SF Corrected |
|---|---|---|---|---|---|---|---|---|---|
| *1* | 200 | *361* | *80* | *102* | *105* | *102* | *28.522* | *1.00* | *1.0000* |
| *2* | 200 | *355* | *120* | *105* | *101* | | | | |
| 3 | 200 | 360 | 53 | 72 | 82 | 69 | 19.171 | 0.6754 | 0.6722 |
| 4 | 200 | 376 | 88 | 92 | 87 | 89 | 23.700 | 0.8711 | 0.8310 |
| 5 | 200 | 349 | 50 | 64 | 64 | 59 | 17.000 | 0.5808 | 0.5960 |
| 6 | 200 | 323 | 67 | 59 | 62 | 63 | 19.400 | 0.6134 | 0.6802 |
| 7 | 200 | 315 | 38 | 45 | 43 | 42 | 13.354 | 0.4111 | 0.4682 |
| 8 | 200 | 336 | 92 | 83 | 81 | 85 | 25.379 | 0.8352 | 0.8898 |
| 9 | 200 | 342 | 52 | 42 | 46 | 47 | 13.645 | 0.4568 | 0.4784 |
| 10 | 200 | 265 | 71 | 60 | 55 | 62 | 23.379 | 0.6069 | 0.8197 |

CoulterSurvival

Experiment: Jan., 15, 2001
Date/Time: Jan., 15, 2001

| Tube # | Coulter count 1st | 2nd | 3rd | Average | Cells/ml | Hemocytometer Count in Grid 1st | 2nd | 3rd | 4th |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 8671 | 8454 | 8777 | 8634 | 3448400 | | | | |
| 2 | 8598 | 8337 | 8448 | 8461 | 3379200 | | | | |
| 3 | 6693 | 6596 | 6618 | 6636 | 2649067 | | | | |
| 4 | 5301 | 5609 | 5520 | 5477 | 2185467 | | | | |
| 5 | 2225 | 2699 | 2464 | 2463 | 979867 | | | | |
| 6 | 6458 | 6859 | 6964 | 6760 | 2698933 | | | | |
| 7 | 6002 | 6437 | 6317 | 6252 | 2495600 | | | | |
| 8 | 9216 | 9905 | 9345 | 9489 | 3790267 | | | | |
| 9 | 7031 | 7112 | 7182 | 7108 | 2838133 | | | | |
| 10 | 6309 | 6002 | 6007 | 6106 | 2437200 | | | | |

| Tube # | Predicted # Cells Seeded | Actual # Cells Seeded | Colony count 1st | 2nd | 3rd | Average | PE (%) | SF Uncorrected | SF Corrected |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 200 | 345 | 121 | 75 | 133 | 128 | 37.495 | 1.00 | 1.0000 |
| 2 | 200 | 338 | 159 | 136 | 144 | | | | |
| 3 | 200 | 265 | 87 | 81 | 52 | 73 | 27.683 | 0.5729 | 0.7383 |
| 4 | 200 | 219 | 83 | 85 | 87 | 85 | 38.893 | 0.6641 | 1.0373 |
| 5 | 200 | 98 | 70 | | | 70 | 71.438 | 0.5469 | 1.9053 |
| 6 | 200 | 270 | 80 | 78 | | 79 | 29.271 | 0.6172 | 0.7807 |
| 7 | 200 | 250 | 55 | 70 | 70 | 65 | 26.046 | 0.5078 | 0.6947 |
| 8 | 200 | 379 | 118 | 118 | 135 | 124 | 32.627 | 0.9661 | 0.8702 |
| 9 | 200 | 284 | 81 | 90 | | 86 | 30.125 | 0.6680 | 0.8035 |
| 10 | 2000 | 2437 | 131 | 123 | 137 | 130 | 5.348 | 0.1018 | 0.1426 |

CoulterSurvival

Experiment: Dec., 26, 2000
Date/Time: Dec., 26, 2000

| Tube # | Coulter count 1st | 2nd | 3rd | Average | Cells/ml | Hemocytometer Count in Grid 1st | 2nd | 3rd | 4th |
|---|---|---|---|---|---|---|---|---|---|
| *1* | *2780* | *2723* | *2534* | *2679* | *1070800* | | | | |
| *2* | *2780* | *2723* | *2534* | *2679* | *1070800* | | | | |
| 3 | 2807 | 2830 | 2783 | 2807 | 1121867 | | | | |
| 4 | 2653 | 2686 | 2607 | 2649 | 1058667 | | | | |
| 5 | 2891 | 2883 | 2822 | 2865 | 1145333 | | | | |
| 6 | 2428 | 2515 | 2576 | 2506 | 1001733 | | | | |
| 7 | 2773 | 2728 | 2783 | 2761 | 1103733 | | | | |
| 8 | 3192 | 3252 | 3131 | 3192 | 1275867 | | | | |
| 9 | 2878 | 2891 | 2747 | 2839 | 1134667 | | | | |
| 10 | 2926 | 2949 | 2908 | 2928 | 1170267 | | | | |

| Tube # | Predicted # Cells Seeded | Actual # Cells Seeded | Colony count 1st | 2nd | 3rd | Average | PE (%) | SF Uncorrected | SF Corrected |
|---|---|---|---|---|---|---|---|---|---|
| *1* | 200 | *107* | *84* | *83* | *88* | *81* | *75.177* | *1.00* | *1.0000* |
| 2 | 200 | *107* | 72 | *80* | 76 | | | | |
| 3 | 200 | 112 | 72 | 73 | 67 | 71 | 62.990 | 0.8778 | 0.8379 |
| 4 | 200 | 106 | 68 | 57 | 63 | 63 | 59.194 | 0.7785 | 0.7874 |
| 5 | 200 | 115 | 92 | 68 | 74 | 78 | 68.102 | 0.9689 | 0.9059 |
| 6 | 200 | 100 | 17 | 21 | 30 | 23 | 22.627 | 0.2816 | 0.3010 |
| 7 | 200 | 110 | 59 | 43 | 53 | 52 | 46.811 | 0.6418 | 0.6227 |
| 8 | 200 | 128 | | | | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 9 | 200 | 113 | 58 | 42 | 45 | 48 | 42.597 | 0.6004 | 0.5666 |
| 10 | 200 | 117 | 64 | 68 | 81 | 71 | 60.670 | 0.8820 | 0.8070 |

# RAW DATA

C's 50% Experiments

exp. # V79, HTaR,

**TABLE-3**

**Expt. # :** 4 6/29/01 **Date/Time :** July 2, 2001

| Tube # | Coulter count for 100 ul cell suspension | Avg. count | Cells/ml [Avg. count x 400 - Bckgr | pCi/cell [uCi/ml x $10^6$ Cells/ml] |
|---|---|---|---|---|
| 1 | 9779, 9910, 9659 | | 3 908 666 | 0,51 |
| 2 | 9996, 10014, 10072 | | 4 006 533 | 0,50 |
| 3 | —NO SAMPLE → | | | → |
| 4 | —NO SAMPLE → | | | → |
| 5 | 9369, 9064, 8934 | | 3 644 533 | 0,56 |
| 6 | 9687, 9505, 9647 | | 3840 800 | 0,53 |
| 7 | 9080, 9335, 9210 | | 3 678 933 | 0,54 |
| 8 | 9613, 9596, 9431 | | 3 814 267 | 0,53 |
| 9 | 8609, 8538, 8421 | | 3 412 667 | 0,59 |
| 10 | 8744, 8699, 8716 | | 3 483 467 | 0,57 |

Bckgr - 11
Media - 500 µl

↑
$2 \times 10^6$/T75 flask for HPRT expression (day 0)

7/9/01



| Tube | Dilution | Colony Counts |
|---|---|---|
| 1 | 200 | 180, 182, 207 |
| 2 | 200 | 178, 134, 179 |
| | | |
| | | |
| 5 | 200 | 125, 109, 133 |
| 6 | 200 | 81, 81, 91 |
| 7 | 200 | 113, 121, 105 |
| 8 | 200 | 117, 100, 104 |
| 9 | 200 | 113, 89, 112 |
| 10 | 200 | 72, 78, 72 |
| | | |
| | | |
| | | |
| | | |

Exp. 3 5/7/2001

Coulter Counts (100Hl cells, Coulter set at 500Hl)

Mutagenesis T75 + 12.5ml + below

| | | | |
|---|---|---|---|
| 1) | 8985, 8822, 8917 | $3.56 \times 10^6$/ml | 0.56ml for $2 \times 10^6$ |
| 2) | 9664, 9567, 9312 | $3.81 \times 10^6$/ml | 0.53 ml for $2 \times 10^6$ |
| 3) | 9476, 9505, 9323 | $3.77 \times 10^6$/ml | 0.53 ml for $2 \times 10^6$ |
| 4) | 7770, 7907, 7819 | $3.13 \times 10^6$/ml | 0.64 ml |
| 5) | 7901, 7830, 7715 | $3.13 \times 10^6$/ml | 0.64 ml |
| 6) | 7279, 7241, 7095 | $2.88 \times 10^6$/ml | 0.69 ml |
| 7) | 7231, 7150, 7128 | $2.87 \times 10^6$/ml | 0.70 ml |
| 8) | 7590, 6689, 7508, 7655 | $3.03 \times 10^6$/ml | 0.66 ml |
| 9) | 6853, 6826, 6447 | $2.68 \times 10^6$/ml | 0.75 ml |
| 10) | 7188, 6886, 6972 | $2.81 \times 10^6$/ml | 0.71 ml |

Background - 8 counts

Mode - 500 µl.

32. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
33. Centrifuge tubes for 10 min at 2000 rpm, 4°C
34. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
35. Centrifuge tubes for 10 min at 2000 rpm, 4°C
36. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 5 cc syringe with 21 gauge needle
37. Determine cell concentration by transferring 100 µl to Coulter cup
38. Vortex tube, transfer 0.5 ml into dilution tube X.5, vortex tube X.5, transfer 0.5 ml into dilution tube X.4, vortex tube X.4 and transfer 0.5 ml to tube X.3, vortex tube X.3 and transfer 0.5 ml to tube X.2 and vortex. Keep tubes on ice.
39. Transfer 1 ml from dilution tubes into dishes labeled X.2, X.3, X.4 (in triplicate). Only X.2 should be seeded for control T-tubes.
40. Transfer 200 µl of cell suspension (in triplicate) to ~~20~~ 7 ml scintillation vial containing 6 ml cocktail (Aquasol)
41. Incubate petri dishes for 1 week
42. Count vials for radioactivity **Date/Time :**
43. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
44. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.

| Tube | Dilution | Colony Counts |
|---|---|---|
| 1 | 1.2 | 161, 143, 123 |
| 2 | 2.2 | 132, 141, 124 |
| 3 | 3.2 | 88, 69, 70 |
| 4 | 4.2 | 77, 65, 55 |
| 5 | 5.2 | 72, 71, ~~80~~ |
| 6 | 6.2 | 62, 73, 58 |
| 7 | 7.2 | 73, 80, 78 |
| 8 | 8.2 | 89, 76, 85 |
| 9 | 9.2 | 58, 74, 85 |
| 10 | 10.2 | 83, 90, 71 |
| | | |
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| | | |
| | | |

Exp. #2 4/23/2001

Coulter Counts after resuspending cluster
500Ml Coulter setting, 100Ml cells in 20ml Estore

1) 7967, 7945, 8022
2) 7404, 7497, 7513
3) 8321, 8426, 8221
4) 7644, 7486, 7622
5) 7535, 7595, 7432
6) 7009, 7128, 7026
7) 7540, 7458, 7458
8) 7117, 7107, 7177
9) 7204, ~~6821~~, 7236, 7144
10) 6896, 6826, 6875

suspensions in 12 ml tubes

30. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***
31. Labeling and preparation of dilution tubes and colony dishes
    - load 66, 60 mm petri dishes with 4 ml MEMA
    - load 40 sterile tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2, X.3, X.4, X.5 etc.
32. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
33. Centrifuge tubes for 10 min at 2000 rpm, 4°C
34. ~~Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA~~
35. ~~Centrifuge tubes for 10 min at 2000 rpm, 4°C~~
36. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
37. Determine cell concentration by transfering 100 µl to Coulter cup
38. Vortex tube, transfer 0.5 ml into dilusion tube X.5, vortex tube X.5, transfer 0.5 ml into dilution tube X.4, vortex tube X.4 and transfer 0.5 ml to tube X.3, vortex tube X.3 and transfer 0.5 ml to tube X.2 and vortex. Keep tubes on ice.
39. Transfer 1 ml from dilution tubes into dishes labeled X.2, X.3, X.4 (in triplicate). Only X.2 should be seeded for control T-tubes.
40. Transfer 200 µl of cell suspension (in triplicate) to 20 ml scintillation vial containing 6 ml cocktail (Aquasol)
41. Incubate petridishes for 1 week
42. Count vials for radioactivity **Date/Time :**
43. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
44. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.

| | |
|---|---|
| 1.2 | 92, 111, 119 |
| 2.2 | 78, 85, 74 |
| 3.2 | 142, 126, 120 |
| 4.2 | 120, 129, 121 |
| 5.2 | 64, 68, 79 |
| 6.2 | 92, 101, 78 |
| 7.2 | 74, 62, 94 |
| 8.2 | 89, 69, 67 |
| 9.2 | 85, 87, 97 |
| 10.2 | 71, 58, 55 |