# V79 COLONY FORMING ASSAY

**Experiment Name :** $^3$HTdR toxicity (cluster, 50% labeling) **Exp. # :** 4

**Investigator:** R Howell **Date:** 6/28/01

**Serum/Lot #s:** Gibco Lot# 1023298 for MEMA and MEMB

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 ~2,000,000 cells/ml in MEMB [Actual count : $2.20 \times 10^6$ cells/ml)

   2584, 2507, 2602 } 100 ml cells, 100 ml Coulter Setting — $5.13 \times 10^6$/ml

   18 ml = ? $\frac{36 \times 10^6}{5.13 \times 10^6}$ = 7.0 ml cells + 11 ml MEMB

   5562, 5411, 5562 } 2.20×
3. Transfer 1 ml of cell suspension into 20 14 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:** 2:00pm
5. Prepare MEMB containing radioactivity in hood

   µl $^3$HTdR (Stock : 1 µCi/µl on 5/30/01 ) + ml MEMB

   Manufacturer: NEN NET-027Z Lot #: 3106-427 Calibration:
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. Also add 1 ml MEMB to each of second set of tubes.

| Tube # | $^3$HTdR µCi/ml | Cells in MEMB (ml) | MEMB (ml) | MEMB+ $^3$HTdR [160µCi/ml] (ml) |
|---|---|---|---|---|
| 1 | 0 | 1.0 | 1.0 | 0 |
| 2 | 0 | 1.0 | 1.0 | 0 |
| 3 | | | | |
| 4 | | | | |
| 5 | 10 | 1.0 | 0.975 | 0.125 |
| 6 | 20 | 1.0 | 0.75 | 0.25 |
| 7 | 30 | 1.0 | 0.625 | 0.375 |
| 8 | 40 | 1.0 | 0.5 | 0.5 |
| 9 | 60 | 1.0 | 0.25 | 0.75 |
| 10 | 80 | 1.0 | 0 | 1.0 |

3ml

Didn't have this much. Had 350-400 ml. Rinsed stock vial to get all of it. Concentrations will be lower than expected.

3.2 ml at 160 µCi/ml

512 µl H-3

7. Return test tubes to roller for 12-14 h. **Date/Time:** 06/28/01; 6-00pm
8. Next day, while test tubes are in roller label 10 gamma-tubes (13 X 100 mm VWR glass test 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:** 8:00 am 6/29/01
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, 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 of wash MEMA~~
16. ~~Centrifuge tubes for 10 min at 2000 rpm, 4°C~~
17. Decant supernatant, click tubes, vortex, resuspend unlabeled cells in 5 ml MEMA.
18. Transfer unlabeled cells to the corresponding tubes containing 2,000,000 labeled cells.
19. Wash unlabeled cell tube with 5 ml MEMA and transfer to corresponding labeled cell tube.
20. ~~Syringe the pooled cells 5 times with 5 ml syringe with 21 G needle.~~
21. Centrifuge tubes for 10 min at 2000 rpm, 4°C.
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 ice cold 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. (Labline shaker w/o shaking) **Date/Time:** 10:10 am 6/29/01
27. Transfer 10 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:** 10:00 am 6/29/01

30

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:** 10:10 am 7/2/01
29. Again add 200 •l 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. 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 (Ecolume)
41. Incubate tissue culture dishes for 1 week
42. Count vials for radioactivity **Date/Time:** 7/3/01 17:02
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.

→ Transfer $2 \times 10^6$ cells to T75 flask w/ 12.5 ml MEMA (same lot) for HPRT expression

7/9/01 RWH



| 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 |
| | | |
| | | |
| | | |
| | | |

Roger's exp. # V79, HTdR,



**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, 8598, 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)

Exp-4 R Howell



USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 TUE 03 JUL 2001 17:02
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
H[illegible] 1 ADC:N DCF:N RCM:N
CHANNEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:@ 1.00000
HALF LIFE(DAYS):N

| SAM | POS | CH | CPM | 2SIG% | TIME | EL TIME | AVG H# | ERR | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | **- 1 | 1 | 12.00 | 57.74 | 1.00 | 1.48 | 86.0 | | 1M |
| 2 | **- 2 | 1 | 13.00 | 55.47 | 1.00 | 3.06 | 85.0 | | |
| 3 | **- 3 | 1 | 11.00 | 60.30 | 1.00 | 4.63 | 85.0 | | |
| 4 | **- 4 | 1 | 16.00 | 50.00 | 1.00 | 6.21 | 85.0 | | 2M |
| 5 | **- 5 | 1 | 11.00 | 60.30 | 1.00 | 7.78 | 85.0 | | |
| 6 | %**- 6 | 1 | 13.00 | 55.47 | 1.00 | 9.34 | 84.0 | | |
| 7 | **- 7 | 1 | 184813.33 | 1.20 | 0.15 | 10.05 | 86.0 | | 5M |
| 8 | **- 8 | 1 | 186820.00 | 1.19 | 0.15 | 10.77 | 84.0 | | |
| 9 | **- 9 | 1 | 182226.66 | 1.21 | 0.15 | 11.48 | 85.0 | | |
| 10 | **-10 | 1 | 362040.00 | 1.05 | 0.10 | 12.20 | 84.0 | | 6M |
| 11 | **-11 | 1 | 355233.31 | 0.87 | 0.15 | 12.93 | 86.0 | | |
| 12 | **-12 | 1 | 357940.00 | 0.86 | 0.15 | 13.65 | 85.0 | | |
| 13 | **-13 | 1 | 550046.62 | 0.70 | 0.15 | 14.39 | 86.0 | | 7M |
| 14 | **-14 | 1 | 541413.31 | 0.70 | 0.15 | 15.13 | 85.0 | | |
| 15 | **-15 | 1 | 543220.00 | 0.70 | 0.15 | 15.88 | 85.0 | | |
| 16 | **-16 | 1 | 718720.00 | 0.61 | 0.15 | 16.63 | 84.0 | | 8M |
| 17 | **-17 | 1 | 723886.62 | 0.61 | 0.15 | 17.39 | 84.0 | | |
| [illegible] | **-18 | 1 | 724306.62 | 0.61 | 0.15 | 18.15 | 85.0 | | |
| [illegible] | **- 1 | 1 | 1086426.62 | 0.50 | 0.15 | 18.99 | 85.0 | | 9M |
| 20 | **- 2 | 1 | 1101986.62 | 0.49 | 0.15 | 19.78 | 84.0 | | |
| 21 | **- 3 | 1 | 1099560.00 | 0.49 | 0.15 | 20.58 | 85.0 | | |
| 22 | **- 4 | 1 | 1445173.25 | 0.43 | 0.15 | 21.39 | 84.0 | | 10M |
| 23 | **- 5 | 1 | 1426913.25 | 0.43 | 0.15 | 22.22 | 83.0 | | |
| 24 | **- 6 | 1 | 1413553.25 | 0.43 | 0.15 | 23.04 | 85.0 | | |
| 25 | **- 7 | 1 | 11.00 | 60.30 | 1.00 | 24.67 | 102.0 | | 1C |
| 26 | **- 8 | 1 | 10.00 | 63.25 | 1.00 | 26.23 | 101.0 | | |
| 27 | **- 9 | 1 | 6.00 | 81.65 | 1.00 | 27.80 | 103.0 | | |
| 28 | **-10 | 1 | 5.00 | 89.44 | 1.00 | 29.41 | 102.0 | | 2C |
| 29 | **-11 | 1 | 7.00 | 75.59 | 1.00 | 30.97 | 101.0 | | |
| 30 | **-12 | 1 | 12.00 | 57.74 | 1.00 | 32.53 | 103.0 | | |
| 31 | **-13 | 1 | 22020.00 | 1.91 | 0.50 | 33.59 | 104.0 | | 5C |
| 32 | **-14 | 1 | 24328.89 | 1.91 | 0.45 | 34.60 | 102.0 | | |
| 33 | **-15 | 1 | 24695.55 | 1.90 | 0.45 | 35.61 | 104.0 | | |
| 34 | **-16 | 1 | 51160.00 | 1.98 | 0.20 | 36.36 | 104.0 | | 6C |
| 35 | **-17 | 1 | 51735.00 | 1.97 | 0.20 | 37.11 | 105.0 | | |
| 36 | **-18 | 1 | 50395.00 | 1.99 | 0.20 | 37.92 | 103.0 | | |
| 37 | **- 1 | 1 | 70573.33 | 1.94 | 0.15 | 38.67 | 102.0 | | 7C |
| 38 | **- 2 | 1 | 72293.33 | 1.92 | 0.15 | 39.37 | 102.0 | | |
| 39 | **- 3 | 1 | 62770.00 | 1.79 | 0.20 | 40.12 | 103.0 | | |
| 40 | **- 4 | 1 | 98573.33 | 1.64 | 0.15 | 40.82 | 102.0 | | 8C |
| 41 | **- 5 | 1 | 94066.66 | 1.68 | 0.15 | 41.52 | 103.0 | | |
| 42 | **- 6 | 1 | 88240.00 | 1.74 | 0.15 | 42.23 | 102.0 | | |
| 43 | **- 7 | 1 | 136593.33 | 1.40 | 0.15 | 42.95 | 103.0 | | 9C |
| [illegible] | **- 8 | 1 | 145240.00 | 1.36 | 0.15 | 43.66 | 105.0 | | |
| 45 | **- 9 | 1 | 145073.33 | 1.36 | 0.15 | 44.37 | 103.0 | | |
| 46 | **-10 | 1 | 187586.66 | 1.19 | 0.15 | 45.07 | 103.0 | | |

30 ML medium

200 ML cells

| SAM | POS | CH | CPM | 2SIG% | TIME | EL TIME | AVG H# | ERR |
|---|---|---|---|---|---|---|---|---|
| [redacted] | **-11 | 1 | 183713.33 | 1.20 | 0.15 | 45.79 | 104.0 | 10C |
| [redacted] | **-12 | 1 | 185440.00 | 1.20 | 0.15 | 46.51 | 104.0 | |
| 49 | **-13 | 1 | 28762.86 | 1.99 | 0.35 | 47.47 | 0.0 | |

Parameters

| | | | |
|---|---|---|---|
| Date | 6/28/2001 | | |
| Experiment No. | 4 | | |
| Investigator | R. Howell | | |
| Cell Line | V79 - Anupam's 10/00 freeze | | |
| Modifier | None | | |
| Radionuclide | H-3 | | |
| Half-life (days) | 4500.45 | I-125=59.408, H-3=4500.45, Po-210=138.376, I-131=8.02 | |
| Radiation Yield | 1 | I-125=1.47, H-3=1.0, Po-210=1.0, I-131=8.02 | |
| Radiochemical | 3HTdR | | |
| Manufacturer/Lot | NEN NET-027Z Lot 3106-427 | | |
| Original Calibration Date/Time | 5/30/2001 12:00 | Original Activity Concentration (MBq/ml) | 37 |
| Present Calibration Date/Time | 6/28/2001 0:00 | Time Elapsed Since Original Calibration (d) | 9 |
| Fraction of Cells Labeled | 0.5 | Present Activity Concentration (MBq/ml) | 36.95 |
| Liquid Scintillation Cocktail | Ecoscint | | |
| Volume of LSC Cocktail (ml) | 6 | | |
| Volume/Type Counting Vial | 7 ml | | |
| Model of Counter | Beckman | | |
| Counting Efficiency | 0.65 | | |
| Activity Added (Date/Time) | 6/28/2001 18:00 | | |
| Cells Washed (Date/Time) | 6/29/2001 8:00 | Time Elapsed Between Add and Wash (hr) | 14.00 |
| Medium Tubes Counted (Date/Time) | 7/3/2001 17:02 | Time Elapsed Between Add and Count (hr) | 59.00 |
| Cell Tubes Counted (Date/Time) | 7/3/2001 17:02 | Time Elapsed Between Wash and Count (hr) | 59.00 |
| Vol. Supernatant Counted (µl) | 30 | | |
| Vol. Suspension Counted Cell Activity (µl) | 200 | | |
| Vol. Suspension Coulter (µl) | 100 | | |
| Coulter Manometer Volume (µl) | 500 | | |
| Average Coulter Background Counts | 11 | | |
| Coulter Calibration Parameter | 400 | | |
| Hemocytometer Counting (Yes or No)? | No | | |

| Background | | |
|---|---|---|
| Coulter 1 | Coulter 2 | Coulter 3 |
| 11 | 11 | 11 |

MediumActivity

Experiment: 4
Date: 6/28/2001

| Tube # | Medium count (CPM) 1st | 2nd | 3rd | CPM Average | CPM corrected for control | DPM CPM/(y e) | At µCi/ml on counting | Ao µCi/ml at addition [At/e-0.693t/T] | Ao kBq/ml at addition |
|---|---|---|---|---|---|---|---|---|---|
| *1* | *12* | *13* | *11* | *13* | *0* | *0* | *0* | *0* | *0* |
| *2* | *16* | *11* | *13* | | *0* | *0* | *0* | *0* | *0* |
| 3 | | | | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 4 | | | | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 5 | 184813 | 186820 | 182226 | 184620 | 184607 | 284011 | 4.2644 | 4.2660 | 157.8435 |
| 6 | 362040 | 355233 | 357940 | 358404 | 358392 | 551372 | 8.2789 | 8.2820 | 306.4336 |
| 7 | 550046 | 541413 | 543220 | 544893 | 544880 | 838277 | 12.5867 | 12.5915 | 465.8860 |
| 8 | 718720 | 723886 | 724306 | 722304 | 722291 | 1111217 | 16.6849 | 16.6913 | 617.5768 |
| 9 | 1086426 | 1101986 | 1099560 | 1095991 | 1095978 | 1686120 | 25.3171 | 25.3267 | 937.0880 |
| 10 | 1445173 | 1426913 | 1413553 | 1428546 | 1428534 | 2197744 | 32.9992 | 33.0117 | 1221.4312 |

CellSuspension

Experiment: 4
Date: 06/28/01

| Tube # | Suspension count (CPM) 1st | 2nd | 3rd | CPM Average | CPM corrected for control | DPM CPM/(y e) | $A_t$ µCi/ml on counting | $A_o$ µCi/ml after uptake | $A_o$ kBq/ml after uptake |
|---|---|---|---|---|---|---|---|---|---|
| *1* | *11* | *10* | *6* | *9* | *0* | *0* | *0.00000* | *0* | *0.0000* |
| *2* | *5* | *7* | *12* | | *0* | *0* | *0.00000* | *0* | *0.0000* |
| 3 | | | | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 4 | | | | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 5 | 22020 | 24328 | 24695 | 23681 | 23673 | 36419 | 0.08203 | 0.08206 | 3.0361 |
| 6 | 51160 | 51735 | 50395 | 51097 | 51088 | 78597 | 0.17702 | 0.17709 | 6.5522 |
| 7 | 70573 | 72293 | 62770 | 68545 | 68537 | 105441 | 0.23748 | 0.23757 | 8.7901 |
| 8 | 98573 | 94066 | 88240 | 93626 | 93618 | 144027 | 0.32439 | 0.32451 | 12.0068 |
| 9 | 136593 | 145240 | 145073 | 142302 | 142294 | 218913 | 0.49305 | 0.49323 | 18.2497 |
| 10 | 187586 | 183713 | 185440 | 185580 | 185571 | 285494 | 0.64300 | 0.64325 | 23.8002 |

riment: 4
e/Time: ######

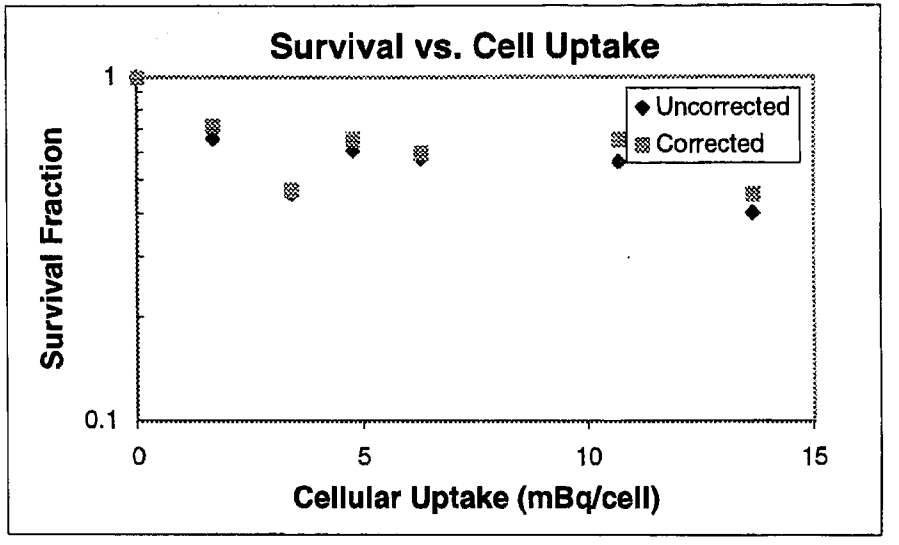
| Tube # | Coulter count 1st | 2nd | 3rd | Average | Cells/ml | Hemocytometer Count in Grid 1st | 2nd | 3rd | 4th |
|---|---|---|---|---|---|---|---|---|---|
| *1* | *9779* | *9910* | *9659* | *9783* | *3908667* | | | | |
| *2* | *9996* | *10014* | *10072* | *10027* | *4006533* | | | | |
| 3 | | | | #DIV/0! | #DIV/0! | | | | |
| 4 | | | | #DIV/0! | #DIV/0! | | | | |
| 5 | 9369 | 9004 | 8934 | 9102 | 3636533 | | | | |
| 6 | 9687 | 9505 | 9647 | 9613 | 3840800 | | | | |
| 7 | 9080 | 9335 | 9210 | 9208 | 3678933 | | | | |
| 8 | 9613 | 9596 | 9431 | 9547 | 3814267 | | | | |
| 9 | 8609 | 8598 | 8421 | 8543 | 3412667 | | | | |
| 10 | 8744 | 8699 | 8716 | 8720 | 3483467 | | | | |

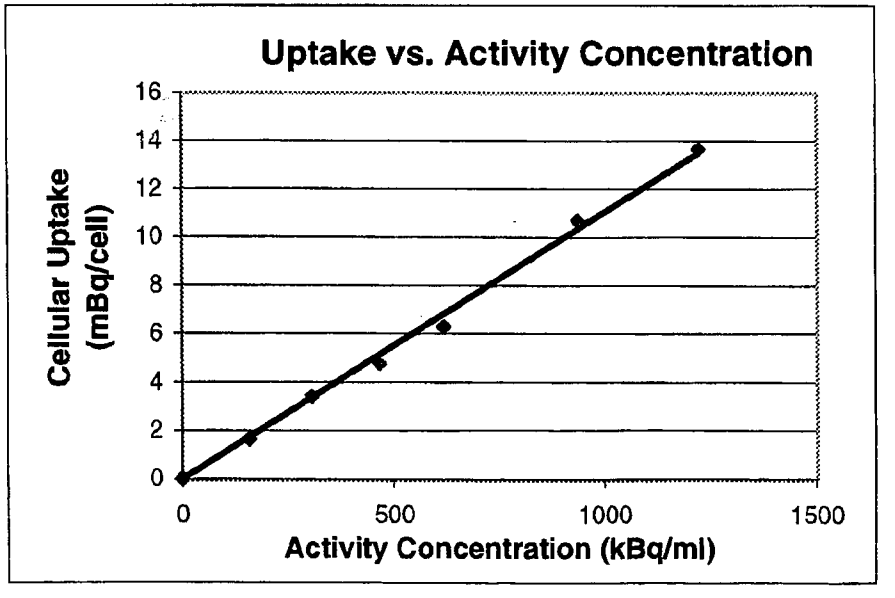
| Tube # | Predicted # Cells Seeded | Actual # Cells Seeded | Colony count 1st | 2nd | 3rd | Average | PE (%) | SF Uncorrected | SF Corrected |
|---|---|---|---|---|---|---|---|---|---|
| *1* | 200 | *391* | *180* | *182* | *207* | *185* | *46.796* | *1.00* | *1.0000* |
| *2* | 200 | *401* | *178* | *179* | | | | | |
| 3 | 200 | #DIV/0! | | | | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 4 | 200 | #DIV/0! | | | | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 5 | 200 | 364 | 125 | 109 | 133 | 122 | 33.640 | 0.6605 | 0.7189 |
| 6 | 200 | 384 | 81 | 81 | 91 | 84 | 21.957 | 0.4554 | 0.4692 |
| 7 | 200 | 368 | 113 | 121 | 105 | 113 | 30.715 | 0.6102 | 0.6564 |
| 8 | 200 | 381 | 117 | 100 | 104 | 107 | 28.053 | 0.5778 | 0.5995 |
| 9 | 200 | 341 | 113 | 89 | 112 | 105 | 30.670 | 0.5652 | 0.6554 |
| 10 | 200 | 348 | 72 | 78 | 72 | 74 | 21.243 | 0.3996 | 0.4540 |

Summary

Experiment: 4
Date/Time: 6/28/2001

| Tube # | Activity Conc. (kBq/ml) | Activity/Cell (mBq/cell) | Survival Uncorrected | Survival Corrected |
|---|---|---|---|---|
| *1* | *0.000* | *0.000* | *1.0000* | *1.0000* |
| *2* | *0.000* | *0.000* | | |
| 3 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 4 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 5 | 157.844 | 1.670 | 0.6605 | 0.7189 |
| 6 | 306.434 | 3.412 | 0.4554 | 0.4692 |
| 7 | 465.886 | 4.779 | 0.6102 | 0.6564 |
| 8 | 617.577 | 6.296 | 0.5778 | 0.5995 |
| 9 | 937.088 | 10.695 | 0.5652 | 0.6554 |
| 10 | 1221.431 | 13.665 | 0.3996 | 0.4540 |




B007430

7/31/01

# Scoring of Plating Efficiency for mutation study

## Exp # 4

| | | | | |
|---|---|---|---|---|
| 1 | 180 | 133 | 167 | |
| 2 | 140 | 140 | 179 | |
| 3 | | | | |
| 4 | | | | |
| 5 | 143 | 149 | 169 | |
| 6 | ~~140~~ | 180 | 175 | 162 |
| 7 | 141 | 147 | 162 | |
| 8 | 123 | 136 | 124 | |
| 9 | 112 | 140 | | |
| 10 | 106 | 105 | 105 | |

HPRT expression → HPRT selection

Fix, count ~ 27 July

Eugen's exp #4, V79 HidR, 50%, Chairil

| Day | 0 | 3 | 6 | 9 | 11 | 13 | Chairil |
|---|---|---|---|---|---|---|---|
| Date | 7/2/15 | 7/8 | 7/11 | 7/13 | 7/15 | 7/17 | |

Colony Counts - Mutant Selection

Exp. 4

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 2 | 0 | 2 | 2 | 1 | 0 | 1 | 2 | 1 |
| 2 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | | | | | | | | | | |
| 4 | | | | | | | | | | |
| 5 | 1 | 2 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| 6 | 1 | 1 | 4 | 1 | 2 | 4 | 2 | 1 | 0 | 1 |
| 7 | 1 | 1 | 1 | 2 | 1 | 2 | 2 | 3 | 2 | |
| 8 | 3 | 3 | 4 | 3 | 0 | 0 | 0 | 2 | 0 | 4 |
| 9 | 0 | 2 | 3 | 0 | 1 | 2 | 2 | 6 | 3 | |
| 10 | 3 | 5 | 5 | 6 | 3 | 4 | 0 | 0 | 3 | |