# V79 COLONY FORMING ASSAY

**Experiment Name :** $^{210}$Po toxicity ($4x10^6$ cell cluster, 10% labeling); **Exp. # :** 1;
**Investigator:** A. Bishayee **Date:** 12/11/98

1. Set two 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 ~400,000 and 3,600,000 cells/ml in MEMB [Actual count: 620,000 and 3,369,333 cells/ml) 15.5%
3. Transfer 1 ml of cell suspension into ten 14 ml tubes (Falcon plastic test tube, 100x80 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:** 12/11/98; 2-00 p.m.
5. Calibrate the stock $^{210}$Po-citrate
6. After 3-4 h, remove first set of test tubes (400,000 cells/ml) from roller and add according to Table below.

**Date/Time:** 12/11/98; 4-00 p.m.

| Tube # | $^{210}$Po-citrate uCi/ml | Cells in MEMB (ml) | MEMB (ul) | Po-citrate (12 uCi/ml) (ul) |
|---|---|---|---|---|
| 1 | 0 | 1.0 | 1000 | 0 |
| 2 | 0 | 1.0 | 1000 | 0 |
| 3 | 0.1 | 1.0 | 985 | 15 |
| 4 | 0.2 | 1.0 | 965 | 35 |
| 5 | 0.3 | 1.0 | 950 | 50 |
| 6 | 0.5 | 1.0 | 915 | 85 |
| 7 | 0.8 | 1.0 | 865 | 135 |
| 8 | 1.0 | 1.0 | 835 | 165 |
| 9 | 1.2 | 1.0 | 800 | 200 |
| 10 | 1.5 | 1.0 | 750 | 250 |

7. Return test tubes to roller for 30 min. **Date/Time:** 12/11/98; 4-15 p.m.

8. After 30 min, centrifuge tubes for 10 min at 2000 rpm, 4°C **Date/Time:** 12/11/98; 4-45 p.m.
9. Collect 150 ul supernatant in separate tubes
10. Resuspend in 10 ml wash MEMA
11. Centrifuge tubes for 10 min at 2000 rpm, 4°C
12. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
13. Centrifuge tubes for 10 min at 2000 rpm, 4°C
14. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
15. Centrifuge tubes for 10 min at 2000 rpm, 4°C
16. Decant supernatant, click tubes, vortex
17. Follow steps 11-16 for second set of tubes containing 3,600,000 cells, suspend in 7 ml of MEMA and transfer cells to the corresponding tubes containing 400,000 cells in step 16
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 (onto a perforated Renin pipet box) at 10°C for 72 h. **Date/Time:** 12/11/98; 6-00 p.m.
23. Transfer 30 ul supernatant in three sets of vials containing 6ml liquid scintillation cocktail (Aquasol) from 150 ul supernatant removed earlier (Step 9) and count them for radioactivity **Date/Time:** 12/15/98; 12-00 noon
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:** 12/14/98; 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 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 test tubes.
36. Transfer 500 µl of cell suspension (in duplicate) to 20 ml scintillation vial containing 6 ml cocktail (Aquasol)
37. Incubate petridishes for 1 week
38. Count vials for radioactivity **Date/Time :** 12/14/98; 3-00 pm.
39. After 1 week, wash colonies 3 times with normal (1X) saline,and 2 times with methanol. Stain colonies with 0.0 5% crystal violet
40. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.

12/11/98

Initial Cell count = 1401, 1417, 1447

Avg. Cell count = 1421

Cell conc. = $1421 \times 4000$

= 5,686,666 cells/ml

Dilution A : 400,000 cells/ml ; 11 ml.

Vol. required = $\frac{4400000}{5,686,666}$ = 0·77 ml

Take 0·8 ml cells + 10·2 ml MEMB = 11 ml.

Final count = 164, 151, 150 ; Cell conc. 620,000 cells/ml

Dilution B : 3,600,000 cells/ml ; 11 ml

Vol. required = $\frac{39600000}{5686666}$ = 6·9 ml

Take 7ml cells + 4ml MEMB = 11 ml.

Final count = 852, 853, 822 ; Cell conc. = 3,369,333 cells/ml

1N HCl : 1N citrate

1ml HCl :
100µl HCl : 100µl citrate
: 200 "
500 "
1ml "

3ml X 2M = 6M X n

2M HCl : 1M Na-Citrate

| | |
|---|---|
| 1 : 1 | 3 |
| 1 : 2 | 4 |
| 1 : 3 | 5 |
| 1 : 4 | 6 |
| 1 : 5 | 6·8 ✓ |
| 1 : 9 | 7 |

1 : 5

4µci

100µci — 6ml

16µci/ml

300µl
1·5ml

3ml X 6M =

1·8ml

Stock 100µci/ml
1 : 5 ⇒ 16·6µci/ml.

1 : 5

① change ratio 2
② ~~low~~ higher survival 2
③

$^{210}$Po- toxicity, 10% labeling

Expt #1

Stock on 10/06/98 = 100 µCi/ml. 12/11/98

$A_t = A_0 \times e^{-\lambda t}$

$= 100 \times e^{-\frac{0.693 \times 65}{138}}$

$= 100 \times 0.72$

$= 72$ µCi/ml. on 12/11/98

After 1:5 dilution : of $^{210}$Po-citrate = 12 µCi/ml.

For 4 tubes: Take 1.5 ml 1M Na-citrate in 1.8 ml Nalgene Cryovial
Add 300 µl of stock Po-cit in 2M HCl) X 3

| | µCi/ml | Cells | MEMB (µl) | Po-citrate (12 µCi/ml) |
|---|---|---|---|---|
| 1 | 0 | 20 | 1000 | 0 |
| 2 | 0 | 20 | 1000 | 0 |
| 3 | 0.1 | 10 | 0.984 | 0.016 |
| 4 | 0.2 | 20 | 0.967 | 0.033 |
| 5 | 0.3 | 30 | 0.950 | 0.05 |
| 6 | 0.5 | 20 | 0.917 | 0.083 |
| 7 | 0.8 | ∞ | 0.867 | 0.133 |
| 8 | 1.0 | ∞ | 0.834 | 0.166 |
| 9 | 1.2 | ∞ | 0.8 | 0.2 |
| 10 | 1.5 | ∞ | 0.75 | 0.25 |

10 µ

210 Po tomicals (10% labeling)

USER: 5 ID:PO-210 PRESET MIN: 1.00 TUE 15 DEC 1998 12:19
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
H#: 1 ADC:N QCF:N RCN:N
CHANNEL 1-LL:600 UL: 900 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR:
DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:0 1.00000
HALF LIFE(DAYS):N

Expt #1

20 ml medium

| SAM | POS | CH | | CPM | 2SIG% | TIME | EL TIME | AVG H# | ERR |
|---|---|---|---|---|---|---|---|---|---|
| 1 | **- 1 | 1 | | 10.00 | 63.25 | 1.00 | 1.60 | 55.0 | |
| 2 | **- 2 | 1 | 1M | 2.00 | 141.4 | 1.00 | 3.28 | 56.0 | |
| 3 | **- 3 | 1 | | 12.00 | 57.74 | 1.00 | 4.97 | 55.0 | |
| 4 | **- 4 | 1 | 2M | 12.00 | 57.74 | 1.00 | 6.65 | 55.0 | |
| 5 | **- 5 | 1 | | 1916.00 | 4.57 | 1.00 | 8.33 | 54.0 | |
| 6 | **- 6 | 1 | 3M-G | 1808.00 | 4.70 | 1.00 | 10.01 | 55.0 | |
| 7 | **- 7 | 1 | | 1718.00 | 4.57 | 1.00 | 11.64 | 61.0 | |
| 8 | **- 8 | 1 | 3M-P | 1801.00 | 4.71 | 1.00 | 13.33 | 59.0 | |
| 9 | **- 9 | 1 | | 3424.00 | 3.42 | 1.00 | 15.00 | 56.0 | |
| 10 | **-10 | 1 | 4M | 3809.00 | 3.24 | 1.00 | 16.68 | 56.0 | |
| 11 | **-11 | 1 | | 5166.00 | 2.78 | 1.00 | 18.32 | 58.0 | |
| 12 | **-12 | 1 | 5M | 5912.00 | 2.60 | 1.00 | 20.00 | 56.0 | |
| 13 | **- 1 | 1 | | 14184.00 | 1.94 | 0.75 | 21.49 | 57.0 | |
| 14 | **- 2 | 1 | 6M ? | 26789.33* | 1.92 | 0.38 | 22.58 | 54.0 | |
| 15 | **- 3 | 1 | | 14722.76 | 1.94 | 0.72 | 24.03 | 56.0 | |
| 16 | **- 4 | 1 | 7M | 16667.69 | 1.92 | 0.65 | 25.36 | 54.0 | |
| 17 | **- 5 | 1 | | 53644.44 | 1.82 | 0.23 | 26.30 | 53.0 | |
| 18 | **- 6 | 1 | 8M | 66993.33 | 2.00 | 0.15 | 27.12 | 56.0 | |
| 19 | **- 7 | 1 | | 12517.58 | 1.97 | 0.82 | 28.67 | 55.0 | |
| 20 | **- 8 | 1 | 9M | 14182.67 | 1.94 | 0.75 | 30.10 | 55.0 | |
| 21 | **- 9 | 1 | | 91206.66 | 1.71 | 0.15 | 30.81 | 53.0 | |
| 22 | **-10 | 1 | 10M | 106085.72 | 1.47 | 0.17 | 31.71 | 54.0 | |

3M-G : 20 ml glass scintillation vial + 6 ml Aquasol

3M-P : 20 ml Plastic " " + " "

* possibly radioactivity added twice

~~(1) error in addition~~

++ error in addition

**TABLE-1**

**Expt. # :** ~~12/15/98~~ 1 **Date/Time :** 12/15/98; 12-20 P.M.

| Tube # | Medium count for ~~30~~ 20 µl (cpm) | Avg. cpm | dpm [cpm/1] | µ Ci/ml ($A_t$) on counting [dpm/~~66600~~ 44400] | µ Ci/ml ($A_o$) on addition [$A_t/e^{-\lambda t}$] |
|---|---|---|---|---|---|
| 1 | See the | | | | |
| 2 | attached Sheet | | | | |
| 3 | | 1859.5 | 1859.5 | 0.0418 | |
| 4 | | 36165 | 36165 | 0.0814 | |
| 5 | | 5539 | 5539 | 0.1247 | |
| 6 | | 14324 | 14324 | 0.3226 | |
| 7 | | 15694 | 15694 | 0.3534 | |
| 8 | | 60318 | 60318 | 1.35 | |
| 9 | | 13349 | 13349 | 0.300* | |
| 10 | | 98645 | 98645 | 2.22 | |

* error in addition

210Po toxicity (10% labeling)

Expt #01

F-451 | Rat/Howell

500µl cells.



USER: 3 ID:PO-210 PRESET TIME: 1.00 MON 14 DEC 1998 14:55
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
H#: 1 AGC:N DCF:N RCM:N
CHANNEL: 1-LL:400 UL: 900 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:Q 1.00000
HALF LIFE(DAYS):N

| SAM | POS | CH | | CPM | 2SIG% | TIME | EL TIME | AVG H# | ERR |
|---|---|---|---|---|---|---|---|---|---|
| 1 | **- 1 | 1 | 1c | 6.00 | 81.65 | 1.00 | 1.55 | 105.0 | |
| 2 | **- 2 | 1 | 2c | 7.00 | 48.51 | 1.00 | 3.27 | 108.0 | |
| 3 | **- 3 | 1 | 3c | 214.00 | 13.67 | 1.00 | 4.91 | 106.0 | |
| 4 | **- 4 | 1 | | 201.00 | 14.11 | 1.00 | 6.63 | 106.0 | |
| 5 | **- 5 | 1 | 4c | 173.00 | 15.21 | 1.00 | 8.37 | 109.0 | |
| 6 | **- 6 | 1 | | 152.00 | 16.22 | 1.00 | 10.04 | 107.0 | |
| 7 | **- 7 | 1 | 5c | 208.00 | 13.87 | 1.00 | 11.72 | 106.0 | |
| 8 | **- 8 | 1 | | 230.00 | 13.19 | 1.00 | 13.35 | 106.0 | |
| 9 | **- 9 | 1 | 6c | 309.00 | 11.36 | 1.00 | 15.03 | 106.0 | |
| 10 | **-10 | 1 | | 329.00 | 11.03 | 1.00 | 16.67 | 102.0 | |
| 11 | **-11 | 1 | 7c | 397.00 | 10.04 | 1.00 | 18.40 | 98.0 | |
| 12 | **-12 | 1 | | 403.00 | 9.96 | 1.00 | 20.08 | 97.0 | |
| 13 | **- 1 | 1 | 8c | 684.00 | 7.65 | 1.00 | 21.87 | 102.0 | |
| 14 | **- 2 | 1 | | 711.00 | 7.50 | 1.00 | 23.61 | 98.0 | |
| 15 | **- 3 | 1 | 9c | 371.00 | 10.38 | 1.00 | 25.24 | 98.0 | |
| 16 | **- 4 | 1 | | 396.00 | 10.05 | 1.00 | 26.87 | 100.0 | |
| 17 | **- 5 | 1 | 10c | 653.00 | 7.83 | 1.00 | 28.56 | 99.0 | |
| 18 | **- 6 | 1 | | 607.00 | 8.12 | 1.00 | 30.24 | 99.0 | |

**TABLE-2**

**Expt. # :** j **Date/Time :** 12/14/98; 3-00 p.m.

| Tube # | Radioactivity for 500 ul cell suspension (cpm) | Avg. cpm | dpm [cpm/1] | μ Ci/ml ($A_t$) on counting [dpm/111x$10^4$] | μ Ci/ml ($A_o$) after 12 h incubation [$A_t/e^{-\lambda t}$] |
|---|---|---|---|---|---|
| 1 | See the attached | | | | |
| 2 | Sheet | | | | |
| 3 | | 207.5 | 207.5 | 0.000186 | |
| 4 | | 162.5 | 162.5 | 0.000146 | |
| 5 | | 219 | 219 | 0.000197 | |
| 6 | | 319 | 319 | 0.000287 | |
| 7 | | 400 | 400 | 0.000360 | |
| 8 | | 698 | 698 | 0.000628 | |
| 9 | | 383.5 | 383.5 | 0.000345 | |
| 10 | | 630 | 630 | 0.000567 | |

**TABLE-3**

**Expt. # :** 1 **Date/Time :** 12/14/98

| Tube # | Coulter count for 100 ul cell suspension | Avg. count | Cells/ml [Avg. count x 4000] | fCi/cell [uCi/ml x$10^9$ Cells/ml] |
|---|---|---|---|---|
| 1 | 543, 516, 541 | 533 | 2133333 | – |
| 2 | 500, 502, 513 | 505 | 2020000 | – |
| 3 | 460, 481, 434 | 458 | 1833333 | 0·1014 |
| 4 | 531, 542, 504 | 525 | 2102666 | 0·0694 |
| 5 | 531, 493, 488 | 504 | 2016000 | 0·0977 |
| 6 | 542, 530, 518 | 530 | 2120000 | 0·1353 |
| 7 | 512, 473, 457 | 480 | 1922666 | 0·1872 |
| 8 | 514, 499, 487 | 500 | 2000000 | 0·314 |
| 9 | 454, 439, 444 | 445 | 1782666 | 0·1935 |
| 10 | 469, 490, 450 | 469 | 1878666 | 0·3018 |

**TABLE-4**

Expt. #: 1

Date: 12/21/98

## Colony Counts and Survival Fraction

| Tube.dilution | Colony 1 | Colony 2 | Colony 3 | Avg Colony | SF |
|---|---|---|---|---|---|
| 1.2 | 156 | 166 | 142 | 146.83 | — |
| 2.2 | 139 | 149 | 129 | | — |
| 3.2 | 137 | 130 | 123 | 130 | 0.8849 |
| 4.2 | 132 | 140 | 151 | 141 | 0.9598 |
| 5.2 | 124 | 125 | 129 | 125 | 0.8509 |
| 6.2 | 108 | 100 | 117 | 100.33 | 0.7374 |
| 7.2 | 83 | 91 | 99 | 91 | 0.6194 |
| 8.2 | 56 | 64 | 52 | 57.33 | 0.3902 |
| 9.2 | 76 | 70 | 82 | 76 | 0.5173 |
| 10. | 79 | 72 | 65 | 72 | 0.4901 |