

V79 COLONY FORMING ASSAY

Experiment Name : ^{210}Po -citrate + 50-200ug/ml MEA ;

Exp. #: 2

Investigator: A. Bishayee

Date: ~~01/15/98~~

01/19/99

1. Set the rocker-roller at 37°C incubator, set the Coulter Counter, wash cells (from 75 cm² flask, subcultured 1:2, 24h before) with PBS, trypsinize cells, resuspend in 7 ml MEMB, 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 ~400,000 cells/ml in MEMB (final volume 11 ml) [Actual count : 392,933 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. Roll the tubes for 3-4 h at 37°C, 5% CO₂ Date/Time: 01/19/99; 3-00 p.m.
5. Calibrate the stock ^{210}Po -citrate for today (12 uCi/ml) on 12/11/98
6. After 3-4 h, remove test tubes from roller and add according to Table below.

Date/Time: 01/19/99; 6-15 p.m.

| Tube # | ^{210}Po -citrate uCi/ml | Cells in MEMB (ml) | MEMB (ml) | Po-citrate (12 uCi/ml) on 12/11 (ml) | MEA in MEMA (200 ug/ml) (ml) | MEMA (ml) | MEA Conc. (ug/ml) |
|--------|--------------------------------------|--------------------------|--------------|--|--|--------------|-------------------------|
| 1 | 0 | 1.0 | 1 | 0 | 0 | 2 | 0 |
| 2 | 0 | 1.0 | 1 | 0 | 0.5 | 1.5 | 50 |
| 3 | 0 | 1.0 | 1 | 0 | 1 | 1 | 100 |
| 4 | 0 | 1.0 | 1 | 0 | 1.5 | 0.5 | 150 |
| 5 | 0 | 1.0 | 1 | 0 | 2 | 0 | 200 |
| 6 | 0.2 | 1.0 | 0.965 | 0.035 | 0 | 2 | 0 |
| 7 | 0.2 | 1.0 | 0.965 | 0.035 | 0.5 | 1.5 | 50 |
| 8 | 0.2 | 1.0 | 0.965 | 0.035 | 1 | 1 | 100 |
| 9 | 0.2 | 1.0 | 0.965 | 0.035 | 1.5 | 0.5 | 150 |
| 10 | 0.2 | 1.0 | 0.965 | 0.035 | 2 | 0 | 200 |

7. Return test tubes to roller for 30 min.

Date/Time: 6-30 p.m. 01/19/99

8. After 30 min, centrifuge tubes for 10 min at 2000 rpm, 4°C

Date/Time: 7-00 p.m. 01/19/99

9. During the centrifugation move roller to 10.5°C

10. Collect 150 ul supernatant in separate tubes
11. Add 8 ml of wash MEMA in each tube containing the pallet
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 the supernatant, click tubes, vortex add MEMA with or without MEA as per Table
18. Transfer tubes at 10°C for 72 h. Date/Time: 01/20/99; 8-15 p.m.
19. Transfer 30 ul of supernatant in triplicate from step 10 into 20 ml scintillation vial containing 6 ml cocktail (Aquasol) and count for radioactivity Date/Time: 01/20/99; 11-00 a.m.
19. After 72 h, add 8 ml wash MEMA in each tube, vortex and centrifuge the tubes for 10 min at 2000 rpm, 4°C (precooled centrifuge) Date/Time: 01/22/99; 11-00 a.m.
20. Labeling and preparation of dilution tubes and colony dishes
 - load 60 mm petri dishes with 4 ml MEMA
 - load 30 test 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.
21. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
22. Centrifuge tubes for 10 min at 2000 rpm, 4°C
23. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
24. Centrifuge tubes for 10 min at 2000 rpm, 4°C
25. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
26. Determine cell concentration by transferring 100 µl to Coulter cup
27. Vortex tube, transfer 0.5 ml into X.4, vortex tube X.4 and transfer 0.5 ml to tube X.3 and vortex tube X.3 and transfer 0.5 ml to tube X.2. Keep tubes on ice.
28. 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.
29. Transfer 500 µl of cell suspension (in duplicate) to 20 ml scintillation vial containing 6 ml cocktail (Aquasol)
30. Incubate petridishes for 1 week
31. Count vials for radioactivity Date/Time : 01/22/99; 1-00 p.m.
32. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
33. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.

Expt #2

01/19/99

Initial count = 10442, 10686, 10581

Avg " = 10569

Cell conc = 4,227,866 cells/ml

$$\text{Vol. required} = \frac{44,000,000}{4,227,866} = 1.04 \text{ ml}$$

Take 1.0 ml Cells + 10ml MEMB = 11ml

Final count = 968, 999, 980

Avg. = 982

Cell conc. = 392,933 cells/ml

F-451

EXC#2

USER: 5 ID:PD-210 PRESET TIME: 1.00
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
T#: 1 AQC:N QCF:N RCM:N

WED 20 JAN 1999 11:00

CHANNEL 1-LL:600 UL: 900 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:0 1.00000
HALF LIFE(DAYS):N

20ul medium

| SAM | POS | CH | CPM | 2SIG% | TIME | EL TIME | AVG H# | ERR |
|-----|-----|----|----------------|-------|------|---------|--------|-----|
| 1 | ** | 1 | 6.00 | 81.65 | 1.00 | 1.55 | 60.0 | |
| 2 | ** | 2 | 1M { 8.00 | 70.71 | 1.00 | 3.23 | 62.0 | |
| 3 | ** | 3 | 2M { 3.00 | 115.5 | 1.00 | 5.12 | 59.0 | |
| 4 | ** | 4 | 23.00 | 41.70 | 1.00 | 6.75 | 60.0 | |
| 5 | ** | 5 | 7.00 | 75.59 | 1.00 | 8.43 | 59.0 | |
| 6 | ** | 6 | 3M { 6.00 | 81.65 | 1.00 | 10.07 | 59.0 | |
| 7 | ** | 7 | 9.00 | 66.67 | 1.00 | 11.85 | 61.0 | |
| 8 | ** | 8 | 4M { 1.00 | 60.30 | 1.00 | 13.48 | 62.0 | |
| 9 | ** | 9 | 7.00 | 75.59 | 1.00 | 15.16 | 61.0 | |
| 10 | ** | 10 | 5M { 9.00 | 66.67 | 1.00 | 17.04 | 61.0 | |
| 11 | ** | 11 | 6M { 8396.00 | 2.18 | 1.00 | 18.72 | 60.0 | |
| 12 | ** | 12 | 9551.00 | 2.05 | 1.00 | 20.35 | 62.0 | |
| 13 | ** | 1 | 7M { 8237.00 | 2.20 | 1.00 | 22.19 | 62.0 | |
| 14 | ** | 2 | 9721.00 | 2.03 | 1.00 | 23.98 | 63.0 | |
| 15 | ** | 3 | 8769.00 | 2.14 | 1.00 | 25.62 | 61.0 | |
| 16 | ** | 4 | 8M { 9531.00 | 2.05 | 1.00 | 27.40 | 61.0 | |
| 17 | ** | 5 | 8660.00 | 2.15 | 1.00 | 29.08 | 60.0 | |
| 18 | ** | 6 | 9M { 9562.00 | 2.05 | 1.00 | 30.72 | 59.0 | |
| 19 | ** | 7 | 8899.00 | 2.12 | 1.00 | 32.50 | 58.0 | |
| 20 | ** | 8 | 110M { 8411.00 | 2.18 | 1.00 | 34.17 | 64.0 | |

TABLE-1

Expt. # : 2

Date/Time : 01/20/99; 11-00 a.m

| Tube # | Medium count for ²⁰ 30 ul (cpm) | Avg. cpm | dpm [cpm/l] | μ Ci/ml (A _c) on counting [dpm/66600] 44400 | μ Ci/ml (A _o) on addition [A _c /e ^{-λt}] |
|--------|---|----------|----------------|--|--|
| 1 | See the attached | | | | |
| 2 | Sheep | | | | |
| 3 | | | | | |
| 4 | | | | | |
| 5 | | | | | |
| 6 | | 8973 | 8973 | 0.2021 | |
| 7 | | 8979 | 8979 | 0.2022 | |
| 8 | | 9150 | 9150 | 0.2061 | |
| 9 | | 9111 | 9111 | 0.2052 | |
| 10 | | 8655 | 8655 | 0.1949 | |

Exp #2

PAGE:

USER: 5 ID:PO-210 PRESET TIME: 1.00 FRI 22 JAN 1999 13:06
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
 H#: 1 AGC:N QCF:N RCM: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:Q 1.00000
 HALF LIFE(DAYS):N

| SAM | POS | CH | CPM | 2SIG% | TIME | EL TIME | AVG H# | E |
|-----|-----|----|---------|-------|------|---------|--------|---|
| 1 | ** | 1 | 14.00 | 53.45 | 1.00 | 1.59 | 116.0 | |
| 2 | ** | 2 | 18.00 | 47.14 | 1.00 | 3.37 | 113.0 | |
| 3 | ** | 3 | 10.00 | 63.25 | 1.00 | 5.16 | 109.0 | |
| 4 | ** | 4 | 9.00 | 66.67 | 1.00 | 6.94 | 112.0 | |
| 5 | ** | 5 | 11.00 | 60.30 | 1.00 | 8.73 | 112.0 | |
| 6 | ** | 6 | 11.00 | 60.30 | 1.00 | 10.51 | 111.0 | |
| 7 | ** | 7 | 9.00 | 66.67 | 1.00 | 12.33 | 118.0 | |
| 8 | ** | 8 | 15.00 | 51.64 | 1.00 | 14.12 | 115.0 | |
| 9 | ** | 9 | 13.00 | 55.47 | 1.00 | 15.89 | 114.0 | |
| 10 | ** | 10 | 12.00 | 57.74 | 1.00 | 17.72 | 116.0 | |
| 11 | ** | 11 | 988.00 | 6.36 | 1.00 | 19.49 | 117.0 | |
| 12 | ** | 12 | 1015.00 | 6.28 | 1.00 | 21.27 | 82.0 | |
| 13 | ** | 1 | 949.00 | 6.49 | 1.00 | 23.11 | 114.0 | |
| 14 | ** | 2 | 998.00 | 6.33 | 1.00 | 24.89 | 81.0 | |
| 15 | ** | 3 | 896.00 | 6.68 | 1.00 | 26.67 | 85.0 | |
| 16 | ** | 4 | 821.00 | 6.59 | 1.00 | 28.39 | 88.0 | |
| 17 | ** | 5 | 799.00 | 6.33 | 1.00 | 30.22 | 79.0 | |
| 18 | ** | 6 | 702.00 | 6.26 | 1.00 | 32.00 | 88.0 | |
| 19 | ** | 7 | 751.00 | 6.49 | 1.00 | 33.77 | 84.0 | |
| 20 | ** | 8 | 899.00 | 6.67 | 1.00 | 35.50 | 78.0 | |

TABLE-2

Expt. # : 2

Date/Time : 01/22/99; 1-06 pm

| Tube # | Radioactivity for 500 ul cell suspension (cpm) | Avg. cpm | dpm [cpm/1] | μ Ci/ml (A_0) on counting [dpm/111x10 ⁴] | μ Ci/ml (A_0) after 12 h incubation [$A_0/e^{-\lambda t}$] |
|--------|--|----------|-------------|--|--|
| 1 | See the attached sheet | | | | |
| 2 | | | | | |
| 3 | | | | | |
| 4 | | | | | |
| 5 | | | | | |
| 6 | | 1001 | 1001 | 0.000902 | |
| 7 | | 973 | 973 | 0.000877 | |
| 8 | | 908 | 908 | 0.000818 | |
| 9 | | 1010 | 1010 | 0.000910 | |
| 10 | | 925 | 925 | 0.000833 | |

TABLE-3

Expt. # : 2

Date/Time : 01/22/99 ; 12:00 noon

| Tube # | Coulter count for 100 ul cell suspension | Avg. count | Cells/ml [Avg. count x 400] | fCi/cell [uCi/ml x 10 ⁹ Cells/ml] |
|--------|--|------------|-----------------------------------|--|
| 1 | See the at 346, 335, 376 | | | |
| 2 | 467, 461, 415 | | | |
| 3 | 380, 384, 377 | | | |
| 4 | 525, 537, 519 | | | |
| 5 | 474, 452, 491 | | | |
| 6 | 434, 413, 448 | 431.6 | 172666 | 5.223 |
| 7 | 465, 445, 466 | 458 | 183466 | 4.787 |
| 8 | 465, 491, 453 | 469 | 187866 | 4.360 |
| 9 | 449, 459, 467 | 458 | 183333 | 4.967 |
| 10 | 411, 426, 445 | 427 | 170933 | 4.878 |

TABLE-4

Expt #: 2 ✓

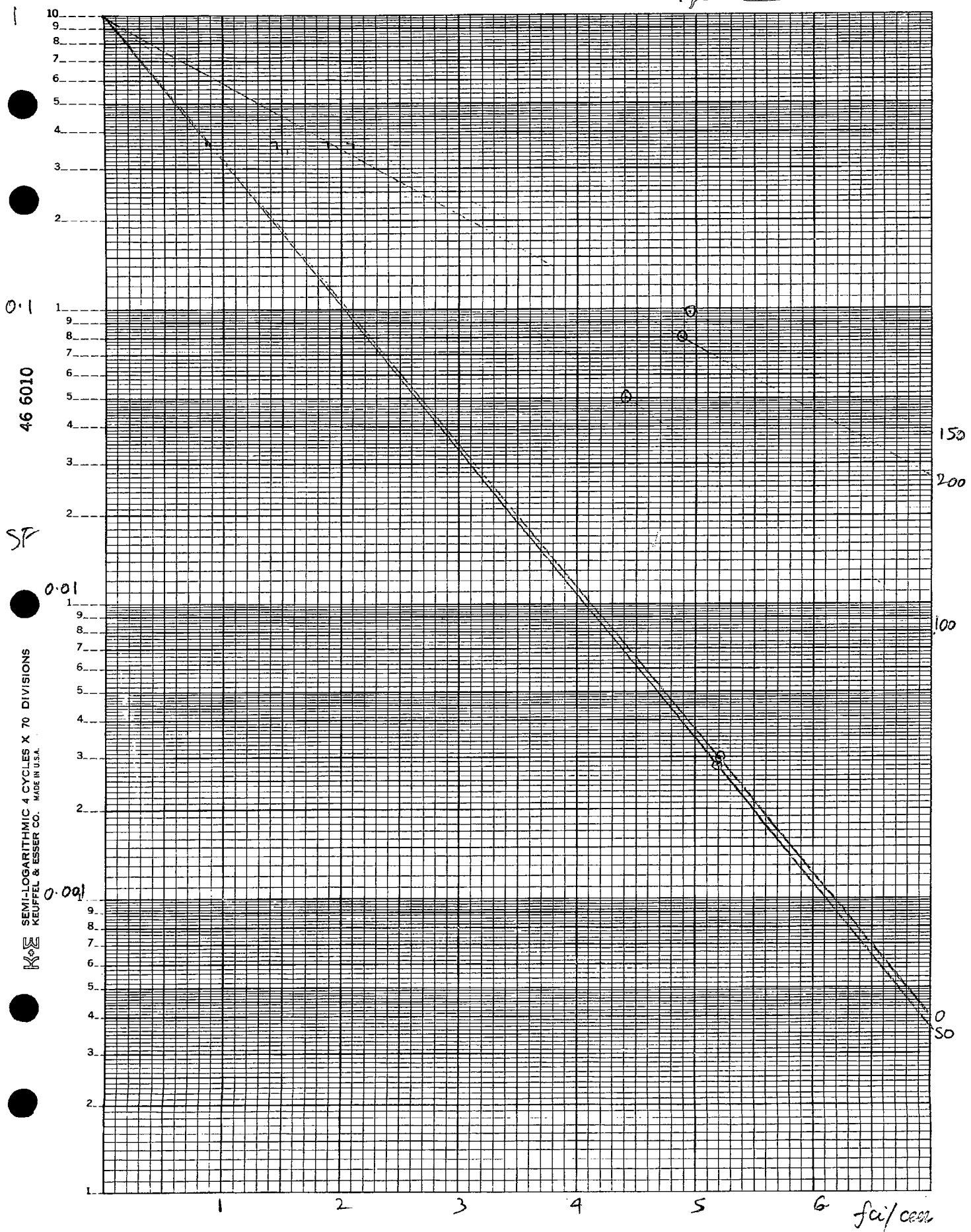
Date: 01/29/99

| Tube.dilution | Colony 1 | Colony 2 | Colony 3 | Avg Colony | SF |
|---------------|----------|----------|----------|------------|--------|
| 1.2 | 152 | 169 | 159 | 160 | - |
| 2.2 | 141 | 146 | 150 | 145.66 | 0.9104 |
| 3.2 | 133 | 138 | 143 | 138 | 0.8625 |
| 4.2 | 110 | 117 | 124 | 117 | 0.7312 |
| 5.2 | 92 | 100 | 107 | 99.66 | 0.6229 |
| 6.4 | 43 | 48 | 54 | 0.48 | 0.003 |
| 7.4 | 36 | 42 | 50 | 0.42 | 0.0029 |
| 8.3 | 63 | 70 | 77 | 7.0 | 0.0507 |
| 9.3 | 116 | 126 | 107 | 11.8 | 0.0994 |
| 10.3 | 73 | 80 | 88 | 8.03 | 0.0806 |

MEA
Concn.
(µg/ml)

DMF

| | |
|-----|------|
| 0 | 1 |
| 50 | 0.97 |
| 100 | 1.61 |
| 150 | 2.33 |
| 200 | 2.11 |



KE SEMI-LOGARITHMIC 4 CYCLES X 70 DIVISIONS
KEUFFEL & ESSER CO. MADE IN U.S.A.