# V79 COLONY FORMING ASSAY

**Experiment Name :** $^3H_2O$ + 10% DMSO; **Exp. # :** 3; **Investigator:** A. Bishayee
**Date:** 07/13/98

1. Set the rocker-roller at 37°C incubator, set the Coulter Counter, wash cells (from 75 $cm^2$ flusk, 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 transfering 100 ul in Coulter cup containing 20 ml isotone (Coulter balanced electrolyte solution)
2. Dilute to ~4,00,000 cells/ml in MEMB (final volume 11 ml) [Actual count : 379733 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% $CO_2$ **Date/Time:** 07/13/98; 4-00 p.m.
5. Obtain $^3H_2O$ from refrigerator (25 mCi/ml) NEN Catalog # NET001C
6. After 3-4 h, remove test tubes from roller and add MEMB and/or $^3H_2O$ according to Table below. **Date/Time:** 07/13/98; 7-45 p.m

| Tube # | $^3H_2O$ mCi/ml | Cells in MEMB (ml) | MEMB (ul) | $^3H_2O$ (ul) [25 mCi/ml] | DMSO (ul) | MEMB (ul) | |
|---|---|---|---|---|---|---|---|
| 1 | 0 | 1.0 | 800 | 0 | 200 | 0 | |
| 2 | 0 | 1.0 | 800 | 0 | 200 | 0 | |
| 3 | 0.25 | 1.0 | 780 | 20 | 200 | 0 | |
| 4 | 0.75 | 1.0 | 740 | 60 | 200 | 0 | |
| 5 | 1.25 | 1.0 | 700 | 100 | 200 | 0 | |
| 6 | 0 | 1.0 | 800 | 0 | 0 | 200 | |
| 7 | 0 | 1.0 | 800 | 0 | 0 | 200 | |
| 8 | 0.25 | 1.0 | 780 | 20 | 0 | 200 | |
| 9 | 0.75 | 1.0 | 740 | 60 | 0 | 200 | |
| 10 | 1.25 | 1.0 | 700 | 100 | 0 | 200 | |

7. Return test tubes to roller for 12 h, increase the elevation angle of the roller.
**Date/Time:** 07/13/98; 8-00 p.m.

8. While test tubes are in roller, obtain sterile DMSO (100%) from refrigerator, thaw it, **move roller to 10.5°C**, obtain ice
9. After ~12 h incubation period, remove tubes from incubator, chill on ice
10. Add DMSO (while vortexing) or MEMB according to the Table, vortex, quickly return to ice

**Date/Time :** 07/14/98; 9-00 a.m.

11. Transfer tubes to roller at 10.5 °C for 72 h. **Date/Time:** 07/14/98; 9-10 a.m.
12. After 72 h, remove tubes, **place on** ice and centrifuge at 2000 rpm at 4°C for 10 min (***precooled centrifuge***) **Date/Time:** 07/17/98; 1-30 a.m.
13. Transfer 10 ul medium to test tubes containing 490 µl MEMB in each
14. Add 8 ml ice-cold wash MEMA, vortex
15. Centrifuge tubes for 10 min at 2000 rpm, 4°C
16. Labeling and preparation of dilution tubes and colony dishes
    - load 48 mm petri dishes with 4 ml MEMA
    - load 30 T-tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 2.2, 2.3, 2.4, X.2, X.3, X.4, etc.
17. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
18. Centrifuge tubes for 10 min at 2000 rpm, 4°C
19. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
20. Centrifuge tubes for 10 min at 2000 rpm, 4°C
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 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
24. Determine cell concentration by transfering 100 µl to Coulter cup
25. Vortex tube, 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.
26. 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.
27. Transfer 100 µl of cell suspension (in triplicate) to prelabelled vial (C) containing 3 ml liquid Scintillation cocktail for each tube and count
28. Incubate petridishes for 1 week
29. ~~Add 490 ul MEMB in tubes containing 10 ul of medium (step 13),~~ vortex, transfer 10 ul in triplicate into prelabelled vials (M). tubes in step 13, then containing 3 ml liquid scintillation cocktail
30. ~~Add 3 ml liquid scintillation cocktail to vials and~~ count for radioactivity
31. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with crystal violet
32. Count colonies (50 or more cells). There must be between 25 and 250 colonies for the dish to be a valid data point.

Expt #3

07/13/98

Initial Cell count = 5327, 5416, 5208
Avg. Cell count = 5317
Cell conc. = 5317 × 400
= 2,126,800 cells/ml

For dilution,

Vol. of cell suspension required = $\frac{4400000}{2126800}$

= 2.06 ml

Take 2.06 ml of Cell Suspension + 8.94 MEMB = 11 ml.

After dilution,

Final cell count = 955, 976, 917
Avg. cell count = 949.3
cell conc. = 949.3 × 400
= 379,733 cells/ml

Expt #3



USER:10 ID:TRITIUM PRESET TIME: 1.00 FRI 17 JUL 1998 16:36
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
[redacted]: 1 AQC:N QCF:N RCM:N 2 PHASE MONITOR: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:Q 1.00000
HALF LIFE(DAYS):N

| SAM | POS | CH | | CPM | 2SIG% | TIME | EL TIME | AVG H# | ERR |
|---|---|---|---|---|---|---|---|---|---|
| 1 | **- 1 | 1 | Back | 21.00 | 43.64 | 1.00 | 1.67 | 54.0 | |
| 2 | **- 2 | 1 | | 25.00 | 40.00 | 1.00 | 3.42 | 55.0 | |
| 3 | **- 3 | 1 | | 43.00 | 30.50 | 1.00 | 5.20 | 56.0 | |
| 4 | **- 4 | 1 | 1M | 27.00 | 38.49 | 1.00 | 6.93 | 57.0 | |
| 5 | **- 5 | 1 | | [illegible].00 | 34.82 | 1.00 | 8.72 | 57.0 | |
| 6 | **- 6 | 1 | | .00 | 33.81 | 1.00 | 10.49 | 58.0 | |
| 7 | **- 7 | 1 | 2M | [illegible].00 | 33.33 | 1.00 | 12.22 | 59.0 | |
| 8 | **- 8 | 1 | | [illegible].00 | 30.50 | 1.00 | 14.06 | 60.0 | |
| 9 | **- 9 | 1 | | 60154.29 | 1.95 | 0.17 | 15.01 | 59.0 | |
| 10 | **-10 | 1 | 13M | 39803.33 | 1.83 | 0.30 | 16.03 | 56.0 | |
| 11 | **-11 | 1 | | 63870.00 | 1.77 | 0.20 | 16.96 | 58.0 | |
| 12 | **-12 | 1 | | 172893.33 | 1.24 | 0.15 | 17.84 | 57.0 | |
| 13 | **-13 | 1 | 14M | 190140.00 | 1.18 | 0.15 | 18.73 | 58.0 | |
| 14 | **-14 | 1 | | 186560.00 | 1.20 | 0.15 | 19.63 | 58.0 | |
| 15 | **-15 | 1 | | 303611.44 | 0.87 | 0.17 | 20.60 | 58.0 | |
| 16 | **-16 | 1 | 15M | [illegible]4125.72 | 0.87 | 0.17 | 21.58 | 59.0 | |
| 17 | **-17 | 1 | | [illegible]725.72 | 0.92 | 0.17 | 22.56 | 58.0 | |
| 18 | **-18 | 1 | | 43.00 | 30.50 | 1.00 | 24.30 | 62.0 | |
| 19 | **- 1 | 1 | 16M | 27.00 | 38.49 | 1.00 | 26.14 | 61.0 | |
| 20 | **- 2 | 1 | | 32.00 | 35.36 | 1.00 | 27.87 | 63.0 | |
| 21 | **- 3 | 1 | | 36.00 | 33.33 | 1.00 | 29.66 | 62.0 | |
| 22 | **- 4 | 1 | 7M | 30.00 | 36.51 | 1.00 | 31.39 | 61.0 | |
| 23 | **- 5 | 1 | | 46.00 | 29.49 | 1.00 | 33.12 | 57.0 | |
| 24 | **- 6 | 1 | | 40730.91 | 1.89 | 0.28 | 34.17 | 61.0 | |
| 25 | **- 7 | 1 | 8M | 54257.78 | .81 | 0.23 | 35.17 | 61.0 | |
| 26 | **- 8 | 1 | | 46024.00 | .86 | 0.25 | 36.14 | 61.0 | |
| 27 | **- 9 | 1 | | 177805.72 | .13 | 0.17 | 37.11 | 63.0 | |
| 28 | **-10 | 1 | 9M | 174646[illegible] | 1.24 | 0.15 | 37.98 | 61.0 | |
| 29 | **-11 | 1 | | 17529[illegible] | 1.14 | 0.17 | 38.95 | 60.0 | |
| 30 | **-12 | 1 | | 30183[illegible] | 0.94 | 0.15 | 39.85 | 60.0 | |
| 31 | **-13 | 1 | 10M | 3027[illegible] | 0.87 | 0.17 | 40.83 | 58.0 | |
| 32 | **-14 | 1 | | 305456.66 | 0.93 | 0.15 | 41.74 | 62.0 | |
| 37 | **- 1 | 1 | | 34.00 | 34.30 | 1.00 | 43.61 | 84.0 | |
| 38 | **- 2 | 1 | 1C | 45.00 | 29.81 | 1.00 | 45.39 | 80.0 | |
| 39 | **- 3 | 1 | | 42.00 | 30.86 | 1.00 | 47.17 | 86.0 | |
| 40 | **- 4 | 1 | | 24.00 | 40.82 | 1.00 | 48.91 | 85.0 | |
| 41 | **- 5 | 1 | 2C | 29.00 | 37.14 | 1.00 | 50.64 | 85.0 | |
| 42 | **- 6 | 1 | | 46.00 | 29.49 | 1.00 | 52.37 | 85.0 | |
| 43 | **- 7 | 1 | | 1553.00 | 5.08 | 1.00 | 54.16 | 85.0 | |
| 44 | **- 8 | 1 | 3C | 1561.00 | 5.06 | 1.00 | 55.94 | 86.0 | |
| 45 | **- 9 | 1 | | 1533.00 | 5.11 | 1.00 | 57.67 | 86.0 | |
| 46 | **-10 | 1 | | 3509.00 | 3.38 | 1.00 | 59.46 | 84.0 | |
| 47 | **-11 | 1 | 4C | 3487.00 | 3.39 | 1.00 | 61.19 | 84.0 | |
| 48 | **-12 | 1 | | 3714.00 | 3.28 | 1.00 | 62.97 | 85.0 | |
| 49 | **-13 | 1 | | 6317.00 | 2.52 | 1.00 | 64.72 | 87.0 | |
| 50 | **-14 | 1 | | 6445.00 | 2.49 | 1.00 | 66.45 | 86.0 | |
| | | | 5C | | | | | | |

| SAM | POS | CH | | CPM | 2SIG% | TIME | EL TIME | AVG H# | ERR |
|---|---|---|---|---|---|---|---|---|---|
| [redacted] | **-15 | 1 | 7C | 6664.00 | 2.45 | 1.00 | 68.18 | 85.0 | |
| 52 | **-16 | 1 | | 33.00 | 34.82 | 1.00 | 69.93 | 83.0 | |
| 53 | **-17 | 1 | 6C | 35.00 | 33.81 | 1.00 | 71.67 | 80.0 | |
| [redacted] | **-18 | 1 | | 28.00 | 37.80 | 1.00 | 73.45 | 73.0 | |
| 55 | **- 1 | 1 | | 32.00 | 35.36 | 1.00 | 75.27 | 86.0 | |
| 56 | **- 2 | 1 | 7C | 38.00 | 32.44 | 1.00 | 77.03 | 86.0 | |
| 57 | **- 3 | 1 | | 41.00 | 31.23 | 1.00 | 78.82 | 86.0 | |
| 58 | **- 4 | 1 | | 1546.00 | 5.09 | 1.00 | 80.55 | 86.0 | |
| 59 | **- 5 | 1 | 8C | 1616.00 | 4.98 | 1.00 | 82.29 | 84.0 | |
| 60 | **- 6 | 1 | | 1446.00 | 5.26 | 1.00 | 84.03 | 81.0 | |
| 61 | **- 7 | 1 | | 4245.00 | 3.07 | 1.00 | 85.82 | 86.0 | |
| 62 | **- 8 | 1 | 9C | 4060.00 | 3.14 | 1.00 | 87.60 | 80.0 | |
| 63 | **- 9 | 1 | | 4167.00 | 3.10 | 1.00 | 89.33 | 84.0 | |
| 64 | **-10 | 1 | | 7050.00 | 2.38 | 1.00 | 91.08 | 82.0 | |
| 65 | **-11 | 1 | | 6969.00 | 2.40 | 1.00 | 92.87 | 79.0 | |
| 66 | **-12 | 1 | 10C | 7213.00 | 2.35 | 1.00 | 94.61 | 83.0 | |

[redacted]

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**TABLE-1**

**Expt. # :** 3 **Date/Time :** 07/17/98; 4-36 p.m.

| Tube # | Medium count for 10 ul of (cpm) 1:50 diluted medium | Avg. cpm | dpm [cpm/0.52] | µCi/ml ($A_t$) on counting [dpm/444] | µCi/ml ($A_o$) ~~on addition [$A_t$/e$^{-\lambda t}$]~~ |
|---|---|---|---|---|---|
| 1 | 20, 4, 10 | 11·3 | 21·7 | 0·04 | 0·00004 |
| 2 | 12, 13, 20 | 15 | 28·8 | 0·06 | 0·00006 |
| 3 | 60131, 39780, 63847 | 54586 | 104973 | 236·4 | 0·236 |
| 4 | 172870, 190117, 186537 | 1831746 | 352258 | 793·3 | 0·793 |
| 5 | 303588, 304102, 268702 | 292130 | 561789·7 | 1265·2 | 1·265 |
| 6 | 20, 4, 9 | 11 | 21·1 | 0·04 | 0·00004 |
| 7 | 13, 7, 23 | 14·3 | 27·5 | 0·06 | 0·00006 |
| 8 | 40707, 54234, 46001 | 46980 | 90347 | 203·4 | 0·203 |
| 9 | 177782, 174623, 175274 | 175893 | 338255 | 761·8 | 0·761 |
| 10 | 301810, 302771, 305443 | 303341 | 583348 | 1313·8 | 1·313 |

200 = 10

**TABLE-2**

**Expt. # :** 3 **Date/Time :** 07/17/98; 4-36 p.m

| Tube # | Radioactivity for 100 ul cell suspension (cpm) | Avg. cpm | dpm [cpm/0.52] | μCi/ml ($A_t$) on counting [dpm/222000] | μCi/ml ($A_o$) after 12 h incubation [$A_t/e^{-\lambda t}$] |
|---|---|---|---|---|---|
| 1 | 11, 22, 19 | | | | |
| 2 | 1, 6, 23 | | | | |
| 3 | 1530, 1538, 1510 | 1526 | 2934.6 | 0.0132 | |
| 4 | 3486, 3464, 3691 | 3547 | 6821.1 | 0.0307 | |
| 5 | 6294, 6422, 6641 | 6452.3 | 12408.3 | 0.0558 | |
| 6 | 10, 12, 5 | | | | |
| 7 | 9, 15, 18 | | | | |
| 8 | 1523, 1593, 1423 | 1513 | 2909.6 | 0.0131 | |
| 9 | 4222, 4037, 4144 | 4134.3 | ~~7950~~ 7950.5 | 0.0358 | |
| 10 | 7027, 6946, 7190 | 7054.3 | 13566 | 0.0611 | |

**TABLE-3**

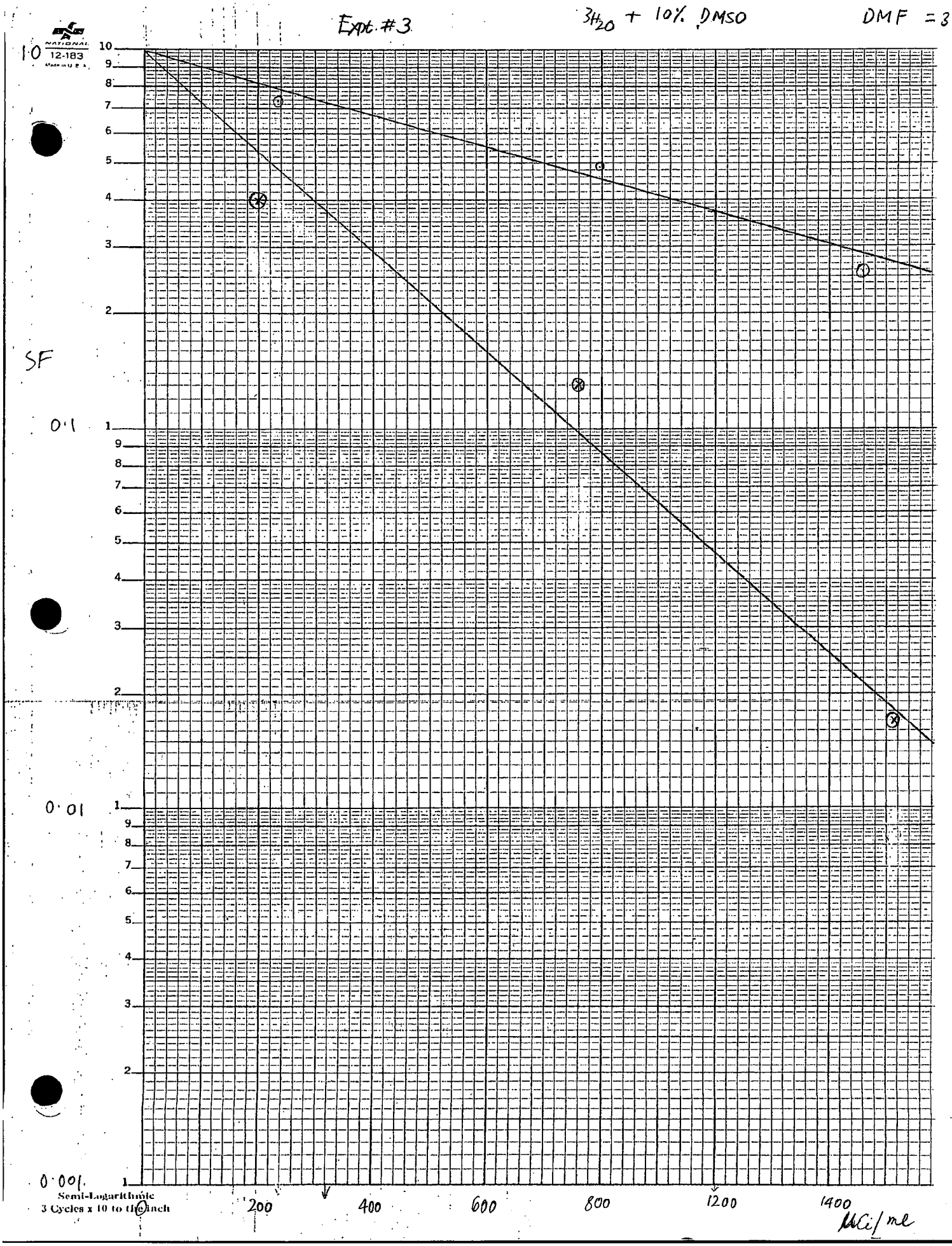
**Expt. # :** 3 **Date/Time :** 07/17/98 ; 3-30 p.m

| 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] |
|---|---|---|---|---|
| 1 | ~~707~~, 636, 607, 620 | 621 | 248400 | |
| 2 | 669, 675, 659 | 667 | 267066 | |
| 3 | 712, 680, ~~633~~, 722 | 704 | 281866 | 0.0468 |
| 4 | 960, 1040, 1021 | 1007 | 402800 | 0.0762 |
| 5 | 1170, 1243, 1077 | 1163 | 465333 | 0.1199 |
| 6 | 1263, 1191, 1202 | 1218 | 487466 | |
| 7 | 861, 842, ~~722~~, ~~764~~, 806 | 836 | 334533 | |
| 8 | 1219, 1249, ~~1348~~ 1269 | 1245.6 | 498240 | 0.0262 |
| 9 | ~~1110, 1013, 1001~~ 880, 880, 866 | 875.3 | 350133 | 0.1022 |
| 10 | 1279, 1266, 1220 | 1255 | 502000 | 0.1217 |

**TABLE-4**

**Expt # :** 3 **Date :** 07/24/98

| Tube.dilution | Colony 1 | Colony 2 | Colony 3 | Avg Colony for ×.2 | SF |
|---|---|---|---|---|---|
| 1·2 | 161 | 169 | 153 | } | |
| 2·2 | 150 | 149 | 156 | } 156·33 | |
| 3·2 | 117 | 120 | 113 | 116·66 | 0·7462 |
| 4·2 | 78 | 70 | 82 | 76·66 | 0·4904 |
| 5·2 | 40 | 45 | 36 | 40·33 | 0·2579 |
| 6·2 | 140 | 149 | 152 | } | |
| 7·2 | 139 | 141 | 143 | } 144 | |
| 8·2 | 57 | 50 | 64 | 57 | 0·3958 |
| 9·3 | 180 | 188 | 196 | 18·8 | 0·1305 |
| 10·4 | 244 | 240 | 248 | 2·44 | 0·0169 |


Expt. #3
$3H_2O$ + 10% DMSO
DMF = 3:1
SF
1.0
0.1
0.01
0.001
10
9
8
7
6
5
4
3
2
1
200
400
600
800
1200
1400
μCi/ml
Semi-Logarithmic
3 Cycles x 10 to the inch