V79 COLONY FORMING ASSAY

Experiment Name : ^{125}IUdR + MEA; **Exp. # :** 2; **Investigator:** A. Bishayee
Date: 04/24/98

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 : 4, 22, 133 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:** 04/27/98; 3-45 p.m.
5. Prepare MEMB containing radioactivity in hood
   3.2 µl ^{125}IUdR (prepared on 04/09/98) + 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:** 04/27/98; 7-15 p.m.

Tube #	^{125}IUdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{125}IUdR (ml) [1.0 uCi/ml]	MEMA+ MEA 100 ug/ml (1.3 mM)	MEMA	
1	0	1.0	1.0	0	2.0	0	
2	0	1.0	1.0	0	2.0	0	
3	0.01	1.0	0.98	0.02	2.0	0	
4	0.02	1.0	0.96	0.04	2.0	0	
5	0.03	1.0	0.94	0.06	2.0	0	
6	0	1.0	1.0	0	0	2.0	
7	0	1.0	1.0	0	0	2.0	
8	0.01	1.0	0.98	0.02	0	2.0	
9	0.02	1.0	0.96	0.04	0	2.0	
10	0.03	1.0	0.94	0.06	0	2.0	

7. Return test tubes to roller for 12 h, increase the elevation angle of the roller.
   Date/Time: 04/27/98; 7-30 p.m.

1.2
2.2
3.2, 3.3
4.2, 4.3
5.3, 5.4
6.2
7.2
8.2, 8.3
9.2, 9.3, 9.4
10.3, 10.4

8. While test tubes are rolling label 40 (4x10) 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:** 04/28/98 9-10 a.m.

MEA (20 ng/ml) = 55 µl
MEMA = 10.945 ml
= 10.945 ml

10. **During centrifugation, move roller to 10°C and obtain ice**
11. Prepare 11 ml of sterile MEA (100 ug/ml) in MEMA , **put on ice**
12. Remove buckets from centrifuge and carefully remove 100 µl of supernatant and place in prelabeled gamma-tube.
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 10 ml wash MEMA
18. Centrifuge tubes for 10 min at 2000 rpm, 4°C
19. Decant supernatant, click tubes, vortex, resuspend in 2 ml ice cold MEMA containing 0 or 100 ug/ml of MEA as per Table. Keep on ice!
20. Transfer tubes to roller at 10°C for 72 h. **Date/Time:** 04/28/98; 11-00 a.m.
21. Transfer 10 ul supernatant in three sets of tubes containing small pieces of tissue paper from 100 ul supernatant removed earlier and count them for radioactivity

    Date/Time: 04/28/98;

21. After 72 h, remove tubes and **place on ice**, add 8 ml ***ice cold*** wash MEMA.

    Date/Time: 05/01/98; 9-10 a.m.

22. Centrifuge tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***
23. Labeling and preparation of dilution tubes and colony dishes
    - load 57 60 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.
24. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
25. Centrifuge tubes for 10 min at 2000 rpm, 4°C
26. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
27. Centrifuge tubes for 10 min at 2000 rpm, 4°C
28. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
29. Determine cell concentration by transfering 100 µl to Coulter cup
30. 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.
31. 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.

32. Transfer 300 µl of cell suspension (in triplicate) to gamma tubes for each tube
33. Incubate petridishes for 1 week
34. Count gamma tubes for radioactivity **Date/Time :** 05/04/98; 12-00 noon
35. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with crystal violet
36. Count colonies (50 or more cells). There must be between 25 and 250 colonies for the dish to be a valid data point.

Expt # 2 04/27/98

Initial cell count = ~~6495~~, 6741, 6880, 6741
Avg. cell count = 6787.3
Cell conc. = 400 × 6787.3
= 2714933.3 cells/ml

For dilution
vol. of cell suspension required = $\frac{4400000}{2714933.3}$
= 1.62

Take 1.62 ml of cells + 9.38 ml MEMB = 11 ml.

After dilution,
Final count = 1127, 1085, 954
Avg. cell count = 1055.3
Cell conc = 400 × 1055.3
= 4, 22, 133 cells/ml

Expt #2

04/27/98

Preparation of ^{125}I UdR in MEMB.

Prepare 5 ml of 1 uci/ml ^{125}I UdR = 5 uCi required.

Stock on 04/09/98 1·93 µCi/µl

on 04/27/98 1·93 × 0·812 µci/µl
= 1·57 µCi/µl.

Stock required = $\frac{5}{1·57}$ = 3·19 µl.

① Take 5 ml of MEMB
② Add 3·2 µl of stock ^{125}I UdR

Expt Name : ^{125}IUdR + 1·3 mM MEA

Expt # : 2

Date / Time : 04/28/98 ; 12-00

10 µl onto time

1	1.00	Blank	20	234
2	1.00		15 (17.5)	229
3	1.00	1M	28	249
4	1.00		17	252
5	1.00		23	239
6	1.00	2M	25	247
7	1.00		18	232
8	1.00		25	247
9	1.00	3M	172	431
10	1.00		195	412
11	1.00		197	384
12	1.00	4M	342	571
13	1.00		317	512
14	1.00		314	526
15	1.00	5M	451	670
16	1.00		487	727
17	1.00		455	656
18	1.00	6M	22	201
19	1.00		17	219
20	1.00		26	221
21	1.00	7M	17	226
22	1.00		24	228
23	1.00		21	255
24	1.00	8M	190	435
25	1.00		182	390
26	1.00		189	382
27	1.00	9M	335	566
28	1.00		309	511
29	1.00		348	578
30	1.00	10M	479	687
31	1.00		511	739
32	1.00		502	706
~~33~~	~~1.27~~		~~8~~	~~68~~

TABLE-1

Expt. # : 2 **Date/Time :** 04/28/98; 12-00

Tube #	Medium count for 10 ul (cpm)	Avg. cpm	dpm [cpm/0.~~142~~ 0.7056]	µCi/ml (A_t) on counting [dpm/22200]	µCi/ml (A_o) on addition [$A_t/e^{-\lambda t}$]
1	11, 0, 6	0	0	0	0
2	8, 1, 8	0	0	0	0
3	155, 178, 180	171	242·3	0·0109	0·0110
4	325, 300, 297	307·3	435·5	0·0196	0·0197
5	434, 470, 438	447·3	633·9	0·0285	0·0287
6	5, 0, 9	0	0	0	0
7	0, 7, 4	0	0	0	0
8	173, 165, 172	170	240·9	0·0108	0·0109
9	318, 292, 331	313·6	444·5	0·0200	0·0201
10	462, 494, 485	480·3	680·7	0·0306	0·0309

04/27/98; 7-30 p.m.

12h + 4.5h

= 16.5h

$e^{-\lambda t}$

$= e^{\frac{0.693 \times 16.5}{1440}}$

$= e^{0.9920}$

Expt Name : ^{125}IUdR + 1·3mM MEA

Expt # : 2

Date : 05/04/98

Time : 12-00 noon

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

300 µl cells.

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

1	1.00	26	31 } Back.	248
2	1.00		22 }	263
3	1.00		18 } 1c	196
4	1.00		13	238
5	1.00		24	230
6	1.00		25 } 2c	223
7	1.00		20	228
8	1.00		30	221
9	1.00		558 } 3c	774
10	1.00		515	745
11	1.00		538	753
12	1.00		910 } 4c	1115
13	1.00		878	1088
14	1.00		894	1129
15	1.00		1635 } 5c	1857
16	1.00		1628	1854
17	1.00		1520	1746
18	1.00		21 } 6c	238
19	1.00		25	229
20	1.00		25	228
21	1.00		18 } 7c	251
22	1.00		18	210
23	1.00		27	222
24	1.00		569 } 8c	754
25	1.00		595	796
26	1.00		642	853
27	1.00		871 } 9c	1089
28	1.00		904	1098
29	1.00		880	1114
30	1.00		1423 } 10c	1614
31	1.00		1480	1695
32	1.00		1564	1779

TABLE-2

Expt. # : 2 **Date/Time :** 05/04/98; 12-00 noon

Tube #	Radioactivity for 300 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/~~0.142~~ 0.7056]	µCi/ml (A_t) on counting [dpm/666000]	µCi/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1	-8, -13, -2	0	0	0	0
2	-1, -6, 4				
3	532, 489, 512	511	724.20	0.00108	0.00116
4	884, 852, 868	868	1230.15	0.00184	0.00198
5	1609, 1602, 1494	1568.3	2222.69	0.00333	0.00358
6	-5, -1, -1	0	0	0	0
7	-8, -8, 1	0	0	0	0
8	543, 569, 616	576	816.32	0.00122	0.00131
9	845, 878, 854	859	1217.40	0.00182	0.00196
10	1397, 1454, 1538	1463	2073.4	0.00311	0.00334

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 147}{1440}}$

$= 0.9317$

04/28/98; 9-10 a.m.

6 × 24h + 3

$= 147h$

TABLE-3

Expt. # : 2 **Date/Time :** 05/01/98; 11-15 a.m.

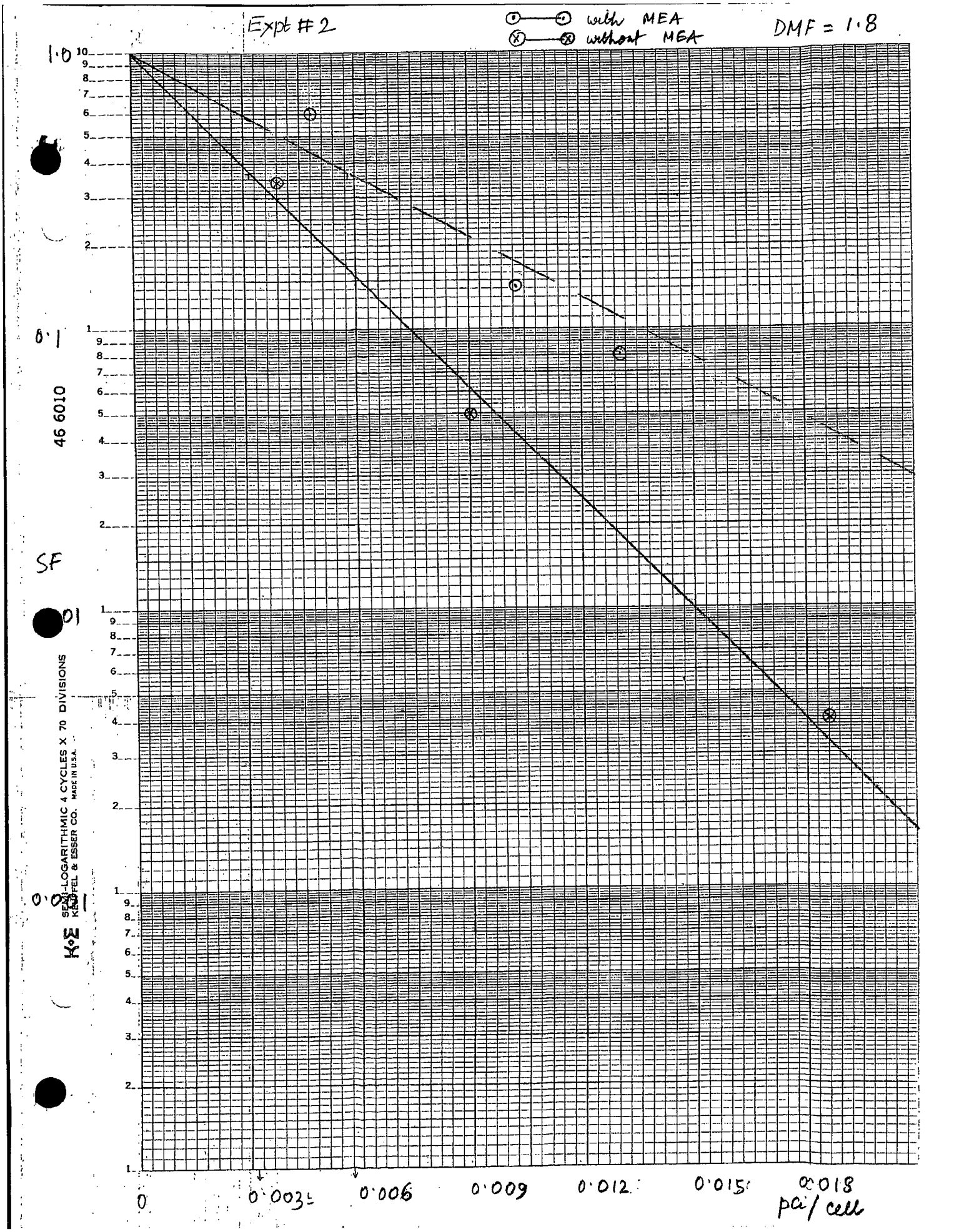
Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [uCi/ml x 10^6 Cells/ml]
1	589, 529, 512	543·3	217333·3	0
2	564, 560, 585	569·6	227866·6	0
3	~~691~~, 627, 605, 593	608·3	243333·3	0·00476
4	518, 461, 460	479·6	191866·6	0·01031
5	695, 667, 695	685·6	274266·6	0·01305 ~~0·06756~~
6	730, 721, 749	733·3	293333·3	0
7.	621, 599, 613	611	244400	0
8	672, 687, 666	675	270000	0·00485
9	~~628~~, 576, 523, 533	544	217600	0·009007
10	458, 421, 475	451·3	180533·3	0·018500?

TABLE-4

Expt. #: 2 Date: 05/08/98

Colony Counts and Survival Fraction

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1·2	58	54	56	} 60·5	
2·2	69	61	65		
3·2	39	33	37	36·33	0·6005
4·3	82	97	91	9·0	0·1487
5·3	58	45	46	4·96	0·0820
6·2	70	75	78	} 71	
7·2	69	68	66		
8·2	23	29	22	24·66	0·3474
9·3	38	40	30	3·6	0·0507
10·4	27	25	36	0·2933	0·0041


Expt #2
with MEA
without MEA
DMF = 1·8
1·0
0·1
0·01
0·001
46 6010
SF
SEMI-LOGARITHMIC 4 CYCLES X 70 DIVISIONS
KEUFFEL & ESSER CO. MADE IN U.S.A.
K·E
0
0·003
0·006
0·009
0·012
0·015
0·018
psi/cell