V79 COLONY FORMING ASSAY

Experiment Name : ^{125}IUdR + 50-200 ug/ml MEA; **Exp. # :** 1; **Investigator:** A.Bishayee
Date: 05/14/98

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 : 415600 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:** 05/14/98; 4-15 p.m.
5. Prepare MEMB containing radioactivity in hood
   1.95 µl ^{125}IUdR (prepared on 04/09/98) + 2.5 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:** 05/14/98; 7-45 p.m.

Tube #	^{125}IUdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{125}IUdR (ml) [1.0 uCi/ml]	MEMA+ MEA (200 ug/ml) (ml)	MEMA (ml)	MEA Conc. (ug/ml)
1	0	1.0	1.0	0	0	2.0	0
2	0	1.0	1.0	0	0.5	1.5	50
3	0	1.0	1.0	0	1	1	100
4	0	1.0	1.0	0	1.5	0.5	150
5	0	1.0	1.0	0	2.0	0	200
6	0.02	1.0	0.96	0.04	0	2.0	0
7	0.02	1.0	0.96	0.04	0.5	1.5	50
8	0.02	1.0	0.96	0.04	1	1	100
9	0.02	1.0	0.96	0.04	1.5	0.5	150
10	0.02	1.0	0.96	0.04	2.0	0	200

7. Return test tubes to roller for 12 h, increase the elevation angle of the roller.
   Date/Time: 05/14/98; 8-00 p.m.

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:** 05/15/98; 9-00 a.m.
10. **During centrifugation, move roller to 10°C and obtain ice** MEMA = 10.89 ml
11. Prepare 11 ml of 200 ug/ml MEA in MEMA , **put on ice** MEA(20mg/ml) = 0.11 ml
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 and/or 200 ug/ml MEA as per Table. Keep on ice!
20. Transfer tubes to roller at 10°C for 72 h. **Date/Time:** 05/15/98; 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: 05/26/98; 11-30 a.m.
21. After 72 h, remove tubes and **place on ice**, add 8 ml ***ice cold*** wash MEMA.

    Date/Time: 05/18/98; 1-00 p.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 :** 05/26/98; 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 flask to be a valid data point.

Expt #1

05/14/98

Initial cell count = 6330, 6320, 6320
Avg. Cell count = 6323.3
Cell conc. = 6323.3 × 400
= 2529333.3 cells/ml

For dilution,

Vol of cell suspension taken = $\frac{4400000}{2529333.3}$
= 1.73 ml

Take 1.73 ml of cells + 9.27 ml MEMB = 11 ml.

After dilution,

Final cell count = 1003, 1058, 1056
Avg. cell count = 1039
Cell conc. = 1039 × 400
= 4,15,600 cells/ml

Expt# 1 05/14/98

Preparation of ^{125}I/UdR in MEMB.

Prepare 2.5 ml of 1 µCi/ml = 2.5 µCi required

Stock

on 04/09/98 = 1.93 µCi/µl

on 05/14/98 = 1.93 × 0.667
= 1.28 µCi/µl.

Stock required = $\frac{2.5}{1.28}$ = 1.95 µl.

① Take 2.5 ml of MEMB
② Add 1.95 µl of ^{125}I/UdR

cpm for 10 µl medium onto time

1	1.00	40	34	266
2	1.00		47 } blank	283
3	1.00	1M	25	261
4	1.00		47	274
5	1.00		30	288
6	1.00	2M	64	287
7	1.00		40	280
8	1.00		49	300
9	1.00	3M	56	326
10	1.00		48	284
11	1.00		33	289
12	1.00	4M	34	290
13	1.00		28	267
14	1.00		38	276
15	1.00	5M	35	296
16	1.00		23	284
17	1.00		54	290
18	1.00	6M	346	602
19	1.00		360	633
20	1.00		368	611
21	1.00	7M	385	645
22	1.00		386	665
23	1.00		430	678
24	1.00	8M	370	617
25	1.00		404	661
26	1.00		339	578
27	1.00	9M	392	657
28	1.00		374	617
29	1.00		418	657
30	1.00	10M	342	602
31	1.00		367	593
32	1.00		312	546

MEA #1

11-30 a.m.

125 I UdR + 50-200 µg/ml MEA

Expt # : 1

Date/Time: 05/26/98

11-30 a.m.

12-00

TABLE-1

Expt. # : 1 **Date/Time :** 05/26/98; 11-30 a.m.

Tube #	Medium count for 10 ul (cpm)	Avg. cpm	dpm [cpm/0.7056]	μ Ci/ml (A_t) on counting [dpm/22200]	μ Ci/ml (A_o) on addition [$A_t/e^{-\lambda t}$]
1	-15, 7, -10	0	0	0	0
2	24, 0, 9	0	0	0	0
3	16, 8, -7	0	0	0	0
4	-6, -12, -2	0	0	0	0
5	-5, -17, 14	0	0	0	0
6	306, 320, 328	318	450·6	0·0203	0·0233
7	345, 346, 390	360·3	510·6	0·023	0·0264
8	330, 364, 249	331	469·1	0·0211	0·0242
9	352, 334, 378	354·6	502·6	0·0226	0·0260
10	302, 327, 272	300·3	425·6	0·0191	0·0220

05/14/98; 8-00 p.m

0·8705

qpw for 300 µl Cells.

Expt Name : 125IUdR + 50-200 µg/ml MEA

Expt # : 1

Date / Time : 05/26/98; 12-00 noon.

1	1.00	32	287
2	1.00	21 (blank)	253
3	1.00	20	266
4	1.00	1C 35	258
5	1.00	31	282
6	1.00	34	246
7	1.00	2C 29	285
8	1.00	22	263
9	1.00	35	248
10	1.00	3C 26	239
11	1.00	22	261
12	1.00	4C 31	245
13	1.00	24	292
14	1.00	18	245
15	1.00	5C 23	246
16	1.00	23	283
17	1.00	28	268
18	1.00	6C 556	815
19	1.00	548	821
20	1.00	582	833
21	1.00	7C 309	567
22	1.00	318	541
23	1.00	265	512
24	1.00	8C 377	649
25	1.00	418	656
26	1.00	348	604
27	1.00	9C 297	556
28	1.00	307	571
29	1.00	346	575
30	1.00	10C 668	917
31	1.00	710	938
32	1.00	667	937
33	1.00	~~27~~	250
34	1.00	~~26~~	266

TABLE-2

Expt. # : 1

Date/Time : 05/26/98; 12-00

Tube #	Radioactivity for 300 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.7056]	μCi/ml (A_t) on counting [dpm/666000]	μCi/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1	-6, 9, 5	0	0	0	0
2	8, 3, -4	0	0	0	0
3	9, 0, -4	0	0	0	0
4	5, -2, -8	0	0	0	0
5	-3, -3, 2	0	0	0	0
6	530, 522, 556	536	759.6	0.00114	0.001296
7	283, 292, 239	271.3	384.5	0.00057	0.000656
8	351, 392, 322	355	503.1	0.00075	0.000859
9	271, 281, 320	290.6	411.9	0.00061	0.000703
10	642, 684, 641	655.66	929.2	0.00139	0.00158

05/15/98; 9-00 a.m

11 days + 3h

= 267

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 267}{1440}}$

$= 0.8794$

TABLE-3

Expt. # : 1 **Date/Time :** 05/18/98

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	480, 490, 499			0
2	392, 415, 442			0
3	311, 325, 309			0
4	375, 390, 392			0
5	350, 329, 341			0
6	425, 419, 433	425.6	170266.6	0.00761
7	280, 270, 262	270.6	108266.6	0.00605
8	355, 342, 352	349.6	139866.6	0.00614
9	285, 270, 272	275.6	110266.6	0.00637
10	475, 490, 411	458.6	183466.6	0.00861

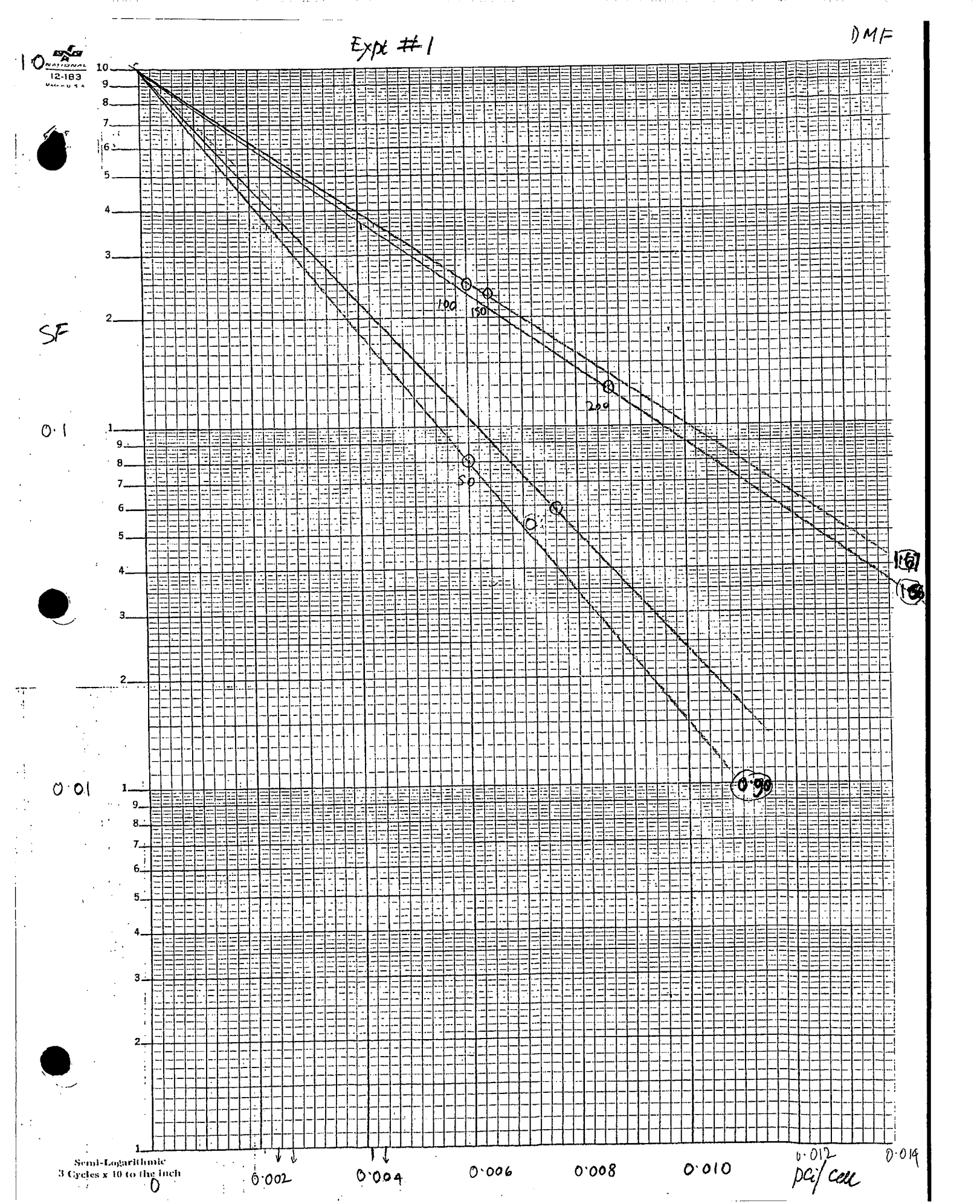
TABLE-4

Expt. #: 1 Date: 05/25/98

Colony Counts and Survival Fraction

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1·2	85	82	87	84·66	
2·2	74	76	78	76	0·8977
3·2	69	75	72	72	0·8504
4·2	58	46	52	52	0·6142
5·2	48	52	40	46·6	0·5512
6·3	56	44	50	5·0	0·059
7·3	58	64	60	6·06	0·0797
8·3	194	160	184	17·9	0·249
9·3	120	140	116	12·5	0·241
10·3	65	62	63	6·33	0·1357

Conc. of MEA (ug/ml)	OMF
50	0·90
100	1·67
150	1·67
200	1·56


Expt #1
DMF
10
12-183
SF
0·1
0·01
100
150
200
50
0
0·90
Semi-Logarithmic
3 Cycles x 10 to the Inch
0·002
0·004
0·006
0·008
0·010
0·012
0·014
psi/cell