

V79 COLONY FORMING ASSAY

Experiment Name : $^3\text{H}_2\text{O}$ + 50-200 ug/ml MEA; **Exp. # :** 2; **Investigator:** A. Bishayee
Date: 07/23/98

1. Set the rocker-roller at 37°C incubator, set the Coulter Counter, wash cells (from 75 cm² 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 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 : 399 333 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:** 07/23/98; 3-30 p.m.
5. Obtain $^3\text{H}_2\text{O}$ from refrigerator (25 mCi/ml) NEN Catalog # NET001C
6. After 3-4 h, remove test tubes from roller and add MEMB and/or $^3\text{H}_2\text{O}$ according to Table below. **Date/Time:** 07/23/98; 7-30 p.m.

Tube #	$^3\text{H}_2\text{O}$ Conc. (mCi/ml)	Cells in MEMB (ml)	MEMB (ul)	$^3\text{H}_2\text{O}$ [25 mCi/ml] (ul)	Sterile MEA in MEMB [2 ug/ul] ²⁰ (ul)	MEMB (ul)	MEA Conc. (ug/ml)
1	0	1.0	750	0	0	250	0
2	0	1.0	750	0	50 ⁵	200 ²⁴⁵	50
3	0	1.0	750	0	100 ¹⁰	150 ²⁴⁰	100
4	0	1.0	750	0	150 ¹⁵	100 ²³⁵	150
5	0	1.0	750	0	200 ²⁰	50 ²³⁰	200
6	0.75	1.0	690	60	0	250	0
7	0.75	1.0	690	60	50 ⁵	200 ²⁴⁵	50
8	0.75	1.0	690	60	100 ¹⁰	150 ²⁴⁰	100
9	0.75	1.0	690	60	150 ¹⁵	100 ²³⁵	150
10	0.75	1.0	690	60	200 ²⁰	50 ²³⁰	200

7. Return test tubes to roller for 12 h, increase the elevation angle of the roller.

Date/Time: 07/23/98; 7-45 p.m.

8. While test tubes are in roller, obtain sterile MEA (²⁰ 2 mg/ml or ²⁰ 2ug/ul) from refrigerator, move roller to 10.5°C, obtain ice
9. After ~12 h incubation period, remove tubes from incubator, chill on ice
10. Add MEA and/or MEMB according to the Table, vortex, quickly return to ice
Date/Time : 07/24/98; 9-00 a.m.
11. Transfer tubes to roller at 10.5 °C for 72 h. Date/Time: 07/24/98; 9-15 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: 07/27/98; 12-15 p.m.
13. Transfer 10 ul medium to test tubes containing 490 ul MEMB (1: 50 dilution)
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
AS - 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 transferring 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. Incubate petridishes for 1 week
28. Transfer 100 µl of cell suspension (in triplicate) to prelabelled vial (C) containing 3 ml liquid scintillation cocktail for each tube
29. Transfer 10 ul (in triplicate) from tubes in step 13 to prelabelled vial (M) containing 3 ml liquid scintillation cocktail for each tube
30. Count vials in steps 28 and 29 for radioactivity in Beckman Scintillation Counter
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

Expt. # 2

07/23/98

Initial Cell Count = 4349, 4435, 4463
Avg Cell Count = 4415.6
Cell conc. = 1766266 Cells/ml

For dilution,

$$\text{Vol. of Cell Suspension required} = \frac{4400000}{1766266} \\ = 2.49 \approx 2.5$$

Take 2.5 ml cells + 8.5 ml MEMB = 11 ml

After dilution,

Final Cell Count = 1016, 990, 989
Avg. Cell count = 998.3
Cell conc. = 399333 Cells/ml

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USER:10 ID:TRITIUM PRESET TIME: 1.00 MON 27 JUL 1998 16:59
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
 H#: 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	17.00	48.51	1.00	1.57	50.0	
2	**	2	21.00	43.64	1.00	3.37	50.0	
3	**	3	26.00	39.22	1.00	5.14	55.0	
4	**	4	31.00	35.92	1.00	6.93	55.0	
5	**	5	43.00	30.50	1.00	8.71	55.0	
6	**	6	25.00	40.00	1.00	10.48	56.0	
7	**	7	17.00	48.51	1.00	12.28	56.0	

8	**	- 8	1		24.00	40.82	1.00	14.01	55.0
9	**	- 9	1		27.00	38.49	1.00	15.75	56.0
10	**	-10	1		33.00	34.82	1.00	17.49	54.0
11	**	-11	1		41.00	31.23	1.00	19.22	56.0
12	**	-12	1	3M	43.00	30.50	1.00	21.00	56.0
13	**	-13	1		25.00	40.00	1.00	22.77	55.0
14	**	-14	1	4M	30.00	36.51	1.00	24.51	56.0
15	**	-15	1		35.00	33.81	1.00	26.30	56.0
16	**	-16	1		35.00	33.81	1.00	28.03	56.0
17	**	-17	1	5M	42.00	30.86	1.00	29.77	58.0
18	**	-18	1		169668.58	1.16	0.17	30.73	57.0
19	**	- 1	1	1CM	168908.58	1.16	0.17	31.78	56.0
20	**	- 2	1		158926.66	1.30	0.15	32.66	56.0
21	**	- 3	1		179850.00	1.49	0.10	33.60	57.0
22	**	- 4	1		175440.00	1.14	0.17	34.56	57.0
23	**	- 5	1	7M	177913.33	1.22	0.15	35.45	57.0
24	**	- 6	1		178605.72	1.13	0.17	36.41	56.0
25	**	- 7	1		164500.00	1.27	0.15	37.28	56.0
26	**	- 8	1	8M	170805.72	1.16	0.17	38.25	55.0
27	**	- 9	1		165988.58	1.17	0.17	39.22	55.0
28	**	-10	1		172240.00	1.15	0.17	40.17	56.0
29	**	-11	1	9M	169560.00	1.16	0.17	41.13	55.0
30	**	-12	1		169240.00	1.26	0.15	42.02	56.0
31	**	-13	1		168251.44	1.17	0.17	42.98	57.0
32	**	-14	1	1CM	162331.44	1.19	0.17	43.94	54.0
37	**	- 1	1		96.00	20.41	1.00	45.85	81.0
38	**	- 2	1	1C	86.00	21.57	1.00	47.63	80.0
39	**	- 3	1		93.00	20.74	1.00	49.42	81.0
40	**	- 4	1		112.00	18.90	1.00	51.15	83.0
41	**	- 5	1	2C	89.00	21.20	1.00	52.88	82.0
42	**	- 6	1		98.00	20.20	1.00	54.67	83.0
43	**	- 7	1		101.00	19.90	1.00	56.41	82.0
44	**	- 8	1		117.00	18.49	1.00	58.20	80.0
45	**	- 9	1	3C	103.00	19.71	1.00	59.98	82.0
46	**	-10	1		136.00	17.15	1.00	61.77	80.0
47	**	-11	1		149.00	16.38	1.00	63.50	80.0
48	**	-12	1	4C	111.00	18.98	1.00	65.29	82.0
49	**	-13	1		124.00	17.96	1.00	67.02	80.0
50	**	-14	1	5C	117.00	18.49	1.00	68.81	80.0

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SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
51	**	15	1	142.00	16.78	1.00	70.53	82.0
52	**	16	1	4909.00	2.85	1.00	72.34	82.0
53	**	17	1	5171.00	2.78	1.00	74.13	81.0
54	**	18	1	5244.00	2.76	1.00	75.93	81.0
55	**	1	1	4497.00	2.98	1.00	77.81	79.0
56	**	2	1	4405.00	3.01	1.00	79.54	81.0
57	**	3	1	4575.00	2.96	1.00	81.34	81.0
58	**	4	1	4619.00	2.94	1.00	83.13	81.0
59	**	5	1	4713.00	2.91	1.00	84.92	81.0
60	**	6	1	4757.00	2.90	1.00	86.64	82.0
61	**	7	1	4286.00	3.05	1.00	88.43	81.0
62	**	8	1	4585.00	2.95	1.00	90.22	83.0
63	**	9	1	4522.00	2.97	1.00	91.96	82.0
64	**	10	1	4275.00	3.06	1.00	93.75	81.0
65	**	11	1	4530.00	2.97	1.00	95.58	81.0
66	**	12	1	4153.00	3.10	1.00	97.38	79.0

TABLE-1

Expt. # : 2

Date/Time : 07/27/98; 5-00 p.m.

Tube #	Medium count for 10 ul (cpm)	Avg. cpm	dpm [cpm/0.52]	$\mu\text{Ci/ml (A)}$ on counting [dpm/444]	$\mu\text{Ci/ml (A}_0)$ on addition [A/e ^{-λt]}
1	7, 12, 24				
2	6, -2, 5				
3	8, 14, 22				
4	24, 6, 11				
5	16, 16, 23				
6	169649, 168889, 158907	165815	318875	718.18	0.7181
7	179831, 175421, 177844	177715	341760	769.73	0.7697
8	178586, 164481, 170786	171284	329392	741.87	0.7418
9	165969, 172221, 169541	169243	325468	733.03	0.7330
10	169221, 168232, 162312	166588	320362	721.53	0.7215

TABLE-2

Expt. # :

Date/Time :

Tube #	Radioactivity for 100 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.52]	μ Ci/ml (A ₀) on counting [dpm/222000]	μCi/ml (A₀) after 12 h incubation [A₀e^{-λt}]
1	77, 67, 74				
2	93, 70, 79				
3	82, 98, 84				
4	117, 130, 92				
5	105, 98, 123				
6	4890, 5152, 5225	5089	9786.5	0.0440	
7	4478, 4386, 4556	4473.3	8602.5	0.0387	
8	4600, 4694, 4738	4677.3	8994.8	0.0405	
9	4267, 4566, 4503	4445.3	8548.7	0.0385	
10	4256, 4511, 4134	4300.3	8269.8	0.0372	

TABLE-3

Expt. # : 2

Date/Time : 07/27/98; 2-20 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	776, 815, 815, 834	821	328533	
2	793, 787, 796	792	316800	
3	720, 738, 738	732	292800	0.1502
4	856 , 777, 822, 774	791	316400	0.1223
5	856, 821, 799	825	330133	
6	831, 853, 824	836	334400	0.1315
7	653, 679, 670	667	266933	0.1449
8	683, 678, 687	682	273066	0.1483
9	779, 759, 768	768	307466	0.1252
10	921, 932, 927	926	370666	0.1003

TABLE-4

Expt. #: 2

Date: 08/03/98

Colony Counts and Survival Fraction

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony for x.2	SF
1.2	150	165	149	154.66	
2.2	132	147	140	139.66	0.9030
3.2	126	135	130	130.33	0.8427
4.2	100	109	92	100.33	0.6487
5.2	90	93	86	89.66	0.5797
6.3	90	95	86	90.3	0.0584
7.3	168	195	180	180.76	0.1343
8.2	48	58	39	48.33	0.3708
9.2	30	39	22	30.33	0.3023
10.3	170	177	169	172	0.1918

MEA Conc.
($\mu\text{g/ml}$)

DMF

50

1.46

100

2.96

150

2.43

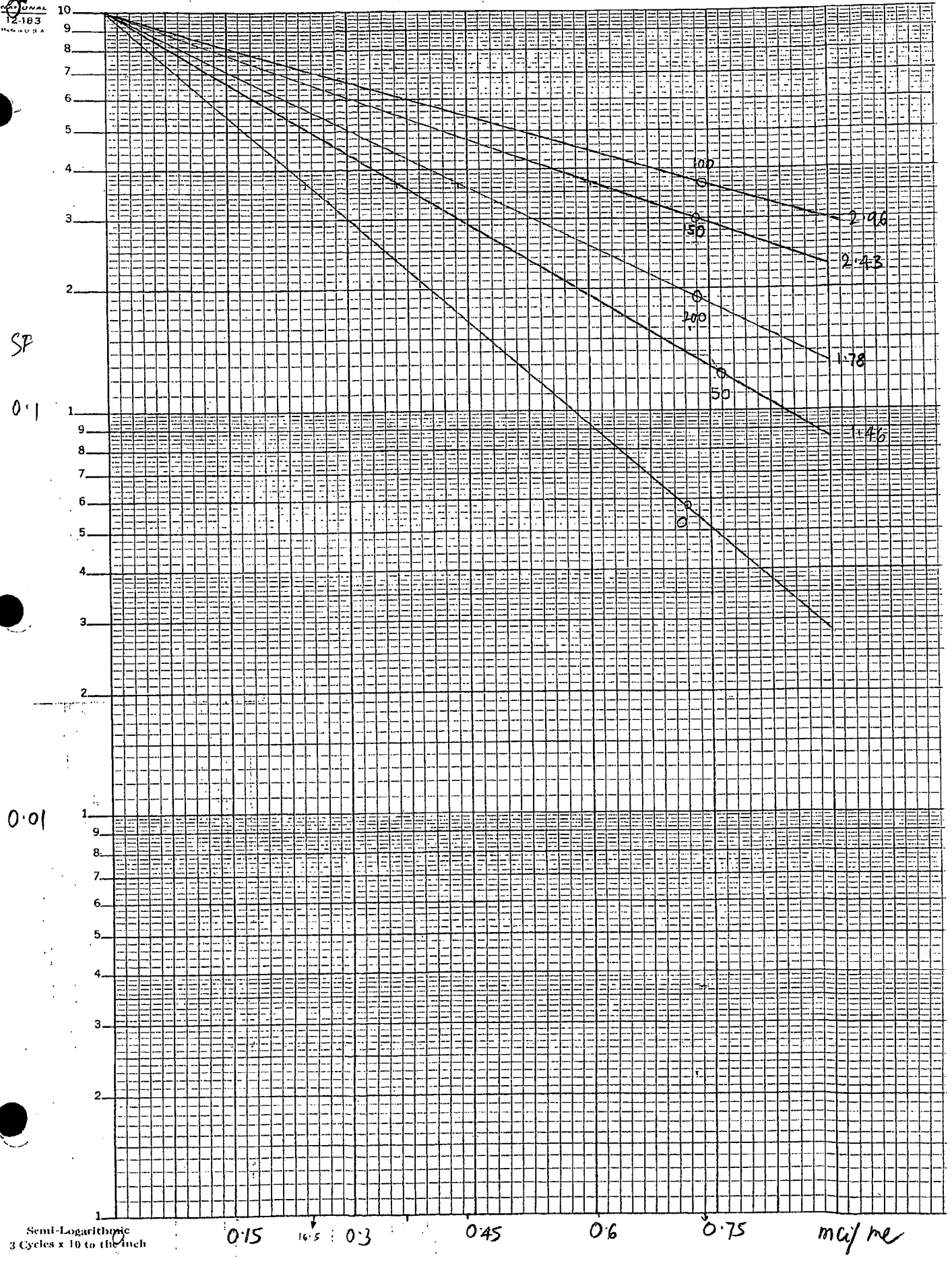
200

1.78



Exp. #2

3H₂O + 50-200 µg/ml MEA



SP

0.1

0.01

Semi-Logarithmic
3 Cycles x 10 to the inch

0.15

0.3

0.45

0.6

0.75

mμ/ml