

V79 COLONY FORMING ASSAY

Experiment Name : ^{210}Po -citrate + 100ug/ml MEA ;

Exp. # : 3;

Investigator: A. Bishayee

Date: 12/08/98

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 : 436,800 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: 12/08/98; 1-00 p.m.
5. Calibrate the stock ^{210}Po -citrate for today ($\mu\text{Ci/ml}$)
6. After 3-4 h, remove test tubes from roller and add according to Table below.

Date/Time: 12/08/98; 3-45 p.m.

Tube #	^{210}Po -citrate uCi/ml	Cells in MEMB (ml)	MEMB (ul)	Po-citrate (4.9 uCi/ml) on 11/20 (ul)	MEA in MEMA (100 ug/ml) (ml)	MEMA (ml)
1	0	1.0	1000	0	2	0
2	0	1.0	1000	0	2	0
3	0.2	1.0	920	80	2	0
4	0.35	1.0	855	145	2	0
5	0.5	1.0	800	200	2	0
6	0	1.0	1000	0	0	2
7	0	1.0	1000	0	0	2
8	0.2	1.0	920	80	0	2
9	0.35	1.0	855	145	0	2
10	0.5	1.0	800	200	0	2

7. Return test tubes to roller for 30 min.

Date/Time: 12/08/98; 4-00 p.m.

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

Date/Time: 12/08/98; 4-30 p.m.

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 2 ml of MEMA with or without 100 ug/ml MEA as per Table

$$\text{MEMA} = \frac{10 \times 10^{-9} \times 945 \text{ ng}}{0.055} = \text{MEMA (20 ng/ml)}$$

18. Transfer tubes at 10°C for 72 h. **Date/Time:** 12/08/98; 5-15 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:** 12/09/98; 1-10 p.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:**
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 :**
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 #3

12/08/98

Initial cell count = 6383, 6245, 6288
Avg. cell count = 6305
Cell conc. = 6305×400
= 2522133 cells/ml

For dilution,

$$\text{Vol. required} = \frac{4400000}{2522133} = 1.74 \text{ ml}$$

Take 1.8 ml Cells + 9.2 ml WEMB = 11 ml

After dil.

Final count = 1123, 1095, 1058
Avg. count = 1092
Cell conc. = 436,800 cells/ml

F-151

²¹⁰Pb + 100 µg/ml KEA
30µl medium

USER: 5 ID:PU-210 PRESET TIME: 1.00 WED 09 DEC 1998 13:12
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
 H#: 1 AOC:N BCF:N RCM:N
 CHANNEL 1-LL:600 UL: 900 SIGMA: 2.00 BKG SUB: 0.00 BKG SIG: 0.00 LSR: 0
 DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:0 1.00000
 HALF LIFE(DAYS):N

SAM	POS	CH	CPM	SIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	4.00	100.0	1.00	1.60	57.0	
2	**	2	12.00	57.74	1.00	3.43	59.0	
3	**	3	17.00	48.51	1.00	5.15	55.0	
4	**	4	7.00	75.59	1.00	6.88	57.0	
5	**	5	9.00	66.67	1.00	8.57	58.0	
6	**	6	10.00	63.25	1.00	10.35	60.0	
7	**	7	10847.37	1.97	0.95	12.09	55.0	
8	**	8	11001.05	1.96	0.95	14.03	59.0	
9	**	9	11508.89	1.97	0.90	15.61	57.0	
10	**	10	21254.00	1.94	0.50	16.78	60.0	
11	**	11	21948.00	1.91	0.50	17.95	58.0	
12	**	12	22425.26	1.94	0.48	19.15	58.0	
13	**	1	30234.29	1.94	0.35	20.22	58.0	
14	**	2	33560.00	1.92	0.33	21.27	52.0	
15	**	3	32617.14	1.87	0.35	22.39	57.0	
16	**	4	8.00	70.71	1.00	24.22	59.0	
17	**	5	8.00	70.71	1.00	25.94	58.0	
18	**	6	16.00	50.00	1.00	27.67	57.0	
19	**	7	9.00	66.67	1.00	29.36	60.0	
20	**	8	17.00	75.59	1.00	31.03	59.0	
21	**	9	9.00	66.67	1.00	32.77	57.0	
22	**	10	10683.08	1.96	0.97	34.47	61.0	
23	**	11	11335.55	1.98	0.90	36.35	58.0	
24	**	12	11304.86	1.96	0.93	38.01	60.0	
25	**	1	24342.22	1.91	0.45	39.19	56.0	
26	**	2	21956.00	1.91	0.50	40.36	57.0	
27	**	3	24602.35	1.96	0.43	41.51	59.0	
28	**	4	23455.58	1.95	0.45	42.62	54.0	
29	**	5	31422.86	1.91	0.35	43.64	58.0	
30	**	6	32428.57	1.88	0.35	44.66	60.0	
31	**	1	4.00	51.65	1.00	46.48	79.0	
32	**	2	4.00	100.0	1.00	48.23	59.0	
33	**	3	5.00	89.44	1.00	50.24	74.0	
34	**	4	8.00	70.71	1.00	52.26	55.0	
35	**	5	4.00	100.0	1.00	53.98	80.0	
36	**	6	5.00	89.44	1.00	55.71	84.0	
37	**	7	7.00	75.59	1.00	57.43	79.0	

TABLE-1

Expt. # : 3

Date/Time : 12/09/98; 1-10 p.m.

Tube #	Medium count for 30 ul (cpm)	Avg. cpm	dpm [cpm/1]	μ Ci/ml (A) on counting [dpm/66600]	μ Ci/ml (A ₀) on addition [A ₁ /e ^{-λt}]
1	See the attached				
2	sheet				
3		11118	11118	0.1669	0.1669
4		21875	21875	0.3284	0.3284
5		32137	32137	0.4825	0.4825
6					
7					
8		11107	11107	0.1667	0.1667
9		23633	23633	0.3548	0.3548
10		31925	31925	0.4793	0.4793

10

240p0 + MEA Expts #3
500 µl cells

USER: 5 ID:FC-210 PRESET TIME: 1.00 MON 14 DEC 1998 14:08
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
H#: 1 AGC:N DCF:N RCM:N
CH: CH1-LL:600 UL: 900 ZSIGMA: 2.00 BKG SUB: 0.00 BKG ZSIG: 0.00 LSR: 0
DA: CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000
HALF LIFE(DAYS):N

SAM	POS	CH	CPM	ZSIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	7.00	75.59	1.00	1.60	110.0	
2	**	2	7.00	75.59	1.00	3.23	99.0	
3	**	3	8.00	70.71	1.00	4.92	107.0	
4	**	4	11.00	60.30	1.00	6.54	107.0	
5	**	5	667.00	7.74	1.00	8.22	107.0	
6	**	6	753.00	7.29	1.00	9.89	110.0	
7	**	7	815.00	7.01	1.00	11.53	106.0	
8	**	8	853.00	6.85	1.00	13.21	108.0	
9	**	9	648.00	7.86	1.00	14.89	108.0	
10	**	10	634.00	7.94	1.00	16.63	106.0	
11	**	11	2.00	141.4	1.00	18.30	107.0	
12	**	12	2.00	57.74	1.00	19.93	108.0	
13	**	1	6.00	81.65	1.00	21.72	105.0	
14	**	2	10.00	63.25	1.00	23.36	103.0	
15	**	3	1186.00	5.81	1.00	25.04	107.0	
16	**	4	1228.00	5.71	1.00	26.67	103.0	
17	**	5	1194.00	5.79	1.00	28.36	103.0	
18	**	6	1251.00	5.65	1.00	30.24	107.0	
19	**	7	1349.00	5.45	1.00	31.87	104.0	
20	**	8	1315.00	5.52	1.00	33.56	108.0	

TABLE-2

Expt. # : 3

Date/Time : 12/14/98; 2-00 p.m.

Tube #	Radioactivity for 500 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/1]	μ Ci/ml (A) on counting [dpm/111x10 ⁴]	μ Ci/ml (A ₀) after 12 h incubation [A ₀ e ^{-λt}]
1	See the attached				
2	Sheet				
3		710	710	0.000639	
4		834	834	0.000751	
5		641	641	0.000577	
6					
7					
8		1207	1207	0.00108	
9		1222	1222	0.001101	
10		1332	1332	0.0012	

TABLE-3

Expt. # :

Date/Time :

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	371, 349, 361	360	144133	-
2	355, 359, 372	362	144800	-
3	340, 325, 322	329	131600	4.85
4	309, 322, 330	320	128133	5.86
5	237, 250, 245	247	107600 97600	5.90
6	319, 333, 323	325	130000	-
7	366, 342, 353	353	141466	-
8	497, 480, 495	490	196266	5.5 5.5
9	445, 460, 432	445	178266	6.17
10	421, 409, 415	415	166000	7.22

TABLE-4

Expt # : 3

Date : 12/17/98

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony Per x.2	SF
1.2	135	145	147	} 133	
2.2	122	130	119		
3.3	31	27	35	3.1	0.023
4.3	20	24	18	2.0	0.015
5.4	123	134	114	1.3	0.010
6.2	165	152	158	} 147	
7.2	137	129	141		
8.4	58	62	54	0.58	0.0039
9.4	25	27	29	0.27	0.0018
10.4	19	22	17	0.19	0.001

1.5

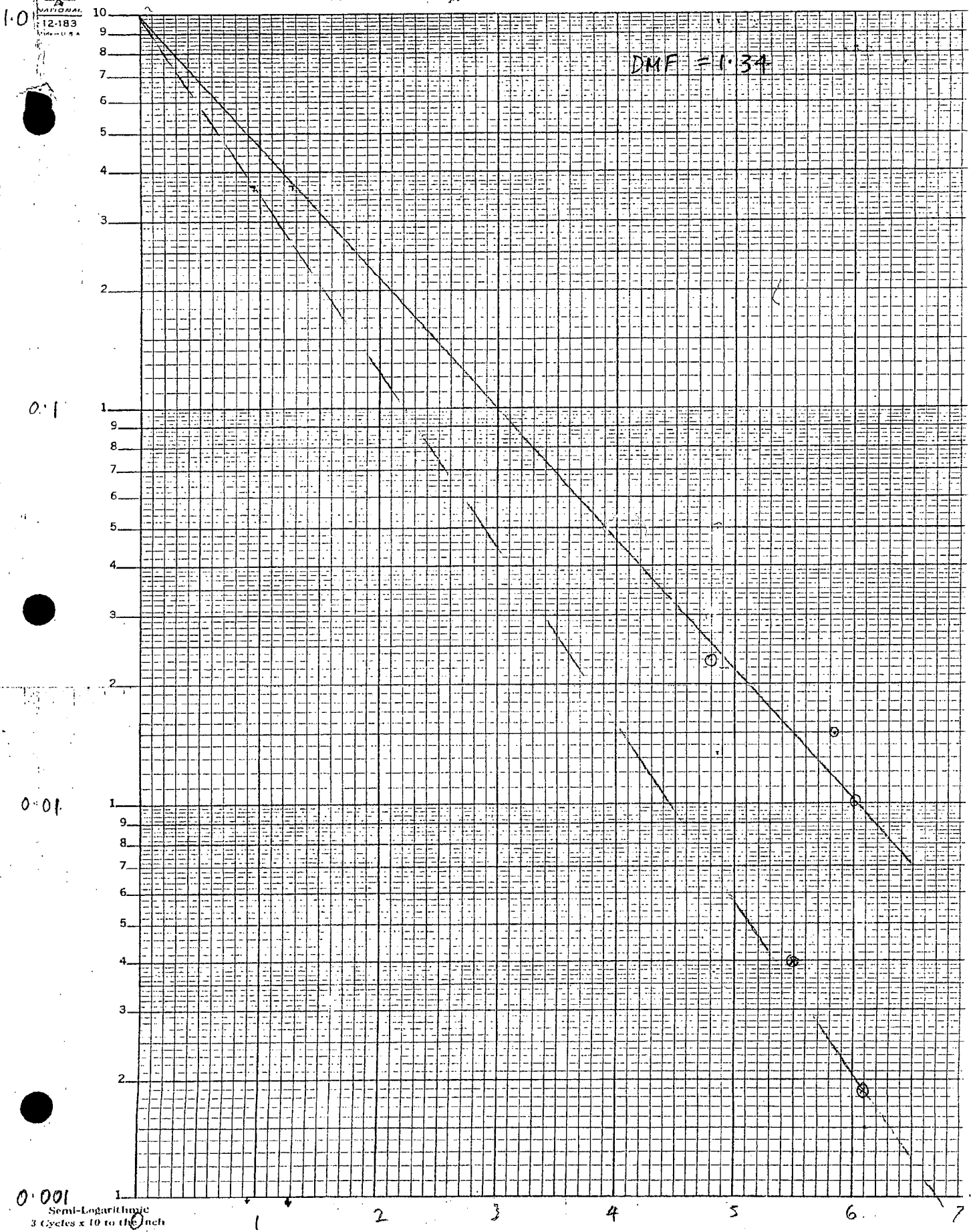


210p₀ + (0.0Mg) ml MEA.

EXPT # 3

NATIONAL
12-183
U.S.A.

DMF = 1.34



0.001
Semi-Logarithmic
3 Cycles x 10 to the Inch