

V79 COLONY FORMING ASSAY

Experiment Name : ^{210}Po -citrate + 50-200ug/ml MEA ;

Exp. # : 1;

Investigator: A. Bishayee

Date: 01/15/98

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 : 408933 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:** 01/15/99 ; 3-00 p.m.
5. Calibrate the stock ^{210}Po -citrate for today (12 µCi/ml) on 12/11/98
6. After 3-4 h, remove test tubes from roller and add according to Table below.

Date/Time: 01/15/99; 6-30 p.m.

Tube #	^{210}Po -citrate µCi/ml	Cells in MEMB (ml)	MEMB (ml)	Po-citrate (12 µCi/ml) on 12/11 (ml)	MEA in MEMA (200 ug/ml) (ml)	MEMA (ml)	MEA Conc. (ug/ml)
1	0	1.0	1	0	0	2	0
2	0	1.0	1	0	0.5	1.5	50
3	0	1.0	1	0	1	1	100
4	0	1.0	1	0	1.5	0.5	150
5	0	1.0	1	0	2	0	200
6	0.2	1.0	0.965	0.035	0	2	0
7	0.2	1.0	0.965	0.035	0.5	1.5	50
8	0.2	1.0	0.965	0.035	1	1	100
9	0.2	1.0	0.965	0.035	1.5	0.5	150
10	0.2	1.0	0.965	0.035	2	0	200

7. Return test tubes to roller for 30 min.

Date/Time: 6-15 p.m. 01/15/99

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

Date/Time: 6-45 p.m. 01/15/99

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 pellet
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 MEMA with or without MEA as per Table
18. Transfer tubes at 10°C for 72 h. Date/Time: 01/15/99; 7-30 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: 01/19/99; 11-45 a.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: 01/18/99; 1-00 p.m.
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 : 01/20/99; 2-00 p.m.
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 # 1

01/15/99

Initial cell count = 6161, 6182, 5955
Avg. cell count = 6099
Cell conc. = 2439733 cells/ml

For dilution,

$$\text{vol required} = \frac{4400000}{2439733} = 1.8 \text{ ml}$$

Take 1.8 ml cells + 9.2 ml MEMB = 11 ml

After dilution,

Final counts = 1081, 993, 993
Avg. count. = 1022
Cell conc. = 408933 cells/ml

2.2
1.8

Exp#1

USER: 5 ID:PO-210 PRESET TIME: 1.00 TUE 19 JAN 1999 11:49
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N

#: 1 AGC:N BCF:N RCM:N
CHANNEL 1-LL:600 UL: 900 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:0 1.00000
LIFE(DAYS):N

20ul medium

AM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	8.00	70.71	1.00	1.71	61.0	
2	**	2	1M { 1.00	60.30	1.00	3.44	61.0	
3	**	3	10.00	63.25	1.00	5.08	60.0	
4	**	4	2M { 9.00	66.67	1.00	6.71	61.0	
5	**	5	9.00	66.67	1.00	8.59	61.0	
6	**	6	3M { 2.00	57.74	1.00	10.42	63.0	
7	**	7	5.00	89.44	1.00	12.34	60.0	
8	**	8	4M { 7.00	75.59	1.00	14.03	62.0	
9	**	9	10.00	63.25	1.00	15.71	63.0	
10	**	10	5M { 7.00	75.59	1.00	17.34	59.0	
11	**	11	11812.94	2.00	0.85	18.97	60.0	
12	**	12	13190.00	1.95	0.80	20.56	60.0	
13	**	1	11665.55	1.95	0.90	22.10	61.0	
14	**	2	17M { 3762.58	1.94	0.77	23.51	61.0	
15	**	3	11574.86	1.99	0.88	25.01	60.0	
16	**	4	12673.62	1.97	0.81	26.65	62.0	
17	**	5	11331.89	1.95	0.93	28.46	61.0	
18	**	6	19M { 3121.25	1.95	0.80	29.94	62.0	
19	**	7	12167.27	2.00	0.82	31.39	61.0	
20	**	8	10M { 2405.88	1.95	0.85	32.92	61.0	
21	**	9	8.00	70.71	1.00	34.75	96.0	
22	**	10	8.00	70.71	1.00	36.58	93.0	
23	**	11	7.00	75.59	1.00	38.22	91.0	
24	**	12	10.00	63.25	1.00	39.85	91.0	
25	**	1	8.00	70.71	1.00	41.54	98.0	
26	**	2	7.00	75.59	1.00	43.32	94.0	
27	**	3	4.00	100.0	1.00	45.11	102.0	
28	**	4	6.00	81.65	1.00	46.94	92.0	
29	**	5	5.00	89.44	1.00	48.57	95.0	
30	**	6	8.00	70.71	1.00	50.25	94.0	
31	**	7	874.00	6.77	1.00	51.93	99.0	
32	**	8	978.00	6.40	1.00	53.72	95.0	
33	**	9	2979.00	3.66	1.00	55.44	98.0	
34	**	10	2957.00	3.68	1.00	57.27	94.0	
35	**	11	4500.00	2.98	1.00	58.90	93.0	
36	**	12	4153.00	3.10	1.00	60.57	99.0	
37	**	1	4264.00	3.06	1.00	62.21	111.0	
38	**	2	4370.00	3.03	1.00	63.99	105.0	
39	**	3	3343.00	3.46	1.00	65.87	103.0	
40	**	4	3392.00	3.43	1.00	67.56	107.0	

TABLE-1

Expt. # : 1

Date/Time : 01/19/99; 11-45 a.m

Tube #	Medium count for 50 ul (cpm)	Avg. cpm	dpm [cpm/1]	μ Ci/ml (A) on counting [dpm/66600] 44400	μ Ci/ml (A ₀) on addition [A ₀ e ^{-λt}]
1	See the attached sheet				
2					
3					
4					
5					
6		12501	12501	0.2815	
7		12713	12713	0.2863	
8		12123	12123	0.2731	
9		12226	12226	0.2754	
10		12286	12286	0.2767	

F-157

Expt #1

SR: 5 ID:PO-210 PRESET TIME: 1.00
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
1 AGC:N QCF:N RCM:N
CHANNEL 1-LL:600 UL: 900 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:0 1.00000
LIFE(DAYS):N *500mc ells*

WED 20 JAN 1999 13:49

POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
**1	1	9.00	66.67	1.00	1.61	102.0	
**2	1	<i>m</i> 11.00	60.30	1.00	3.19	100.0	
**3	1	5.00	89.44	1.00	4.77	96.0	
**4	1	<i>m</i> 13.00	55.47	1.00	6.50	97.0	
**5	1	15.00	51.64	1.00	8.18	99.0	
**6	1	<i>3m</i> 19.00	66.67	1.00	9.82	98.0	
**7	1	11.00	60.30	1.00	11.60	109.0	
**8	1	<i>4m</i> 17.00	75.59	1.00	13.28	109.0	
**9	1	11.00	60.30	1.00	14.97	104.0	
**10	1	<i>5m</i> 11.00	60.30	1.00	16.60	105.0	
**11	1	935.00	6.54	1.00	18.23	110.0	
**12	1	<i>6m</i> 900.00	6.67	1.00	19.92	104.0	
**1	1	995.00	6.34	1.00	21.70	83.0	
**2	1	<i>7m</i> 54.00	6.48	1.00	23.33	83.0	
**3	1	912.00	6.62	1.00	25.12	78.0	
**4	1	<i>8m</i> 991.00	6.35	1.00	26.85	77.0	
**5	1	976.00	6.40	1.00	28.68	75.0	
**6	1	<i>9m</i> 919.00	6.27	1.00	30.51	78.0	
**7	1	932.00	6.55	1.00	32.18	81.0	
**8	1	<i>10m</i> 942.00	6.52	1.00	33.92	79.0	

TABLE-2

Expt. # : i

Date/Time : 01/20/99 ; 1-50 pm.

Tube #	Radioactivity for 500 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/1]	μ Ci/ml (A_t) on counting [dpm/111x10 ⁴]	μ Ci/ml (A_0) after 12 h incubation [$A_t e^{-\lambda t}$]
1	<i>See the attached sheet</i>				
2					
3					
4					
5					
6		917	917	0.000826	
7		975	975	0.000878	
8		952	952	0.000858	
9		997	997	0.000899	
10		937	937	0.000844	

TABLE-3

Expt. # : i

Date/Time : 01/18/99 ; 1-30 p.m.

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	505, 478, 482			
2	438, 424, 432			
3	435, 397, 381			
4	379, 394, 422			
5	418, 417, 397			
6	365, 345, 313	341	136400	6.056
7	⁴⁷⁶ 547, 542 ⁴⁹⁵ , 462	477	191066	4.595
8	478, 439, 449	455	182133	4.711
9	511, 488, 490	496	198400	4.531
10	467, 427, 399	431	172400	4.895

TABLE-4

Expt #: 1

Date: 01/25/99

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony <i>Ex. 2</i>	SF
1.2	176	162	155	164.33	-
2.2	135	145	155	145	0.8823
3.2	128	135	142	135	0.8215
4.2	110	127	97	111.31	0.6775
5.2	96	108	85	96.3	0.5862
6.4	44	46	39	0.43	0.0061
7.4	155	162	148	1.55	0.01068
8.3	63	70	56	6.3	0.0466
9.3	120	112	104	11.2	0.1006
10.3	77	68	60	6.83	0.0709

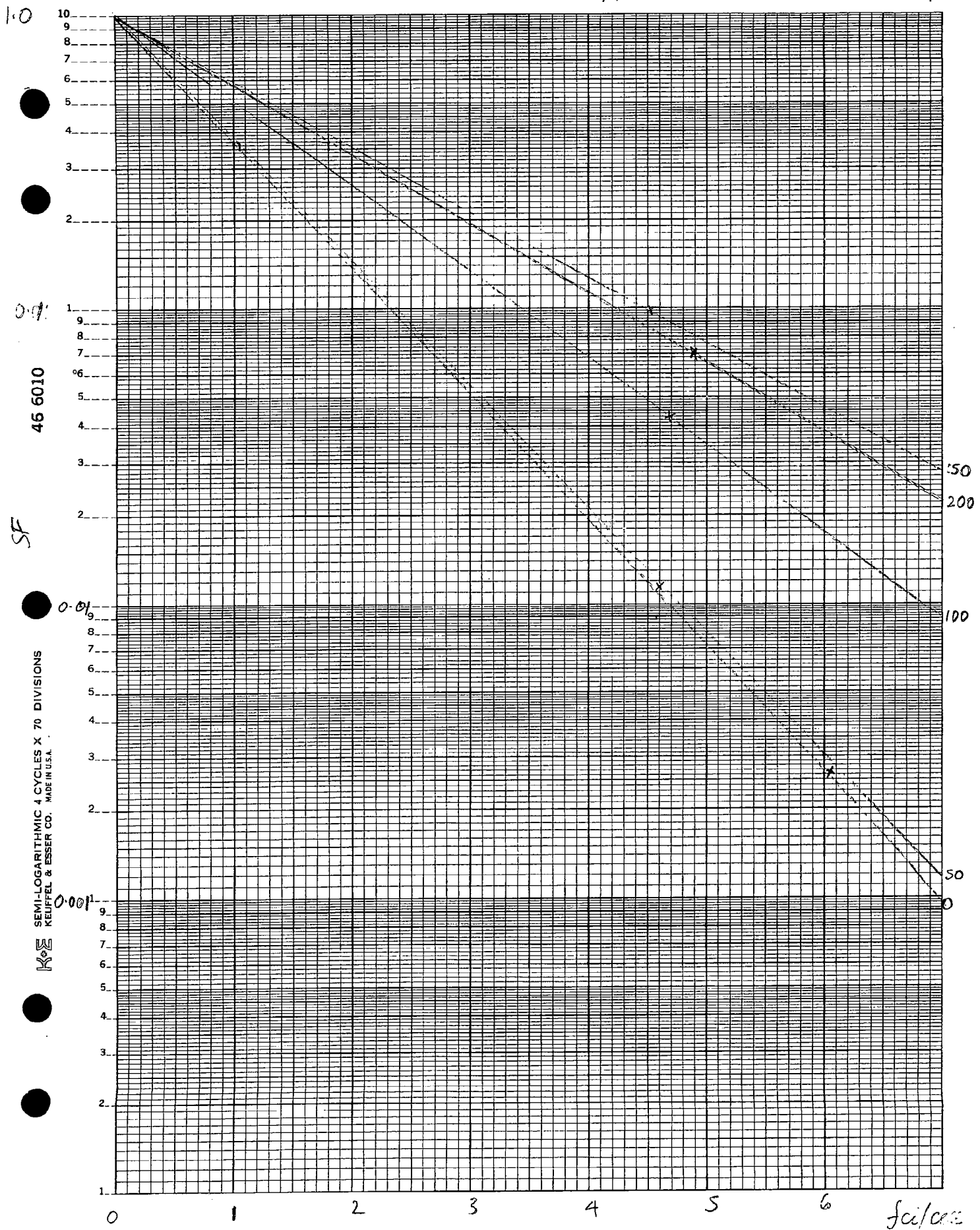
Conc. (mg/ml)

DMF

0	1
50	1.03
100	1.50
150	1.90
200	1.80

^{210}Po + MEA (50-200 $\mu\text{g}/\text{ml}$) Exp #1

$\mu\text{g}/\text{ml}$



1.0
0.1
46 6010
SF
0.01
0.001

SEMI-LOGARITHMIC 4 CYCLES X 70 DIVISIONS
KEUFFEL & ESSER CO. MADE IN U.S.A.
K&E

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