

V79 COLONY FORMING ASSAY

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

Exp. # : 1;

Investigator: A. Bishayee

Date: 11/10/98

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 : 450,933 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: 11/10/98; 3-00 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:

Tube #	^{210}Po -citrate uCi/ml	Cells in MEMB (ml)	MEMB (ul)	Po-citrate 6.23 ^{5.9} uCi/ml or 10/20/98 (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	935	65	2	0
4	0.35	1.0	885	115	2	0
5	0.5	1.0	840	160	2	0
6	0	1.0	1000	0	0	2
7	0	1.0	1000	0	0	2
8	0.2	1.0	935	65	0	2
9	0.35	1.0	885	115	0	2
10	0.5	1.0	840	160	0	2

$1000 - \frac{5.9}{1000} \times 1000$

7. Return test tubes to roller for 30 min. Date/Time: 11/10/98; 4-30 p.m.
8. After 30 min, centrifuge tubes for 10 min at 2000 rpm, 4°C Date/Time: 11/10/98; 5-00 p.m.
9. During the centrifugation move roller to 10.5°C
10. Collect 150 ul supernatant in separate tubes

1000-01-10

1000-01-10

1000-01-10

1.2
2.2
3.2, 3.3

4.2, 4.3, 4.4	5.2, 5.3, 5.4	6.2	7.2	8.2, 8.3	9.2, 9.3, 9.4	10.2, 10.3, 10.4

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
- $MEMA = 10.89 \mu\text{C}$
 $MEA (20 \text{ mg}/\mu\text{L}) = 0.11 \mu\text{L}$
18. Transfer tubes at 10°C for 72 h. Date/Time: 11/10/98; 6-30 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: 11/11/98; 10-45 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: 11-13-98; 1-30 a.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 : 11/13/98; 4:00 pm
 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.

MEA + P0-210

Exp. # 1

11/10/98

Initial cell count = 9801, 9562, 9573, 9704
Avg cell count = 9613
Cell conc. = 3,845,200 cells/ml

For dilution,

$$\text{Vol. required} = \frac{4400000}{3845200} = 1.14 \text{ ml}$$

Take 1.2 ml cells + 9.8 ml MEMB = 11 ml

After dilution,

Final cell count = 1163, 1251, 1069, 1150
Avg cell count = 1127.3
Cell conc. = 450.933 cells/ml

F-451

²¹⁰Po + 100ug/ml NEA : Expt. #1

30 ul medium

USER: 5 ID:PO-210 PRESET TIME: 1.00 WED 11 NOV 1998 10:42
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
 H# ABC:N QCF: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
 HALF LIFE(DAYS):N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	8.00	70.71	1.00	1.60	55.0	
2	**	2	3.00	115.5	1.00	3.28	56.0	
3	**	3	4.00	100.0	1.00	4.97	57.0	
4	**	4	10.00	63.25	1.00	6.70	57.0	
5	**	5	14.00	53.45	1.00	8.68	56.0	
6	**	6	9.00	66.67	1.00	10.37	57.0	
7	**	7	9894.00	2.01	1.00	12.24	59.0	
8	**	8	11088.52	1.99	0.92	13.99	57.0	
9	**	9	12242.35	1.96	0.85	15.53	55.0	
10	**	10	18707.83	1.93	0.57	16.83	51.0	
11	**	11	19392.73	1.94	0.55	18.05	57.0	
12	**	12	20937.87	1.93	0.51	19.38	56.0	
13	**	1	27042.50	1.92	0.40	20.62	55.0	
14	**	2	30469.33	1.87	0.38	21.71	57.0	
15	**	3	30024.66	1.91	0.36	22.89	55.0	
16	**	4	6.00	81.65	1.00	24.67	59.0	
17	**	5	9.00	66.67	1.00	26.34	57.0	
18	**	6	6.00	81.65	1.00	28.07	57.0	
19	**	7	5.00	89.44	1.00	29.89	57.0	
20	**	8	10.00	63.25	1.00	31.77	57.0	
21	**	9	10.00	63.25	1.00	33.60	56.0	
22	**	10	10722.05	1.96	0.97	35.30	55.0	
23	**	11	11874.12	1.99	0.85	36.83	58.0	
24	**	12	12616.25	1.99	0.80	38.30	58.0	
25	**	1	19072.38	2.00	0.52	39.61	58.0	
26	**	2	19634.29	1.97	0.52	40.86	56.0	
27	**	3	19666.67	1.97	0.52	42.11	57.0	
28	**	4	29102.86	1.98	0.35	43.22	56.0	
29	**	5	28965.33	1.92	0.38	44.32	55.0	
30	**	6	29034.29	1.98	0.35	45.63	56.0	

TABLE-1

Expt. # : {

Date/Time : 11/11/98; 10-45 a.m

Tube #	Medium count for 30 ul (cpm)	Avg. cpm	dpm [cpm/1]	μ Ci/ml (A _c) on counting [dpm/66600]	μ Ci/ml (A _o) on addition [A _c /e ^{-λt}]
1	<i>See the</i>				
2	<i>attached sheet</i>				
3		11074.9	11074.9	0.1662	
4		19679.4	19679.4	0.2954	
5		29178.8	29178.8	0.4381	
6					
7					
8		11737.4	11737.4	0.1762	
9		19457.7	19457.7	0.2921	
10		29034	29034	0.4359	

F-451/Howell

210Po f 100ug/ml MEA

USER: 5 ID:PO-210 PRESET TIME: 1.00 Exp #1 FRI 13 NOV 1998 15:47
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
 H# 1 AGC:N GCF:N RCM:N
 CHANNEL 1-LL:600 UL: 900 ZSIGMA: 2.00 BKG SUB: 0.00 BKG ZSIG: 0.00 LSR: 0
 DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR: 1.00000
 HALF LIFE(DAYS):N

SAM	POS	CH	CPM	ZSIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	6.00	81.65	1.00	1.65	121.0	
2	**	2	6.00	81.65	1.00	3.57	112.0	
3	**	3	6.00	81.65	1.00	5.25	109.0	
4	**	4	11.00	60.30	1.00	6.93	108.0	
5	**	5	675.00	7.70	1.00	8.62	106.0	
6	**	6	665.00	7.76	1.00	10.55	108.0	
7	**	7	1425.00	5.30	1.00	12.23	107.0	
8	**	8	1472.00	5.21	1.00	14.05	103.0	
9	**	9	1279.00	5.59	1.00	15.78	100.0	
10	**	10	1174.00	5.84	1.00	17.47	106.0	
11	**	11	6.00	81.65	1.00	19.15	101.0	
12	**	12	12.00	57.74	1.00	20.83	103.0	
13	**	1	8.00	70.71	1.00	22.57	107.0	
14	**	2	5.00	89.44	1.00	24.26	104.0	
15	**	3	448.00	9.45	1.00	26.18	102.0	
16	**	4	467.00	9.25	1.00	27.87	102.0	
17	**	5	1018.00	6.27	1.00	29.55	101.0	
18	**	6	980.00	6.39	1.00	31.23	98.0	
19	**	7	1014.00	6.28	1.00	32.92	101.0	
20	**	8	949.00	6.49	1.00	34.65	104.0	

500ul Cells

TABLE-2

Expt. # : 1

Date/Time : 11/13/98; 4:00 p.m.

Tube #	Radioactivity for 500 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/1]	μ Ci/ml (A_t) on counting [dpm/111 \times 10 ⁴]	μ Ci/ml (A_0) after 12 h incubation [$A_t/e^{-\lambda t}$]
1	6, 6				
2	6, 11				
3	675, 665	670	670	0.000603	
4	1279, 1174	1226	1226	0.001104	
5	1425, 1472	1448	1448	0.001304	
6	6, 12				
7	8, 5				
8	448, 467	457.5	457.5	0.000412	
9	1014, 949	981.5	981.5	0.000884	
10	1018, 980	999	999	0.0009	

TABLE-3

Expt. # : 1

Date/Time : 11/13/98; 2:00 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	484, 471, 465	473.3	189333	-
2	520, 490, 550 507	505.6	202266	-
3	592 , 539, 541, 535	538.3	215333	2.80
4	487 , 583, 570, 541	564.6	225866	4.88
5	541, 548, 567	552	220800	5.90
6	488, 481, 445	471	188533	-
7	533, 584, 591	569	227733	-
8	432, 432, 426	430	172000	2.39
9	644, 669, 626	646.3	258533	3.42
10	649, 624, 638	637	254800	3.53

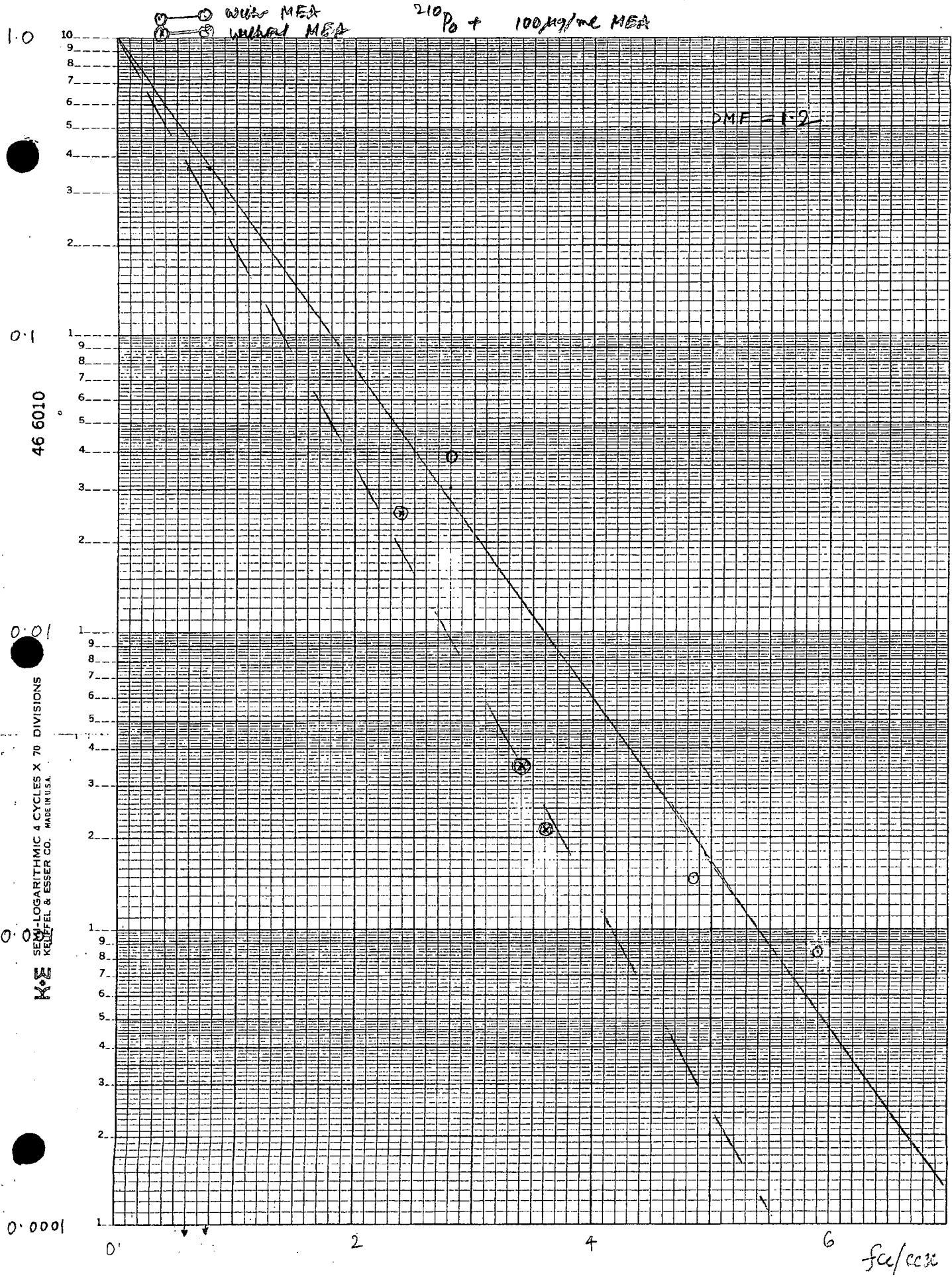
TABLE-4

Expt #: 1/11/20/98

Date: 11/20/98

Colony Counts and Survival Fraction

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1.2	145	159	152	} 146.33	
2.2	135	147	140		
3.3	58	250	69	58	0.0395
4.4	23	24	22	0.23	0.0015
5.4	11	12	14	0.12	0.00084
6.2	162	169	170	} 161.66	
7.2	159	149	161		
8.3	38	44	40	4.06	0.0251
9.4	56	66	52	0.58	0.0035
10.4	39	38	34	0.34	0.0021



46 6010

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