V79 COLONY FORMING ASSAY

Experiment Name : ^{210}Po-citrate + 100ug/ml MEA ; **Exp. #:** 2;
Investigator: A. Bishayee **Date:** 11/20/98

1. Set the rocker-roller at 37°C incubator, set the Coulter Counter, wash cells (from 75 cm^2 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 transfering 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: 458,533 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:** 11/20/98; 1-45 p.m.
5. Calibrate the stock ^{210}Po-citrate for today (4.9 µCi/ml)
6. After 3-4 h, remove test tubes from roller and add according to Table below.

Date/Time: 11/20/98; 4-00 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:** 11/20/98; 4-15 p.m.
8. After 30 min, centrifuge tubes for 10 min at 2000 rpm, 4°C **Date/Time:** 11/20/98; 4-45 p.m
9. During the centrifugation move roller to 10.5°C

1·2

2·2

3·2, 3·3

4·3, 4·4

5·3, 5·4

6·2

7·2

8·2, 8·3

9·3, 9·4

10·3, 10·4

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

10.945
MEMA = ~~10.890~~
MEA (20mg/ml) = ~~0.110~~ 0.055

18. Transfer tubes at 10°C for 72 h. **Date/Time:** 11/20/98; 6-00 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:** 11/23/98; 14-15 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:** 11/23/98; 11-15 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 transfering 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 :** 11/23/98; 2-30 p.m.
32. After 1 week, wash colonies 3 times with normal (1X) saline,and 2 times with methanol. Stain colonies with 0.0 5% crystal violet
33. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.

Expt #2

11/20/98

Initial Cell Count = 5582, 5729, 5749
Avg. Cell count = 5686
Cell conc. = 2274666 cells/ml

For dilution Vol. required = $\frac{4400000}{2274666}$ = 1.9 ml

Take ~2 ml cells + 9 ml MEMB = 11 ml.

After dilution,
Final Cell count = 1174, 1169, 1096
Avg. Cell count = 1146
Cell conc. = 458533 cells/ml

Expt #2

11/20/98

Stock Po-citrate = on 10/20/98 = 5.9 μCi/mL

on 10/20/98 = 5.9 X 0.8305
= 4.9 μCi/mL.

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 25}{138.9}}$

$= 0.8305$

4.9 μCi = 1000 mL

1 μCi = $\frac{1000}{4.9}$

82

143

204

USER: 5 ID:PO-210 PRESET TIME: 1.00 MON 26 NOV 1998 14:28
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
H#: 1 AQC: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

[illegible]	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**- 1	1	14.00	53.45	1.00	1.80	108.0	
2	**- 2	1	5.00	89.44	1.00	3.68	103.0	
3	**- 3	1	8.00	70.71	1.00	5.37	103.0	
4	**- 4	1	10.00	63.25	1.00	7.10	100.0	
5	**- 5	1	540.00	8.61	1.00	8.83	82.0	
6	**- 6	1	563.00	8.43	1.00	10.56	93.0	
7	**- 7	1	972.00	6.42	1.00	12.29	102.0	
8	**- 8	1	968.00	6.43	1.00	14.08	97.0	
9	**- 9	1	939.00	6.53	1.00	15.95	81.0	
10	**-10	1	928.00	6.57	1.00	17.69	86.0	
11	**-11	1	7.00	75.59	1.00	19.32	95.0	
12	**-12	1	8.00	70.71	1.00	21.00	70.0	
13	**- 1	1	5.00	89.44	1.00	22.68	95.0	
14	**- 2	1	6.00	81.65	1.00	24.41	86.0	
15	**- 3	1	540.00	8.61	1.00	26.29	92.0	
16	**- 4	1	515.00	8.81	1.00	28.02	90.0	
17	**- 5	1	877.00	6.75	1.00	29.90	92.0	
18	**- 6	1	776.00	7.18	1.00	31.83	91.0	
19	**- 7	1	984.00	6.38	1.00	33.57	90.0	
20	**- 8	1	1399.00	5.35	1.00	35.54	92.0	
21	**- 9	1	7.00	75.59	1.00	37.42	59.0	
22	**-10	1	6.00	81.65	1.00	39.16	59.0	
23	**-11	1	5.00	89.44	1.00	40.78	58.0	
[illegible]	**-12	1	5.00	89.44	1.00	42.47	59.0	
25	**- 1	1	8.00	70.71	1.00	44.21	59.0	
26	**- 2	1	11.00	60.30	1.00	45.93	60.0	
27	**- 3	1	14243.36	1.98	0.71	47.48	57.0	
28	**- 4	1	13954.67	1.95	0.75	49.02	57.0	
29	**- 5	1	14885.71	1.96	0.70	50.39	59.0	
30	**- 6	1	26337.50	1.95	0.40	51.47	58.0	
31	**- 7	1	15245.71	1.94	0.70	52.85	56.0	
32	**- 8	1	29165.71	1.98	0.35	53.87	59.0	
33	**- 9	1	36363.33	1.91	0.30	54.83	59.0	
34	**-10	1	34858.46	1.88	0.33	56.03	57.0	
35	**-11	1	41370.91	1.88	0.28	57.17	62.0	
36	**-12	1	5.00	89.44	1.00	58.79	59.0	
37	**- 1	1	7.00	75.59	1.00	60.53	59.0	
38	**- 2	1	11.00	60.30	1.00	62.22	59.0	
39	**- 3	1	5.00	89.44	1.00	63.95	60.0	
40	**- 4	1	4.00	100.0	1.00	65.87	60.0	
41	**- 5	1	5.00	89.44	1.00	67.50	59.0	
42	**- 6	1	15220.74	1.97	0.68	68.90	56.0	
43	**- 7	1	16475.38	1.93	0.65	70.22	61.0	
44	**- 8	1	16868.80	1.95	0.62	71.57	57.0	
45	**- 9	1	26910.00	1.93	0.40	72.65	57.0	
46	**-10	1	28728.00	1.93	0.38	73.90	56.0	

M	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
7	**-11	1	30581.33	1.87	0.38	75.00	60.0	
8	**-12	1	34960.00	1.88	0.33	75.94	59.0	
9	**- 1	1	38403.33	1.86	0.30	76.98	59.0	
0	**- 2	1	39072.73	1.93	0.28	77.97	61.0	

B007805

TABLE-1

Expt. # : 2 **Date/Time :** 11/23/98; 2-30 p.m.

Tube #	Medium count for 30 ul (cpm)	Avg. cpm	dpm [cpm/1]	µ Ci/ml (A_t) on counting [dpm/66600]	µ Ci/ml (A_o) on addition [$A_t/e^{-\lambda t}$]
1	See the				
2	attached sheet				
3		14361	14361	0·2156	
4		23582	23582	0·3540	
5		37530	37530	0·5635	
6					
7					
8		16187	16187	0·2430	
9		28739	28739	0·4315	
10		37478	37478	0·5627	

TABLE-2

Expt. # : 2 **Date/Time :** 11/23/98; 2-30 p.m

Tube #	Radioactivity for 500 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/1]	μCi/ml (A_t) on counting [dpm/111x10^4]	μCi/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1	See the				
2	attached Sheet				
3		551.5	551.5	0.000496	0.000496
4		970	970	0.000873	0.000873
5		933.5	933.5	0.000840	0.000840
6					
7					
8		527.5	527.5	0.000475	0.000475
9		826.5	826.5	0.000744	0.000744
10		1191.5	1191.5	0.001073	0.001073

TABLE-3

Expt. # : 2 **Date/Time :** 11/23/98; 12-00 noon

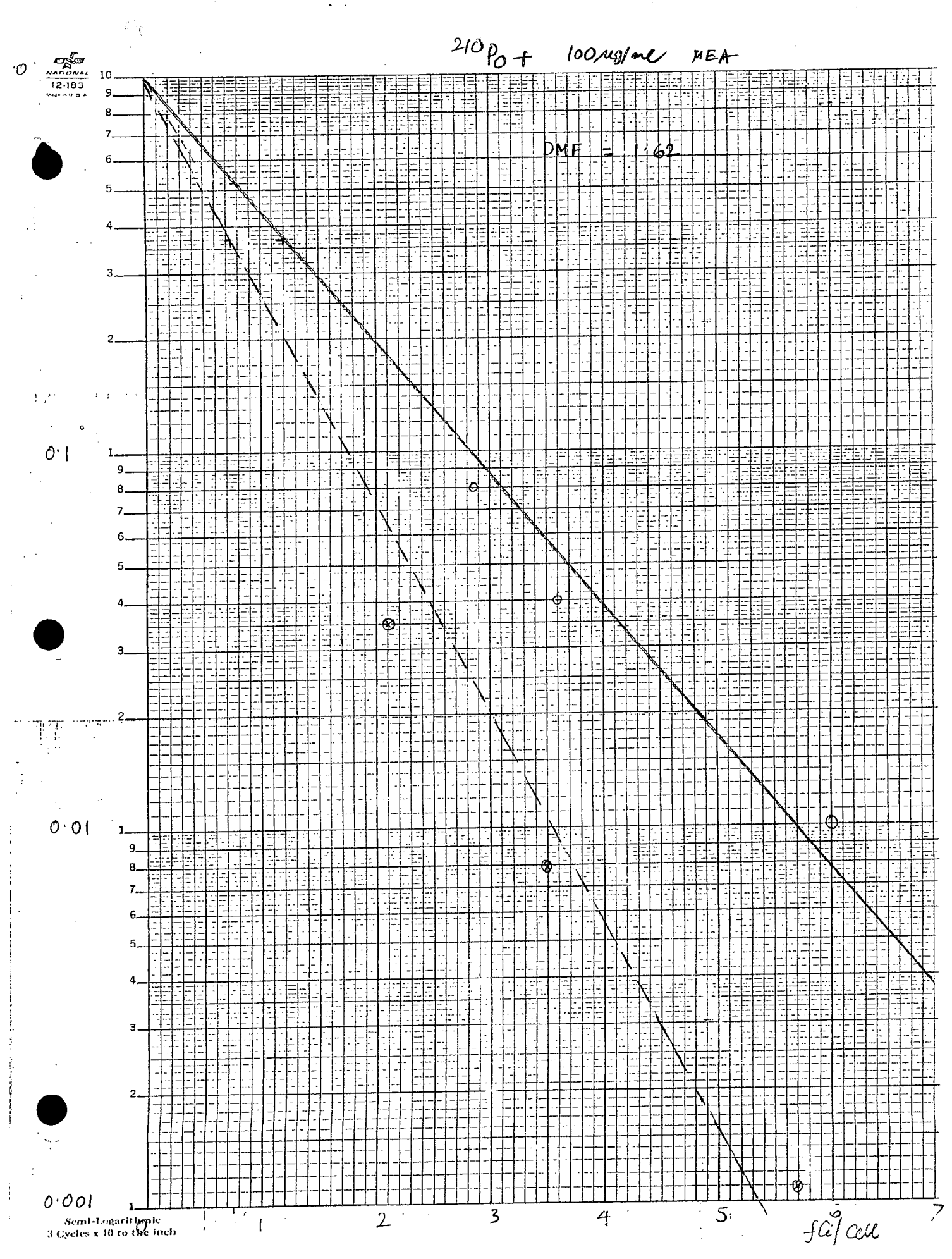
Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 400]	fCi/cell [uCi/ml x 10^9 Cells/ml]
1	501, 524, 474	499	199866	–
2	475, 472, 441	462	185066	–
3	437, 458, 424	439	175866	2.82
4	~~482, 467, 437~~ 616, 630, 622	~~462~~ 622	~~184800~~ 249066	~~4.72~~ 3.50
5	~~367, 354, 358~~	346	1385333	6.06
6	447, 441, 432	440	176000	–
7	530, 511, 520	520	208133	–
8	552, 549, 564	545	218000	2.17
9	512, 490, 537	513	205200	3.62
10	464, 466, 452	467	186933	5.74

859, 773,

TABLE-4

Expt # : 2 **Date :** 10/30/98

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1·2	129	121	135	122.5	
2·2	112	129	109		
3·3	107	99	92	9.9	0·0807
4·3	50	57	42	4·9	0·0405
5·4	123	130	116	1·23	0· 0100
6·2	145	155	139	137.83	
7·2	121	132	135		
8·3	57	48	46	5·0	0·0362
9·4	120	109	99	1·09	0·008
10·4	15	19	13	0·15	0·0011


210 Po + 100 µg/ml MEA
DMF = 1·62
0·1
0·01
0·001
Semi-Logarithmic
3 Cycles x 10 to the inch
fci/cell