V79 COLONY FORMING ASSAY

Experiment Name : ^{210}Po toxicity ($4x10^6$ cell cluster, 10% labeling); **Exp. #** : 2;
Investigator: A. Bishayee **Date:** 01/05/98

1. Set two rocker-roller at 37^{0}C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 150 cm^2 flusk, subcultured 1:2, 24h before) with PBS, trypsinize cells, each resuspend in 9 ml MEMB, pool, 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 and 3,600,000 cells/ml in MEMB [Actual count : 572,000 and 3,394,666 cells/ml) 14.4
3. Transfer 1 ml of cell suspension into ten 14 ml tubes (Falcon plastic test tube, 100x80 mm) labeled 1-10 both on cap and wall
4. Keep the tubes in the roller for 3-4 h at 37°C, 5% CO_2 **Date/Time:** 1/5/99; 3-00 p.m.
5. Calibrate the stock ^{210}Po-citrate
6. After 3-4 h, remove first set of test tubes (400,000 cells/ml) from roller and add according to Table below.

Date/Time: 1/5/99; 7-00 p.m.

Tube #	^{210}Po-citrate uCi/ml	Cells in MEMB (ml)	MEMB (ul)	Po-citrate (12 uCi/ml) (ul)
1	0	1.0	1000	0
2	0	1.0	1000	0
3	0.1	1.0	985	15
4	0.2	1.0	965	35
5	0.3	1.0	950	50
6	0.5	1.0	915	85
7	0.8	1.0	865	135
8	1.0	1.0	835	165
9	1.2	1.0	800	200
10	1.5	1.0	750	250

7. Return test tubes to roller for 30 min. **Date/Time:** 1/5/98, 7-00 p.m.

8. After 30 min, centrifuge tubes for 10 min at 2000 rpm, 4°C **Date/Time:** 1/5/99; 7-40 p.m.
9. Collect 150 ul supernatant in separate tubes
10. Resuspend in 10 ml wash MEMA
11. Centrifuge tubes for 10 min at 2000 rpm, 4°C
12. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
13. Centrifuge tubes for 10 min at 2000 rpm, 4°C
14. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
15. Centrifuge tubes for 10 min at 2000 rpm, 4°C
16. Decant supernatant, click tubes, vortex
17. Follow steps 11-16 for second set of tubes containing 3,600,000 cells, suspend in 7 ml of MEMA and transfer cells to the corresponding tubes containing 400,000 cells in step 16
18. Centrifuge tubes for 10 min at 2000 rpm, 4°C
19. Decant supernatant, click tubes,vortex, transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips
20. Again add 200 ul ice cold MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
21. Centrifuge tubes for 5 min at 1000 rpm, 4°C
22. Transfer tubes (onto a perforated Renin pipet box) at 10°C for 72 h. **Date/Time:** 1/5/99; 8-30 p.m
23. Transfer 30 ul supernatant in three sets of vials containing 6ml liquid scintillation cocktail (Aquasol) from 150 ul supernatant removed earlier (Step 9) and count them for radioactivity

Date/Time: 1/6/99; 10-00 a.m.

24. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the content to ten 12 ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA by using pasteur pipet

Date/Time: 1/8/99; 11-00 a.m.

25. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes
26. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***
27. Labeling and preparation of dilution tubes and colony dishes
    - load 60 mm petri dishes with 4 ml MEMA
    - load 40 sterile 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.
28. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
29. Centrifuge tubes for 10 min at 2000 rpm, 4°C
30. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
31. Centrifuge tubes for 10 min at 2000 rpm, 4°C
32. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times

through 3 cc syringe with 21 gauge needle

33. Determine cell concentration by transfering 100 µl to Coulter cup
34. Vortex tube, transfer 0.5 ml into dilusion tube X.5, vortex tube X.5, 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.
35. 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 test tubes.
36. Transfer 500 µl of cell suspension (in duplicate) to 20 ml scintillation vial containing 6 ml cocktail (Aquasol)
37. Incubate petridishes for 1 week
38. Count vials for radioactivity **Date/Time :** 1/08/99; 2-00 P.m.
39. After 1 week, wash colonies 3 times with normal (1X) saline,and 2 times with methanol. Stain colonies with 0.0 5% crystal violet
40. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.

Expt #2 01/05/99

Initial cell count = 672, 620, 647
Avg cell count = 646
cell conc. = 2,585,333 cells/ml

Dil. A we need 400,000 cells/ml ; 11 ml

vol required = $\frac{4400000}{2585333}$ = 1.70 ml

Take 1.7ml cells + 9.3 ml MEMB = 11 ml

Final count : 148, 142, 139 (143) Cell conc. = 572,000 cells/ml

Dil. B : we need 3,600,000 cells/ml ; 11 ml

vol required = $\frac{39600000}{}$ = ml.

In 888, 888, 720
Avg 848
Cell Conc = 3,394,666 cells/ml

²¹⁰Po toxicity (10% labeling) E-451 Howell Exp (#2)

SER: 5 ID:PO-210 PRESET TIME: 1.00 WED 06 JAN 1999 10:14
AMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
%: 1 AQC:N GCF:N RCM:N
[illegible] 1-UL 400 U: 900 2SIGMA: 2.00 BKG [illegible] ~~.00 BKG 2SIG:~~ 0.00 LSR: 0
[illegible] CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:0 1.00000
ALF LIFE(DAYS):N

20 µl medium

AM	POS	CH		CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**- 1	1	1M	6.00	81.65	1.00	1.49	58.0	
2	**- 2	1		8.00	70.71	1.00	3.17	59.0	
3	**- 3	1	2M	6.00	81.65	1.00	4.80	61.0	
4	**- 4	1		6.00	81.65	1.00	6.48	58.0	
5	**- 5	1	3M	6058.00	2.57	1.00	8.12	58.0	
6	**- 6	1		6534.00	2.47	1.00	9.85	59.0	
7	**- 7	1	4M	12640.00	1.96	0.82	11.31	57.0	
8	**- 8	1		14100.69	1.98	0.72	12.66	58.0	
9	**- 9	1	5M	18392.73	1.99	0.55	13.88	59.0	
10	**-10	1		20028.57	1.95	0.52	15.03	59.0	
11	**-11	1	6M	29024.00	1.92	0.38	16.03	60.0	
12	**-12	1		34146.66	1.98	0.30	16.99	59.0	
13	**- 1	1	7M	48736.00	1.81	0.25	17.97	59.0	
14	**- 2	1		53915.55	1.82	0.23	18.81	62.0	
15	**- 3	1	8M	57045.00	1.87	0.20	19.68	61.0	
16	**- 4	1		64777.14	1.88	0.18	20.47	56.0	
17	**- 5	1	9M	69573.33	1.96	0.15	21.28	60.0	
18	**- 6	1		78977.14	1.70	0.18	22.08	61.0	
19	**- 7	1	10M	66177.14	1.86	0.17	22.97	61.0	
20	**- 8	1		69285.71	1.82	0.18	23.76	59.0	

B007833

TABLE-1

Expt. # : 2 **Date/Time :** 01/06/99; 10-15 a.m.

Tube #	Medium count for 20 ul (cpm)	Avg. cpm	dpm [cpm/1]	µ Ci/ml (A_t) on counting [dpm/44400]	µ Ci/ml (A_o) on addition [$A_t/e^{-\lambda t}$]
1	See the				
2	attached sheet				
3		6296	6296	0·1418	
4		13370	13370	0·3011	
5		19210	19210	0·4326	
6		31585	31585	0·7113 ~~1·4057~~	
7		51325	51325	1·1559	
8		60911	60911	1·371	
9		74275	74275	1·672	
10		67717	67717	1·525	

F-451

500 µl cells

USER: 5 ID:PO-210 PRESET TIME: 1.00 FRI 08 JAN 1999 13:51
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
R#: 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: 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	1	4.00	100.0	1.00	1.55	99.0	
2	**- 2	1	7.00	66.67	1.00	3.17	90.0	
3	**- 3	1	7.00	75.59	1.00	4.81	91.0	
4	**- 4	1	8.00	70.71	1.00	6.44	83.0	
5	**- 5	1	290.00	11.74	1.00	8.07	93.0	
6	**- 6	1	296.00	11.62	1.00	9.65	87.0	
7	**- 7	1	647.00	7.86	1.00	11.28	92.0	
8	**- 8	1	643.00	7.89	1.00	12.92	93.0	
9	**- 9	1	768.00	7.22	1.00	14.55	94.0	
10	**-10	1	736.00	7.37	1.00	16.18	91.0	
11	**-11	1	1054.00	6.16	1.00	17.81	91.0	
12	**-12	1	23.00	3.97	1.00	19.49	104.0	
13	**- .	1	1537.00	5.10	1.00	21.17	94.0	
14	**- 2	1	1564.00	5.06	1.00	22.81	92.0	
15	**- 3	1	1654.00	4.92	1.00	24.44	97.0	
16	**- 4	1	1652.00	4.92	1.00	26.12	82.0	
17	**- 5	1	1864.00	4.63	1.00	27.80	95.0	
18	**- 6	1	1932.00	4.55	1.00	29.48	88.0	
19	**- 7	1	2124.00	4.34	1.00	31.17	97.0	
20	**- 8	1	2105.00	4.36	1.00	32.80	94.0	

1c, 2c, 3c, 4c, 5c, 6c, 7c, 8c, 9c, 10c

^{210}Po toxicity (10% labeling)

Expt #2

484
2622

TABLE-2

Expt. # : 2 **Date/Time :** 01/08/99; 2-00 pm

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		293	293	0·000264	
4		645	645	0·000581	
5		752	752	0·000677	
6		1088	1088	0·000980	
7		1550	1550	0·00139	
8		1653	1653	0·00148	
9		1898	1898	0·00170	
10		2115	2115	0·00190	

TABLE-3

Expt. # : 2 **Date/Time :** 01/08/99;

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	fCi/cell [uCi/ml x10^9 Cells/ml]
1	425, 454, 451	443	1773333	—
2	446, 417, 425	429	1717333	—
3	463, 463, 456	460	1842666	0·1432
4	412, 429, 430	423	1694666	0·3428
5	404, 429, 411	414	1658666	0·4081
6	420, 417, 401	412	1650666	0·5936
7	430, 421, 413	421	1685333	0·8247
8	386, 388, 393	389	1556000	0·9511
9	443, 464, 437	448	1792000	0·9486
10	453, 469, 479	467	1868000	1·017

TABLE-4

Expt # : 2 **Date :** 01/15/99

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF		
1·2	172	161	167	161	–		
2·2	156	149	161		–		
3·2	133	140	147	140	0·8695	0·1	0·1
4·2	101	112	90	101	0·6273	0·2	0·25
5·2	80	86	92	86	0·5341	0·3	0·5
6·2	60	69	65	646	04016	0·5	0·75
7·2	25	32	40	32·3	0·2008	0·8	1
8·2	26	29	23	26	0·1614	1·0	1·5
9·2	19	22	15	18·6	0·1159	1·2	2
10·3	158	162	140	15·3	0·0952	1·5	2·5