

V79 COLONY FORMING ASSAY

Experiment Name : ^{210}Po toxicity (4×10^6 cell cluster, crossed dose); Exp. # : 3;

Investigator: A. Bishayee

Date: 12/04/98

1. Set the rocker-roller at 37°C incubator with 5% CO_2 , set the Coulter Counter, wash cells (from two 150 cm^2 flask, 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 transferring 100 ul in Coulter cup containing 20 ml isotone (Coulter balanced electrolyte solution)
2. Dilute to $\sim 4,000,000$ cells/ml in MEMB [Actual count : 4,122,666 cells/ml]
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: 12/04/98; 1-00 p.m.
5. Calibrate the stock ^{210}Po -citrate
6. After 3-4 h, remove test tubes from roller and add according to Table below.

Date/Time: 12/04/98; 4-20 p.m.

Tube #	^{210}Po -citrate uCi/ml	Cells in MEMB (ml)	MEMB (ul)	Po-citrate (5.9 uCi/ml) (ul)
1	0	1.0	1000	0
2	0	1.0	1000	0
3	0.01	1.0	997	3
4	0.025	1.0	992	8
5	0.05	1.0	983	17
6	0.1	1.0	967	33
7	0.2	1.0	933	67
8	0.3	1.0	900	100
9	0.4	1.0	865	135
10	0.5	1.0	830	170

on 12/26/98

7. Return test tubes to roller for 30 min.

Date/Time: 12/04/98; 4-30 p.m.

8. After 30 min, centrifuge tubes for 10 min at 2000 rpm, 4°C Date/Time: 12/04/98; 5-00 p.m.

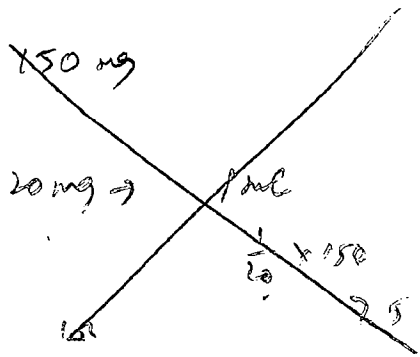
9. Collect 150 ul supernatant in separate tubes
10. Resuspend in 10 ml wash MEMA
11. Centrifuge tubes for 10 min at 2000 rpm, 4°C
12. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
13. Centrifuge tubes for 10 min at 2000 rpm, 4°C
14. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
15. Centrifuge tubes for 10 min at 2000 rpm, 4°C
16. Decant supernatant, click tubes, vortex, resuspend in 7 ml of MEMA
17. Centrifuge tubes for 10 min at 2000 rpm, 4°C
18. 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
19. Again add 200 ul ice cold MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
20. Centrifuge tubes for 5 min at 1000 rpm, 4°C
21. Transfer tubes (onto a perforated Renin pipet box) at 10°C for 72 h. Date/Time: 12/09/98;
22. 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 6-00 p.m.
Date/Time: 12/07/98; 1-40 p.m.
23. 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: 12/07/98; 9-30 a.m.
24. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes
25. Centrifuge the tubes for 10 min at 2000 rpm, 4°C (precooled centrifuge)
26. 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.
27. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
28. Centrifuge tubes for 10 min at 2000 rpm, 4°C
29. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
30. Centrifuge tubes for 10 min at 2000 rpm, 4°C
31. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
32. Determine cell concentration by transferring 100 µl to Coulter cup
33. Vortex tube, transfer 0.5 ml into dilution 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.

34. 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.
35. Transfer 500 μ l of cell suspension (in duplicate) to 20 ml scintillation vial containing 6 ml cocktail (Aquasol)
36. Incubate petridishes for 1 week
37. Count vials for radioactivity **Date/Time : 12/07/98; 1-40 p.m.**
38. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
39. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.

Exp #3

12/04/98



MS = Total.

Initial cell count = 1036, 1044, 1012

Avg. Count = 1030

Cell conc. = 1030×4000

= 4,122,666 Cells/ml

TABLE-1

Expt. # : 3

Date/Time : 12/07/98; 1-40 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_0) on addition [$A_t/e^{-\lambda t}$]
1	See the attached				
2	Sheet				
3		451	451	0.00677	
4		1325.6	1325.6	0.0199	
5		2378.3	2378.3	0.0357	
6		4804.3	4804.3	0.0721	
7		10108.3	10108.3	0.1517	
8		15520	15520	0.2330	
9		21053	21053	0.3161	
10		28244	28244	0.4240	

TABLE-2

Expt. # : 3

Date/Time : 12/07/98; 1-40 p.m.

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					
2					
3		119.5	119.5	0.000107	
4		264.5	264.5	0.000238	
5		492.5	492.5	0.000443	
6		1070	1070	0.000963	
7		1926	1926	0.001735	
8		2853	2853	0.00257	
9		3199	3199	0.002881	
10		3296	3296	0.002969	

F-451

210

Po toxicity
Expt # 3

USER: 5 ID:FD-210 PRESET TIME: 1.00 MON 07 DEC 1998 13:40
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
 H₀:1 AGC:N QCF:N RCM:N
 CHANNEL 1-LL:600 UL: 900 ZSIGMA: 2.00 BKG SUR: 0.00 BKG 2SIG: 0.00 LSR: 0
 DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:Q 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	1.85	57.0	
2	**	2	5.00	89.44	1.00	3.47	57.0	
3	**	3	8.00	70.71	1.00	5.16	59.0	
4	**	4	6.00	81.65	1.00	6.83	56.0	
5	**	5	13.00	55.47	1.00	8.52	59.0	
6	**	6	6.00	81.65	1.00	10.20	57.0	
7	**	7	457.00	9.36	1.00	11.93	57.0	
8	**	8	453.00	9.40	1.00	13.67	56.0	
9	**	9	443.00	9.50	1.00	15.39	57.0	
10	**	10	1252.00	5.65	1.00	17.23	57.0	
11	**	11	1325.00	5.49	1.00	18.95	59.0	
12	**	12	1400.00	5.35	1.00	20.57	57.0	
13	**	1	2232.00	4.23	1.00	22.31	55.0	
14	**	2	2395.00	4.09	1.00	23.99	53.0	
15	**	3	2508.00	3.99	1.00	25.72	54.0	
16	**	4	4685.00	2.92	1.00	27.46	57.0	
17	**	5	5254.00	2.76	1.00	29.19	57.0	
18	**	6	4474.00	2.99	1.00	30.87	59.0	
19	**	7	9901.00	2.01	1.00	32.76	59.0	
20	**	8	10225.00	1.98	1.00	34.49	57.0	
21	**	9	10199.00	1.98	1.00	36.17	59.0	
22	**	10	15008.57	1.95	0.70	37.54	58.0	
23	**	11	16142.40	1.99	0.62	38.89	56.0	
24	**	12	15412.03	1.98	0.67	40.38	56.0	
25	**	1	20658.25	1.94	0.51	41.77	59.0	
26	**	2	22906.67	1.97	0.45	42.89	58.0	
27	**	3	19596.36	1.93	0.55	44.21	64.0	
28	**	4	26347.50	1.95	0.40	45.27	58.0	
29	**	5	29280.00	1.98	0.35	46.40	60.0	
30	**	6	29106.85	1.94	0.36	47.57	56.0	
31	**	7	8.00	70.71	1.00	49.36	94.0	
32	**	8	9.00	66.67	1.00	51.09	95.0	
33	**	9	14.00	53.45	1.00	52.77	86.0	
34	**	10	2.00	141.4	1.00	54.46	92.0	
35	**	11	123.00	18.03	1.00	56.14	96.0	
36	**	12	16.00	18.57	1.00	57.82	94.0	
37	**	1	257.00	12.48	1.00	59.57	98.0	
38	**	2	272.00	12.13	1.00	61.30	98.0	
39	**	3	493.00	9.01	1.00	63.02	91.0	
40	**	4	492.00	9.02	1.00	64.75	92.0	
41	**	5	1062.00	6.14	1.00	66.47	96.0	
42	**	6	1078.00	6.09	1.00	68.16	94.0	
43	**	7	1948.00	4.53	1.00	70.04	98.0	
44	**	8	1904.00	4.58	1.00	71.73	97.0	
45	**	9	2922.00	3.70	1.00	73.51	98.0	
46	**	10	2785.00	3.79	1.00	75.19	101.0	

SAN	FDS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
42	**	-11	1	3271.00	3.50	1.00	76.88	102.0
	**	-12	1	3127.00	3.58	1.00	78.56	103.0
49	**	-1	1	3457.00	3.40	1.00	80.35	105.0
50	**	-2	1	3136.00	3.57	1.00	82.18	102.0

TABLE-3

Expt. # : 3

Date/Time : 12/07/98; 11-00 a.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	452, 422, 439	437.6	1750666	-
2	492, 475, 444	470.3	1881333	-
3	513, 496, 488	499	1996000	0.0536
4	443, 432, 430	435	1740000	0.1367
5	549, 568, 570	562	2249333	0.1970
6	549, 566, 571	561	2246666	0.4286
7	515, 506, 472	497	1990666	0.8715
8	490, 548, 543	527	2108000	1.2191
9	412, 395, 393	400	1600000	1.8006
10	451, 438, 426	438	1753333	1.6933

TABLE-4

Expt # : 3

Date : 12/14/98

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1.2	183	194	201	} 173.66	
2.2	142	160	162		
3.2	114	116	110	113.33	0.6525
4.2	75	84	91	83.33	0.4798
5.2	54	50	45	49.66	0.2859
6.3	99	120	119	11.20	0.0648
7.3	32	20	26	2.62	0.0151
8.4	52	44	49	0.48	0.0028
9.4	21,	20	17	0.19	0.0011
10.4	20	15	14	0.15	0.0009