V79 COLONY FORMING ASSAY

Experiment Name : ^{210}Po toxicity ($4x10^6$ cell cluster, crossed dose); Growing in rocker-roller **Exp. # :** 2;

Investigator: A. Bishayee **Date:** 11/06/98

1. Set the rocker roller at 37°C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 150 cm^2 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 ~4,000,000 cells/ml in MEMB [Actual count : 3,650,666 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:** 11/06/98; 3-30 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: 11/06/98; 5-00 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

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7. Return test tubes to roller for 30 min. **Date/Time:** 11/06/98; 5-10 p.m.
8. After 30 min, centrifuge tubes for 10 min at 2000 rpm, 4°C **Date/Time:** 11/06/98; 5-40 p.m.

9. Collect 150 ul supernatant in separate tubes
10. Resuspend in 8 ml wash MEMA
11. Centrifuge tubes for 10 min at 2000 rpm, 4°C
12. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
13. Centrifuge tubes for 10 min at 2000 rpm, 4°C
14. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
15. Centrifuge tubes for 10 min at 2000 rpm, 4°C
16. Decant supernatant, click tubes, vortex, resuspend in 2 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:** 11/6/98; 7-15 p.m.
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

    Date/Time: 11/09/98; 1-30 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: 11/09/98; ~~1:00 P.M.~~ 10-00 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 transfering 100 µl to Coulter cup
33. 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.

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 µl 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 :** 11/09/98; 1-30 pm.
38. After 1 week, wash colonies 3 times with normal (1X) saline,and 2 times with methanol. Stain colonies with 0.0 5% crystal violet
39. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.

Expt #2

11/06/98

Mono select : = 50 ml
2 x 150 cm² flasks; 80-85% confluent
Total volume = 14 ml

= 923, 903, 912
= 912
= 912 × 4000
= 3, 650, 666 Cells/ml

For datum we need = 44000000 / 3,650,666 =

^{210}Po in 4×10^6 cells cluster : Expt #2

NSD F451

USER: 5 ID:PO-210 PRESET TIME: 1.00 MON 09 NOV 1998 13:36
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
H#: 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
DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:@ 1.00000
HALF LIFE(DAYS):N

SAM	POS	CH		CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**- 1	1		5.00	89.44	1.00	1.55	62.0	
2	**- 2	1	1M	2.00	141.4	1.00	3.28	57.0	
3	**- 3	1		5.00	89.44	1.00	5.02	55.0	
4	**- 4	1		8.00	70.71	1.00	6.70	61.0	
5	**- 5	1	2M	8.00	70.71	1.00	8.33	53.0	
6	**- 6	1		10.00	63.25	1.00	10.02	55.0	
7	**- 7	1		440.00	9.53	1.00	11.74	54.0	
8	**- 8	1	3M	589.00	8.24	1.00	13.48	56.0	
9	**- 9	1		542.00	8.59	1.00	15.14	58.0	
10	**-10	1		1240.00	5.68	1.00	16.92	56.0	
11	**-11	1	4M	1633.00	4.95	1.00	18.65	58.0	
12	**-12	1		1541.00	5.09	1.00	20.37	58.0	
13	**- 1	1		2822.00	3.76	1.00	22.17	57.0	
14	**- 2	1	5M	3018.00	3.64	1.00	23.85	57.0	
15	**- 3	1		3076.00	3.61	1.00	25.53	56.0	
16	**- 4	1		4555.00	2.96	1.00	27.22	54.0	
17	**- 5	1	6M	5989.00	2.58	1.00	28.91	57.0	
18	**- 6	1		5985.00	2.59	1.00	30.59	56.0	
19	**- 7	1		11363.33	1.98	0.90	32.17	56.0	
20	**- 8	1	7M	12875.15	1.94	0.82	33.73	55.0	
21	**- 9	1		12433.94	1.97	0.82	35.28	55.0	
22	**-10	1		17509.57	1.99	0.57	36.58	56.0	
23	**-11	1	8M	17664.35	1.98	0.57	37.88	56.0	
24	**-12	1		17716.52	1.92	0.57	39.18	53.0	
25	**- 1	1		17626.67	1.94	0.60	40.52	54.0	
26	**- 2	1	9M	24472.94	1.96	0.43	41.66	55.0	
27	**- 3	1		24677.78	1.90	0.45	42.78	56.0	
28	**- 4	1		29020.00	1.98	0.35	43.80	60.0	
29	**- 5	1	10M	31374.29	1.91	0.35	44.82	54.0	
30	**- 6	1		29474.29	1.97	0.35	45.82	56.0	
31	**- 7	1		6.00	81.65	1.00	47.51	106.0	
32	**- 8	1	1C	11.00	60.30	1.00	49.18	108.0	
33	**- 9	1		8.00	70.71	1.00	50.87	105.0	
34	**-10	1	2C	9.00	66.67	1.00	52.55	108.0	
35	**-11	1		89.00	21.20	1.00	54.18	107.0	
36	**-12	1	3C	68.00	24.25	1.00	55.87	109.0	
37	**- 1	1		203.00	14.04	1.00	57.61	108.0	
38	**- 2	1	4C	202.00	14.07	1.00	59.29	108.0	
39	**- 3	1		286.00	11.83	1.00	60.97	111.0	
40	**- 4	1	5C	311.00	11.34	1.00	62.65	108.0	
41	**- 5	1		623.00	8.01	1.00	64.33	102.0	
42	**- 6	1	6C	542.00	8.59	1.00	66.02	107.0	
43	**- 7	1		884.00	6.73	1.00	67.70	107.0	
44	**- 8	1	7C	898.00	6.67	1.00	69.38	106.0	
45	**- 9	1		1177.00	5.83	1.00	71.12	107.0	
46	**-10	1	8C	1179.00	5.82	1.00	72.84	108.0	

30 µl medium

500 µl cells

SAM	POS	CH		CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
[redacted]	**-11	1	9c	1608.00	4.99	1.00	74.52	104.0	
48	**-12	1		1673.00	4.89	1.00	76.20	108.0	
49	**- 1	1	1ac	2349.00	4.13	1.00	77.93	102.0	
50	**- 2	1		2205.00	4.26	1.00	79.67	107.0	

TABLE-1

Expt. # : 2 **Date/Time :** 11/09/98; 1-30 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	4			
2	attached Sheet	8.6			
3		523.6	523.6	0.0078	
4		1471.3	1471.3	0.0220	
5		2972	2972	0.0446	
6		5509.6	5509.6	0.0827	
7		12224	12224	0.1835	
8		18030	18030	0.2707	
9		22258	22258	0.3342	
10		29956	29956	0.4497	

TABLE-2

Expt. # : 2 **Date/Time :** 11/09/98; 1-30 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_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1	See the attached				
2	Sheet				
3		78·5	78·5	0·0000707	
4		202·5	202·5	0·000182	
5		298·5	298·5	0·000268	
6		582·5	582·5	0·000524	
7		891	891	0·000802	
8		1178	1178	0·00106	
9		1640·5	1640·5	0·00147	
10		2277	2277	0·00205	

TABLE-3

Expt. # : 2 **Date/Time :** 11/09/98; 11-00 a.m

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000	fCi/cell [uCi/ml x 10^9 Cells/ml]
1	415, 404, 383	400	1602666	—
2	383, 352, 373	369.3	1477333	—
3	346, 337, 356	346.3	1385333	0.0510
4	363, 361, 372	365.3	1461333	0.1245
5	362, 365, 395	374	1496000	0.1791
6	354, ~~330~~, 395, 377	375.3	1501333	0.3490
7	359, 366, 344	356.3	1425333	0.5626
8	~~418~~, 385, 368, 353	368.6	1474666	0.7188
9	368, 350, 362	360	1440000	1.0208
10	444, 455, 473	457.3	1829333	1.1206

TABLE-4

Expt # : 2 **Date :** 11/16/98

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1·2	98	118	110	125·16	—
2·2	142	154	129		—
3·2	96	91	90	92·33	0·7376
4·2	67	71	69	69	0·5512
5·2	44	46	42	44	0·3515
6·2	23	30	28	27	0·2157
7·3	70	63	68	6·7	0·0535
8·3	36	33	30	3·3	0·0263
9·4	91	96	94	0·93	0·0074
10·4	51	46	42	0·46	0·0037