V79 COLONY FORMING ASSAY

Culturing in shaker

Experiment Name : ^{210}Po toxicity ($4x10^6$ cell cluster, crossed dose); **Exp. # :** 1;

Investigator: A. Bishayee **Date:** 10/26/98

1. Set the shaker 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 : 3430666 cells/ml)
3. Transfer 1 ml of cell suspension into ten 6 ml tubes (Falcon plastic test tube, 12x75 mm) labeled 1-10 both on cap and wall
4. Keep the tubes in the shaker (shaking mode : interval) for 3-4 h at 37℃, 5% CO_2

   Date/Time: 10/26/98; 2-30 p.m.
5. After 3-4 h, remove test tubes from roller and add according to Table below.

   Date/Time: 10/26/98; 4-15 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	3.3
4	0.025	1.0	992	8.4
5	0.05	1.0	983	17
6	0.1	1.0	967	33.8
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

6. Return test tubes to shaker for 30 min. **Date/Time:** 10/26/98; 4-30 p.m
7. After 30 min, centrifuge tubes for 10 min at 2000 rpm, 4℃ **Date/Time:** 10/26/98; 5-00 p.m.

~~8. During the centrifugation move roller to 10.5°C~~

1.2

2.2

3.2

4.2

5.2, 5.3

6.2, 6.3

7.2, 7.3, 7.4

8.2, 8.3, 8.4

~~9.2~~, 9.3, 9.4

10.3, 10.4

9. Collect 150 ul supernatant in separate tubes
10. Resuspend in 3 ml wash MEMA
11. Centrifuge tubes for 10 min at 2000 rpm, 4℃
12. Decant supernatant, click tubes, vortex, resuspend in 3 ml wash MEMA
13. Centrifuge tubes for 10 min at 2000 rpm, 4℃
14. Decant supernatant, click tubes, vortex, resuspend in 3 ml wash MEMA
15. Centrifuge tubes for 10 min at 2000 rpm, 4℃
16. Decant supernatant, click tubes, vortex, resuspend in 2 ml of MEMA
17. Centrifuge tubes for 10 min at 2000 rpm, 4℃
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℃
21. Transfer tubes at 10℃ for 72 h. **Date/Time:** 10/26/98; 6-45 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: 11/03/98; 4-20 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: 10/30/98; 1-00 p.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℃ ***(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℃
29. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
30. Centrifuge tubes for 10 min at 2000 rpm, 4℃
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 :** 11/03/98; 4-20 p.m.
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 #1. 10/26/98

Mono. select = 50 ml.
2 × 150 cm^2 Flask, 80-90% confluent.
Total volume 12 ml.

Initial Cell count = 1609, 1656, 1579
Avg. Cell count = 1615
Cell conc. = 6,458,666 Cells/ml.

For dilution,

We need = $\frac{44000000}{6458666}$ = ~~0.819~~ ml 6.8 ml

6.8 4.2
Take ~~0.7~~ ml cells + ~~10.3~~ ml HEMB = 11 ml.

After dilution,

Final Cell count = 860, 860, 853
Avg count = 857.6
Cell conc. = 3430666 Cells/ml

10/26/98

0.0033

Stock Po-citrate

on 10/16/98 ⇒ 6.23 μCi/ml

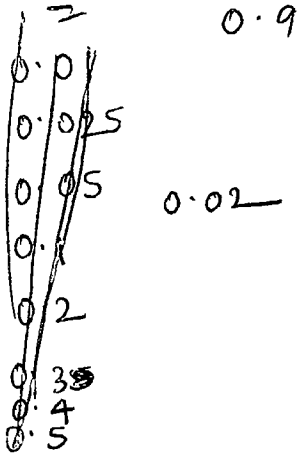
on 10/26/98 ⇒ 6.23 × 0.95

= 5.9 μCi/ml.

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 10}{138}}$

$= 0.9510$



^{210}Po in $4X10^6$ cells cluster Expt#1

TUE 03 NOV 1998 16:14

ER: 5 ID:PO-210 PRESET TIME: 1.00
LE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
AQC:N QCF:N RCM:N
HANNEL 1-LL:600 UL: 900 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
ATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:0 1.00000
ALF LIFE(DAYS):N

AM	POS	CH		CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**- 1	1	1M	6.00	81.65	1.00	1.60	56.0	
2	**- 2	1		7.00	75.59	1.00	3.27	56.0	
3	**- 3	1	2M	3.00	115.5	1.00	5.01	59.0	
4	**- 4	1		10.00	63.25	1.00	6.69	59.0	
5	**- 5	1	3M	923.00	6.58	1.00	8.63	57.0	
6	**- 6	1		1032.00	6.23	1.00	10.35	58.0	
7	**- 7	1	4M	2545.00	3.96	1.00	12.03	57.0	
8	**- 8	1		2728.00	3.83	1.00	13.92	59.0	
9	**- 9	1	5M	4892.00	2.86	1.00	15.80	58.0	
10	**-10	1		5266.00	2.76	1.00	17.53	60.0	
11	**-11	1		4343.00	3.03	1.00	19.26	58.0	
12	**-12	1	6M	15826.15	1.97	0.65	20.57	53.0	
13	**- 1	1	7M	9556.00	2.05	1.00	22.32	58.0	
14	**- 2	1		9440.00	2.06	1.00	24.06	60.0	
15	**- 3	1	8M	29765.33	1.89	0.38	25.15	58.0	
16	**- 4	1		29500.00	1.97	0.35	26.27	60.0	
17	**- 5	1	9M	37906.66	1.88	0.30	27.23	58.0	
18	**- 6	1		45853.33	1.97	0.23	28.17	58.0	
19	**- 7	1	10M	49017.78	1.90	0.23	29.12	57.0	
20	**- 8	1		49800.00	1.89	0.23	30.06	59.0	
21	**- 9	1	1c	7.00	75.59	1.00	31.74	105.0	
22	**-10	1		5.00	89.44	1.00	33.67	105.0	
23	**-11	1	2c	6.00	81.65	1.00	35.50	103.0	
24	**-12	1		7.00	75.59	1.00	37.17	103.0	
25	**- 1	1	3c	133.00	17.34	1.00	38.97	103.0	
26	**- 2	1		146.00	16.55	1.00	40.70	104.0	
27	**- 3	1	4c	356.00	10.60	1.00	42.43	102.0	
28	**- 4	1		367.00	10.44	1.00	44.32	91.0	
29	**- 5	1	5c	674.00	7.70	1.00	46.20	102.0	
30	**- 6	1		706.00	7.53	1.00	48.08	99.0	
31	**- 7	1	6c	1325.00	5.49	1.00	49.86	103.0	
32	**- 8	1		1466.00	5.22	1.00	51.54	101.0	
33	**- 9	1	7c	2175.00	4.29	1.00	53.32	102.0	
34	**-10	1		2046.00	4.42	1.00	55.26	96.0	
35	**-11	1	8c	2947.00	3.68	1.00	56.93	100.0	
36	**-12	1		2784.00	3.79	1.00	58.57	106.0	
37	**- 1	1	9c	4361.00	3.03	1.00	60.36	103.0	
38	**- 2	1		4322.00	3.04	1.00	62.03	98.0	
39	**- 3	1	10c	5155.00	2.79	1.00	63.97	106.0	
40	**- 4	1		5028.00	2.82	1.00	65.71	102.0	

30 µl medium

500 µl cells

TABLE-1

Expt. # : i **Date/Time :** 11/03/98 ; 4-20 p.m

Tube #	Medium count for 30 ul (cpm)	Avg. cpm	dpm [cpm/1]	μCi/ml (A_t) on counting [dpm/66600]	μCi/ml (A_o) on addition [$A_t/e^{-\lambda t}$]
1	See the				
2	attached				
3	sheet	997.5	997.5	0.0146	
4		2636.5	2636.5	0.0395	
5		5079	5079	0.0762	
6		10084	10084	0.1514	
7		9498	9498	0.1426*	
8		29632	29632	0.4449	
9		41879	41879	0.6288	
10		49408	49408	0.7418	

* errore in taking samples for Counting

TABLE-2

Expt. # : 1 **Date/Time :** 11/03/98; 2-40 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_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1	See the				
2	attached sheet				
3		139.5	139.5	0.000125	
4		361.5	361.5	0.000325	
5		690	690	0.000621	
6		1395.5	1395.5	0.001257	
7		2110.5	2110.5	0.001901	
8		2865.5	2865.5	0.002581	
9		4341.5	4341.5	0.003911	
10		5091.5	5091.5	0.004586	

TABLE-3

Expt. # : 1 **Date/Time :** 11/03/98; 2-c

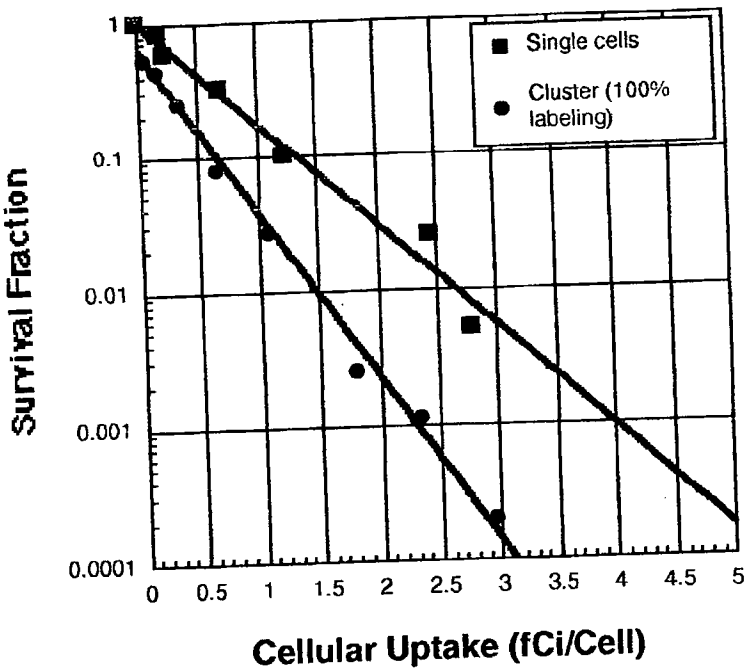
Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	fCi/cell [uCi/ml x 10^9 Cells/ml]
1	418, 422, 425	421	1686666	–
2	501, 447, 503	483	1934666	–
3	487, 473, 463	474	1897333	0·0658
4	461, 454, 466	460	1841333	0·1765
5	478, 448, 450	458	1834666	0·3384
6	460, 488, 475	474	1897333	0·6625
7	434, 442, 428	434	1738666	1·0933
8	376, 344, 352	357	1429333	1·8057
9	414, 422, 420	418	1674666	2·3353
10	385, 373, 402	386·6	1546666	2·9650

TABLE-4

Expt. #: 1 Date: 11/06/98

Colony Counts and Survival Fraction

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1·2	124	128	123	144.33	
2·2	164	153	174		
3·2	74	85	72	77	0·5334
4·2	55	57	70	60·66	0·4203
5·2	33	34	42	36·33	0·2517
6·3	108	115	111	11·13	0·0771
7·3	34	42	40	3·86	0·0267
8·4	30	39	43	0·37	0·0025
9·4	14	18	19	0·17	0·0011
10·4	2	3	4	0·03	0·0002


Survival Fraction
1
0.1
0.01
0.001
0.0001
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
Cellular Uptake (fCi/Cell)
Single cells
Cluster (100% labeling)

	Label	A	B	C	D
Label		Single cells	SF	Cluster (100%	SF
1		0	1	0	1
2		0.176	0.8047	0.0658	0.5334
3		0.2357	0.5936	0.1765	0.4203
4		0.6913	0.3245	0.3384	0.2517
5		1.2251	0.1002	0.6625	0.0771
6		2.4493	0.025	1.0933	0.0267
7		2.7779	0.0052	1.8057	0.0025
8				2.3353	0.0011
9				2.965	0.0002