

V79 COLONY FORMING ASSAY

Experiment Name : $^3\text{HTdR}$ toxicity (cluster, 100% labeling);

Exp. #: 1;

Experiment performed by: A. Bishayee

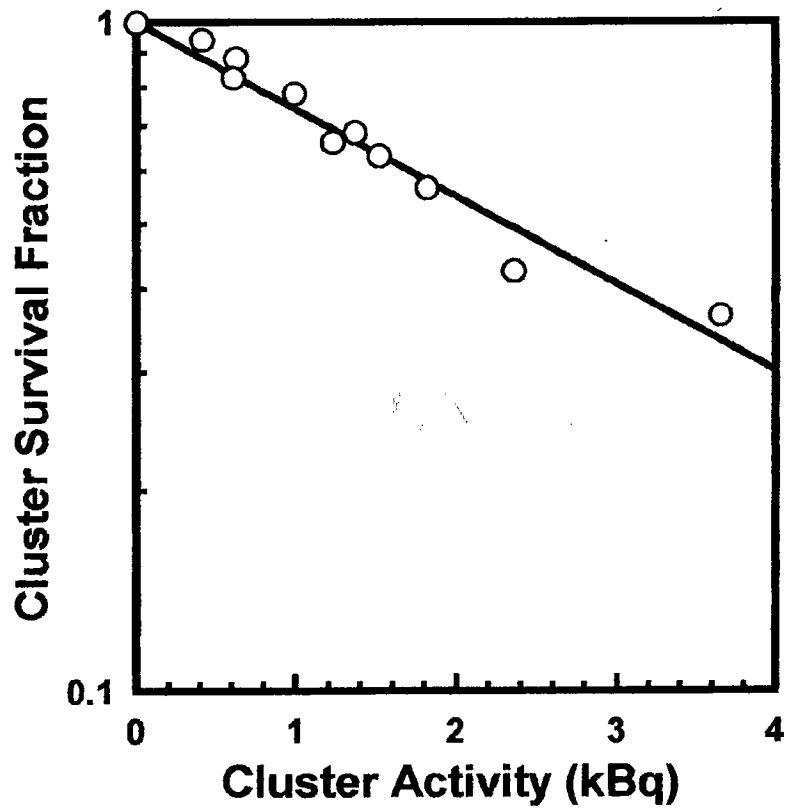
Date: 02/24/00

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 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: $4,028,000$ 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. Keep the tubes in the roller for 3-4 h at 37°C , 5% CO_2 Date/Time: 02/24/00; 4:00 pm
5. Prepare MEMB containing radioactivity in hood
 $29\ \mu\text{l } ^3\text{HTdR}$ (Stock: $1\ \mu\text{Ci}/\mu\text{l}$ on 01/30) + 7 ml MEMB
6. After 3-4 h, remove test tubes from roller and add MEMB with or without radioactivity according to Table below. Date/Time: 02/24/00; 7:00 pm

Tube #	$^3\text{HTdR}$ uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ $^3\text{HTdR}$ [4uCi/ml] (ml)
1	0	1.0	1.0	0
2	0	1.0	1.0	0
3	0.2	1.0	0.9	0.1
4	0.4	1.0	0.8	0.2
5	0.6	1.0	0.7	0.3
6	0.8	1.0	0.6	0.4
7	1.0	1.0	0.5	0.5
8	1.2	1.0	0.4	0.6
9	1.4	1.0	0.3	0.7
10	1.6	1.0	0.2	0.8
11	1.8	1.0	0.1	0.9
12	2.0	1.0	0	1

7. Return test tubes to roller for 12 h. Date/Time: 02/24/00, 7-15 PM
8. Next day, while test tubes are in roller label 10 gamma-tubes (13 X 100 mm VWR glass test tube)
9. After ~12 h incubation period, remove tubes and centrifuge at 2000 rpm at 4°C for 10 min (precooled centrifuge). Date/Time: 02/25/00, 9-00 am
10. Remove buckets from centrifuge and carefully remove 150 µl of supernatant and place in prelabeled gamma-tube.
11. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
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 supernatant, click tubes, vortex, resuspend in 7 ml of MEMA
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 at 10°C for 72 h. Date/Time: 02/25/00, 11-30 a.m.
23. Transfer 30 ul supernatant in three sets of 20 ml scintillation vials containing 6 ml liquid scintillation cocktail (Aquasol) from 150 ul supernatant removed earlier (Step 10) and count them for radioactivity *Ecolund* Date/Time: 02/25/00, 12-45 PM
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: 02/28/00, 8-00 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 66, 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 transferring 100 μ l to Coulter cup
34. 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.
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 T-tubes.
36. Transfer 200 μ l of cell suspension (in triplicate) to 20 ml scintillation vial containing 6 ml cocktail (Aquasol) *Feedure*
37. Incubate petridishes for 1 week
38. Count vials for radioactivity Date/Time : 02/28/00, 2-30 pm
39. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
40. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.



$$A_0 = 3.34 \text{ kBq/cluster}$$

30 µl medium

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 FRI 25 FEB 2000 12:44
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
H#: 1 AQC:N OCF:N RCM:N
CHANNEL 1-LL: 0 UL: 400 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

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	49.00	28.57	1.00	1.85	93.0	
2	**	2	71.00	23.74	1.00	4.08	92.0	
3	**	3	60.00	25.82	1.00	6.42	92.0	
4	**	4	69.00	24.08	1.00	8.35	95.0	
5	**	5	81.00	22.22	1.00	10.33	93.0	
6	**	6	63.00	25.20	1.00	12.32	94.0	
7	**	7	3121.00	3.58	1.00	14.45	95.0	
8	**	8	3404.00	3.43	1.00	16.63	95.0	
9	**	9	3270.00	3.50	1.00	18.70	94.0	
10	**	10	6561.00	2.47	1.00	20.73	96.0	
11	**	11	7031.00	2.39	1.00	22.71	96.0	
12	**	12	7112.00	2.37	1.00	24.74	96.0	
13	**	13	10231.00	1.98	1.00	26.92	95.0	
14	**	14	10583.59	1.97	0.97	29.27	96.0	
15	**	15	10721.65	1.96	0.97	31.57	94.0	
16	**	16	13688.89	1.95	0.76	33.37	96.0	
17	**	17	14282.52	1.98	0.71	35.11	97.0	
18	**	18	14760.87	1.98	0.69	36.72	95.0	
19	**	1	15936.84	1.94	0.67	38.47	94.0	
20	**	2	17118.70	1.95	0.62	40.10	96.0	
21	**	3	16900.81	1.96	0.62	41.74	95.0	
22	**	4	18864.91	1.93	0.57	43.62	95.0	
23	**	5	19142.34	1.94	0.56	45.41	98.0	
24	**	6	20062.00	2.00	0.50	46.88	95.0	
25	**	7	22570.53	1.93	0.48	48.42	94.0	
26	**	8	22868.89	1.97	0.45	49.84	96.0	
27	**	9	23935.48	1.90	0.47	51.32	96.0	
28	**	10	25746.15	2.00	0.39	52.63	94.0	
29	**	11	26848.00	1.99	0.38	54.07	95.0	
30	**	12	26335.90	1.97	0.39	55.58	95.0	
31	**	13	28962.16	1.93	0.37	57.27	95.0	
32	**	14	30768.57	1.93	0.35	58.59	95.0	
33	**	15	32831.43	1.87	0.35	59.91	95.0	
34	**	16	31768.25	2.00	0.31	61.24	97.0	
35	**	17	34510.77	1.89	0.33	62.94	97.0	
36	**	18	32506.25	1.96	0.32	64.58	93.0	

TABLE-1

Expt. # : 1

Date/Time : 02/25/00; 12-44 pm

Tube #	Medium count for 30 ul (cpm)	Avg. cpm	dpm 0.58 [cpm/ 0.65]	μ Ci/ml (A_0) on counting [dpm/66600]	μ Ci/ml (A_0) on addition [$A_1/e^{-\lambda t}$]
1					
2					
3		3265	5629	0.0845	
4		6901	11898	0.1787	
5		10511	18123	0.2721	
6		14243	24557	0.3687	
7		16651	28709	0.4311	
8		19356	33372	0.5011	
9		23124	39869	0.5986	
10		26309	45361	0.6811	
11		30853	53195	0.7987	

200µl cells

PAGE: 1

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 TUE 29 FEB 2000 14:29
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
H#: 1 AQC:N QCF:N RCM:N
CHANNEL 1-LL: 0 UL: 400 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	16.00	50.00	1.00	2.15	109.0	
2	**	2	16.00	50.00	1.00	4.53	110.0	
3	**	3	14.00	53.45	1.00	6.46	110.0	
4	**	4	22.00	42.64	1.00	8.38	108.0	
5	**	5	18.00	47.14	1.00	10.36	107.0	
6	**	6	19.00	45.88	1.00	12.59	107.0	
7	**	7	1581.00	5.03	1.00	14.78	105.0	
8	**	8	1459.00	5.24	1.00	17.21	96.0	
9	**	9	1567.00	5.05	1.00	19.19	100.0	
10	**	10	1594.00	5.01	1.00	21.22	96.0	
11	**	11	2579.00	3.94	1.00	23.20	110.0	
12	**	12	2177.00	4.29	1.00	25.03	109.0	
13	**	13	1997.00	4.48	1.00	27.27	100.0	
14	**	14	2574.00	3.94	1.00	29.35	100.0	
15	**	15	2237.00	4.23	1.00	31.32	100.0	
16	**	16	3179.00	3.55	1.00	33.26	100.0	
17	**	17	3203.00	3.53	1.00	35.23	99.0	
18	**	18	3817.00	3.24	1.00	37.32	101.0	
19	**	1	4380.00	3.02	1.00	39.70	99.0	
20	**	2	4289.00	3.05	1.00	41.98	101.0	
21	**	3	9002.00	2.11	1.00	43.96	106.0	
22	**	4	5460.00	2.71	1.00	45.89	100.0	
23	**	5	4832.00	2.88	1.00	47.86	104.0	
24	**	6	4704.00	2.92	1.00	49.84	99.0	
25	**	7	5561.00	2.68	1.00	51.97	100.0	
26	**	8	4453.00	3.00	1.00	54.42	99.0	
27	**	9	5416.00	2.72	1.00	56.70	97.0	
28	**	10	5366.00	2.73	1.00	58.57	100.0	
29	**	11	5412.00	2.72	1.00	60.56	97.0	
30	**	12	5043.00	2.82	1.00	62.39	96.0	
31	**	13	5314.00	2.74	1.00	64.67	97.0	
32	**	14	9040.00	2.10	1.00	67.06	100.0	
33	**	15	8716.00	2.14	1.00	68.89	101.0	
34	**	16	11682.22	1.95	0.90	70.77	106.0	
35	**	17	9660.00	2.03	1.00	72.76	102.0	
36	**	18	10139.39	2.00	0.99	74.88	103.0	

TABLE-2

Expt. # : 1

Date/Time : 02/29/00 ; 2-30 pm

Tube #	Radioactivity for 200 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.65]	μ Ci/ml (A_1) on counting [dpm/444000]	μ Ci/ml (A_0) after 12 h incubation [$A_1 e^{-\lambda t}$]
1					
2					
3		1535	2647	0.0059	
4		2116	3649	0.0082	
5		2269	3912	0.0088	
6		3399	5861	0.0132	
7		4334	7473	0.0168	
8		4998	8618	0.0194	
9		5143	8867	0.0199	
10		5274	9093	0.0205	

11 7690 13258 0.0298

12 10493 18092 0.0407

TABLE-3

Expt. # : |

Date/Time : 02/28/00

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [uCi/ml x 10 ⁶ Cells/ml]
1	511, 526, 532			
2	546, 530, 519			
3	520, 540, 543	534	2137333	0.0027
4	475, 495, 482	484	1936000	0.0042 0.5165
5	531, 521, 547	533	2132000	0.0041
6	480, 499, 501	493	1973333	0.0067
7	507, 519, 489	505	2020000	0.0083
8	465, 472, 482	473	1892000	0.0102
9	555, 532, 521	536	2144000	0.0092
10	411, 408, 429	416	1664000	0.0123

KBr/dm
C pCi/cell
148
0.4088
~~0.4088~~
~~0.418~~
~~0.1385~~

11 460, 469, 472 467 1868000 0.0159 2.36
12 422, 410, 404 412 1648000 0.0246 3.65

TABLE-4

Expt # : 1

Date : 03/06/00

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1.2	126	139	119	} 121.33	
2.2	109	120	115		
3.2	104	115	124	114.33	0.9423
4.2	98	107	117	107.33	0.8846
5.2	100	109	92	100.33	0.8269
6.2	95	88	102	95	0.7829
7.2	80	89	72	80.33	0.6621
8.2	67	87	76	76.66	0.6319
9.2	76	83	90	83	0.6841
10.2	68	79	59	68.66	0.5659
11.2	43	51	61	51.66	0.4258
12.2	37	44	52	44.33	0.3654