

V79 COLONY FORMING ASSAY

Experiment Name : $^3\text{HTdR}$ toxicity (cluster, 100% labeling, DMSO, lindane); Exp. #: 1;

Investigator: A. Bishayee

Date: 02/25/99

1. Take 150-cm² flasks with cells and perform cell count
2. Dilute to ~4,000,000 cells/ml in MEMB [Actual count : 4,005,333 cells/ml]
3. Transfer 1 ml of cell suspension into 15 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% CO₂
5. Prepare MEMB containing radioactivity in hood

Date/Time: 02/25/99; 2-30 p.m.

72 μl $^3\text{HTdR}$ (Stock : 1 $\mu\text{Ci}/\mu\text{l}$ on 2/11/99) + 6 ml MEMB

6. After 3-4 h, remove first set of 10 test tubes from roller and add MEMB with or without radioactivity according to Table below.

Date/Time: 02/25/99; 7-15 p.m.

Tube #	$^3\text{HTdR}$ uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ $^3\text{HTdR}$ (ml) 12uCi/ml	MEMA (ml) for wash	0.58% DMSO in MEMA (ml) for wash	0.58% DMSO- 100uM Lindane in MEMA (ml) for wash
1	0	1.0	1	0	8	0	0
2	0	1.0	1	0	8	0	0
3	1	1.0	0.835	0.165	8	0	0
4	3	1.0	0.5	0.5	8	0	0
5	6	1.0	0	1	8	0	0
6	0	1.0	1	0	0	8	0
7	0	1.0	1	0	0	8	0
8	1	1.0	0.835	0.165	0	8	0
9	3	1.0	0.5	0.5	0	8	0
10	6	1.0	0	1	0	8	0
11	0	1.0	1	0	0	0	8
12	0	1.0	1	0	0	0	8
13	1	1.0	0.835	0.165	0	0	8
14	3	1.0	0.5	0.5	0	0	8
15	6	1.0	0	1	0	0	8

7. Return test tubes to roller for 12 h.

Date/Time: 02/25/99; 7-30 p.m.

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: 02/26/99; 9-00 a.m.
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 8 ml MEMA with or without DMSO or Lindane as per the Table
16. Centrifuge tubes for 10 min at 2000 rpm, 4°C
17. Decant supernatant, click tubes, vortex, resuspend in 0.4 ml MEMA with or without DMSO or Lindane as per the Table
18. Transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips
19. Centrifuge tubes for 5 min at 1000 rpm, 4°C
20. Transfer tubes at 10°C for 72 h. Date/Time: 02/26/99; 11-30 a.m.
21. 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 Date/Time: 02/26/99; 16-30 p.m.
22. 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: ^{03/01/99} 02/27/99; 12:30 p.m.
23. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes
24. Centrifuge the tubes for 10 min at 2000 rpm, 4°C (precooled centrifuge)
25. 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.
26. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
27. Centrifuge tubes for 10 min at 2000 rpm, 4°C
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 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
31. Determine cell concentration by transferring 100 μ l to Coulter cup
32. 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.
33. 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.
34. Transfer 200 μ l of cell suspension (in triplicate) to 20 ml scintillation vial containing 6 ml cocktail (Aquasol)
35. Incubate petridishes for 1 week
36. Count vials for radioactivity Date/Time : 03/01/99; 2-55 p.m.
37. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
38. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.

Expt #1.

02/25/99

we need $4,000,000 \text{ cells/ml} \times 17 \text{ ml} = 68,000,000 \text{ cells.}$

Initial count = 1217, 1212, 1141
Avg. count = 1190
Cell conc. = 4,760,000 cells/ml

$$\text{Vol. required} = \frac{68,000,000}{4,760,000}$$
$$= 14.3 \text{ ml.}$$

Take 14.3 ml cells + 2.7 ml MEMB = 17 ml

Final count = 998, 1011, 995
Avg. count = 1001.3
Cell conc. = 4,005,333 cells/ml

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 FRI 26 FEB 1999 16:3
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
 H#: 1 AQC:N GCF:N RCM:N
 CHANNEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR:
 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#	
1	**	1	1M { 9.00	66.67	1.00	1.75	60.0	<i>Expt #1</i> <i>30 µl medium</i>
2	**	2	6.00	81.65	1.00	3.63	57.0	
3	**	3	12.00	57.74	1.00	5.47	59.0	
4	**	4	2M { 3.00	115.5	1.00	7.29	61.0	
5	**	5	2590.00	3.93	1.00	9.12	61.0	
6	**	6	3M { 2628.00	3.90	1.00	11.04	62.0	
7	**	7	10212.00	1.98	1.00	12.92	62.0	
8	**	8	4M { 11221.86	1.97	0.92	14.67	60.0	
9	**	9	18231.30	1.95	0.57	16.12	64.0	
10	**	10	5M { 19613.33	1.97	0.52	17.52	63.0	
11	**	11	6M { 11.00	60.30	1.00	19.40	66.0	
12	**	12	7.00	75.59	1.00	21.28	60.0	
13	**	1	11.00	60.30	1.00	23.22	61.0	
14	**	2	7M { 9.00	66.67	1.00	25.04	59.0	
15	**	3	2735.00	3.82	1.00	26.87	64.0	
16	**	4	8M { 3183.00	3.54	1.00	28.75	64.0	
17	**	5	8907.00	2.12	1.00	30.63	58.0	
18	**	6	9M { 8896.00	2.12	1.00	32.51	61.0	
19	**	7	20314.56	1.96	0.51	33.84	58.0	
20	**	8	10M { 17979.13	1.97	0.57	35.29	67.0	
21	**	9	15.00	51.64	1.00	37.17	64.0	
22	**	10	11M { 8.00	70.71	1.00	39.05	65.0	
23	**	11	11.00	60.30	1.00	40.88	60.0	
24	**	12	12M { 10.00	63.25	1.00	42.76	63.0	
25	**	1	13M { 3077.00	3.61	1.00	44.65	59.0	
26	**	2	14M { 2889.00	3.72	1.00	46.47	57.0	
27	**	3	9717.00	2.03	1.00	48.34	59.0	
28	**	4	14M { 10847.57	2.00	0.93	50.14	65.0	
29	**	5	15M { 20203.88	1.96	0.51	51.47	60.0	
30	**	6	20068.57	1.95	0.52	52.87	59.0	
31	**	7	1C { 7.00	75.59	1.00	54.69	72.0	<i>Expt #1</i> <i>200 µl cells</i>
32	**	8	14.00	53.45	1.00	56.52	78.0	
33	**	9	3.00	115.5	1.00	58.40	80.0	
34	**	10	2C { 5.00	89.44	1.00	60.28	80.0	
35	**	11	10.00	63.25	1.00	62.11	77.0	
36	**	12	3C { 10.00	63.25	1.00	63.98	78.0	
37	**	1	2.00	141.4	1.00	65.87	74.0	
38	**	2	4C { 12.00	57.74	1.00	67.69	77.0	
39	**	3	3.00	115.5	1.00	69.56	64.0	
40	**	4	5C { 3.00	55.47	1.00	71.38	69.0	
41	**	5	97240.00	1.81	0.12	72.37	66.0	
42	**	6	16C { 97548.67	1.90	0.11	73.31	72.0	
43	**	7	102097.34	1.86	0.11	74.24	70.0	
44	**	8	17C { 106946.91	1.82	0.11	75.17	68.0	
45	**	9	100955.76	1.87	0.11	76.11	65.0	
46	**	10	19C { 100469.03	1.88	0.11	77.04	68.0	

TABLE-1

Expt. #: 1

Date/Time: 02/26/99; 4-30 P.M.

Tube #	Medium count for 30 ul (cpm)	Avg. cpm	dpm [cpm/0.65]	μ Ci/ml (A) on counting [dpm/66600]	μ Ci/ml (A ₀) on addition [A _t /e ^{-λt}]
1	<i>See the attached</i>				
2	<i>Sheet</i>				
3		2609	4013.8	0.0603	
4		10716	16486	0.2475	
5		18922	29110	0.4371	
6					
7					
8		2959	4552	0.0684	
9		8901	13694	0.2056	
10		19146	29456	0.4422	

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2983 4589 0.0689

14

10282 15818 0.2375

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20135 30977 0.4651

Exp#1

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
 H#: 1 AOC:N OCF:N RCM:N
 CHANNEL 1-LL: 0 UL: 400 ZSIGMA: 2.00 BKG SUB: 0.00 BKG ZSIG: 0.00 LSR: 0
 DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000
 HALF LIFE(DAYS): N

MON 01 MAR 1999 14:55

200 µl cell suspension

SAM	POS	CH	CPM	ZSIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	6.00	81.65	1.00	1.74	79.0	
2	**	2	14.00	53.45	1.00	3.62	73.0	
3	**	3	15.00	51.64	1.00	5.50	76.0	
4	**	4	8.00	70.71	1.00	7.37	79.0	
5	**	5	38148.28	1.90	0.29	8.57	78.0	
6	**	6	40552.73	1.89	0.28	9.71	80.0	
7	**	7	103544.00	1.76	0.12	10.71	78.0	
8	**	8	105272.00	1.74	0.12	11.71	77.0	
9	**	9	210432.00	1.23	0.12	12.73	79.0	
10	**	10	202984.00	1.26	0.12	13.74	76.0	
11	**	11	29.00	37.14	1.00	15.63	77.0	
12	**	12	12.00	57.74	1.00	17.50	78.0	
13	**	1	13.00	55.47	1.00	19.38	80.0	
14	**	2	5.00	89.44	1.00	21.27	79.0	
15	**	3	36981.82	1.98	0.28	22.41	80.0	
16	**	4	34227.69	1.90	0.33	23.60	78.0	
17	**	5	110792.00	1.70	0.12	24.60	79.0	
18	**	6	108297.10	1.64	0.14	25.67	79.0	
19	**	7	210292.03	1.30	0.11	26.62	79.0	
20	**	8	211522.12	1.29	0.11	27.57	81.0	
21	**	9	14.00	53.45	1.00	29.44	78.0	
22	**	10	13.00	55.47	1.00	31.32	78.0	
23	**	11	12.00	57.74	1.00	33.14	83.0	
24	**	12	27.00	38.49	1.00	35.02	80.0	
25	**	1	34276.92	1.89	0.33	36.26	82.0	
26	**	2	38800.00	1.97	0.26	37.34	82.0	
27	**	3	92768.12	1.77	0.14	38.39	80.0	
28	**	4	99210.15	1.71	0.14	39.45	83.0	
29	**	5	203944.00	1.25	0.12	40.47	84.0	
30	**	6	219312.00	1.21	0.12	41.48	86.0	

TABLE-2

Expt. # : 1

Date/Time : 03/01/99; 2-55 p.m.

Tube #	Radioactivity for 200 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.65]	μ Ci/ml (A_t) on counting [dpm/444000]	μ Ci/ml (A_0) after 12 h incubation [$A_t/e^{-\lambda t}$]
1	See the attached				
2	Sheet				
3		39350	60538	0.1363	
4		104408	160627	0.3618	
5		206708	318012	0.7162	
6					
7					
8		35604	54775	0.1234	
9		109544	168530	0.3796	
10		210907	324472	0.7308	

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36538	56212	0.1266
95989	147675	0.3326
211628	325581	0.7333

TABLE-3

Expt. # : [

Date/Time : 03/01/99

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	640, 666, 685			
2	701, 722, 735			
3	735, 742, 722	733	2932000	0.0465
4	760, 750, 742	750	3002666	0.1205
5	775, 790, 769	778	3112000	0.2301
6	711, 729, 731			
7	699, 719, 698			
8	765, 777, 759	767	3068000	0.0402
9	745, 755, 761	753	3014666	0.1259
10	762, 772, 760	764	3058666	0.2389

11 685, 672, 669

12 701, 735, 723

13 795, 780, 776 783 3134666 0.0404

14 740, 722, 729 730 2921333 0.1138

15 811, 788, 792 797 3188000 0.23

TABLE-4

Expt # : 1

Date : 03/08/99

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1.2	155	160	149	} 150	
2.2	145	139	152		
3.2	21	20	17	19.33	0.1288
4.4	23	26	21	0.23	0.0015
5.4	2	2	1	0.016	0.00012
6.2	174	185	170	} 174.5	
7.2	169	172	177		
8.2	25	27	29	27	0.1547
9.4	26	30	35	0.3	0.0017
10.4	2,	3,	3	0.026	0.00016

11.2	182	169	178	} 171.66	
12.2	177	161	163		
13.2	26	31	37	31.33	0.1825
14.4	22	26	29	0.25	0.0015
15.4	2	2	2	0.02	0.00011