

### V79 COLONY FORMING ASSAY

Experiment Name :  $^3\text{HTdR}$  toxicity (cluster, 100% labeling, DMSO, lindane); Exp. #: 2;

Investigator: A. Bishayee

Date: 03/01/99

1. Take 150-cm<sup>2</sup> flasks with cells and perform cell count
2. Dilute to ~4,000,000 cells/ml in MEMB [Actual count : 4,033,333 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% CO<sub>2</sub>

Date/Time: 03/01/99; 4:00pm

5. Prepare MEMB containing radioactivity in hood

72  $\mu\text{l}$   $^3\text{HTdR}$  (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: 03/01/99; 7:30 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: 03/01/99; 7:45 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: 03/02/99; 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 µl) using 200 µl pipet tips
19. Centrifuge tubes for 5 min at 1000 rpm, 4°C
20. Transfer tubes at 10°C for 72 h. Date/Time: 03/02/99; 11-00 a.m.
21. Transfer 30 µl supernatant in three sets of 20 ml scintillation vials containing 6 ml liquid scintillation cocktail (Aquasol) from 150 µl supernatant removed earlier (Step 10) and count them for radioactivity Date/Time:
22. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 µl 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: 03/05/99; 10-00 a.m.
23. Again add 200 µl 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  $\mu$ 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  $\mu$ 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 : 03/05/99; 12-30 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 #2

03/01/99

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

Initial count = 1004, 1025, 996

Avg count = 1008

Cell conc = 4,033,333 cells/ml

~~vol. required =  $\frac{68,000,000}{4,760,000}$~~

~~Take ml cells + ml MEM B = 17 ml~~

~~Final count =~~

~~Avg. count =~~

~~Cell conc. =~~

Expt # 2

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 TUE 02 MAR 1999 11:28  
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N  
M#: 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: 0 1.00000  
HALF LIFE(DAYS):N *30 µl medium*

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	13.00	55.47	1.00	1.75	55.0	
2	**	2	11.00	60.30	1.00	3.67	58.0	
3	**	3	10.00	63.25	1.00	5.56	57.0	
4	**	4	14.00	53.45	1.00	7.44	60.0	
5	**	5	6460.00	2.49	1.00	9.36	59.0	
6	**	6	6763.00	2.43	1.00	11.24	58.0	
7	**	7	18768.70	1.93	0.57	12.69	59.0	
8	**	8	19422.86	1.98	0.52	14.09	60.0	
9	**	9	34895.38	1.88	0.33	15.28	59.0	
10	**	10	38952.73	1.93	0.28	16.42	58.0	
11	**	11	6.00	81.65	1.00	18.34	58.0	
12	**	12	12.00	57.74	1.00	20.22	58.0	
13	**	1	18.00	47.14	1.00	22.17	57.0	
14	**	2	8.00	70.71	1.00	24.05	56.0	
15	**	3	5830.00	2.62	1.00	25.93	58.0	
16	**	4	6199.00	2.54	1.00	27.87	61.0	
17	**	5	17376.00	1.92	0.62	29.37	59.0	
18	**	6	19792.38	1.96	0.52	30.77	60.0	
19	**	7	36273.84	1.84	0.33	31.96	62.0	
20	**	8	42583.33	1.98	0.24	33.12	60.0	
21	**	9	7.00	75.59	1.00	35.03	59.0	
22	**	10	7.00	75.59	1.00	36.97	60.0	
23	**	11	8.00	70.71	1.00	38.84	59.0	
24	**	12	10.00	63.25	1.00	40.72	61.0	
25	**	1	6714.00	2.44	1.00	42.69	60.0	
26	**	2	7105.00	2.37	1.00	44.57	64.0	
27	**	3	19396.29	1.95	0.54	46.03	62.0	
28	**	4	19896.29	1.93	0.54	47.49	64.0	
29	**	5	39543.40	1.95	0.26	48.57	60.0	
30	**	6	43090.91	1.84	0.28	49.71	64.0	

TABLE-1

Expt. #: 2

Date/Time: 03/02/99; 11-30 a.m.

Tube #	Medium count for 30 ul (cpm)	Avg. cpm	dpm [cpm/0.65]	$\mu\text{Ci/ml (A)}$ on counting [dpm/66600]	$\mu\text{Ci/ml (A}_0)$ on addition [A/e <sup>-<math>\lambda</math>t</sup> ]
1	See the attached				
2	Sheet				
3		6611	10171	0.1527	
4		19095	29376	0.4410	
5		36923	56805	0.8529	
6					
7					
8		6014	9253	0.1389	
9		18584	28590	0.4293	
10		39428	60658	0.9108	

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6909	10630	0.1596
19646	30224	0.4538
41316	<del>82633</del>	<del>1.2407</del>
	63563	0.9544

Exp#2

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 FRI 05 MAR 1999 12:37  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N  
 AOC:N BCF: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	8.00	70.71	1.00	1.75	78.0	
2	**	2	22.00	42.64	1.00	3.63	86.0	
3	**	3	11.00	60.30	1.00	5.52	61.0	
4	**	4	3.00	115.5	1.00	7.40	59.0	
5	**	5	30757.33	1.86	0.38	8.64	84.0	
6	**	6	19763.81	1.96	0.52	10.04	62.0	
7	**	7	56471.11	1.77	0.23	11.13	63.0	
8	**	8	57957.89	1.91	0.19	12.24	62.0	
9	**	9	34231.89	1.47	0.14	13.31	68.0	
10	**	10	37280.00	1.53	0.12	14.31	61.0	
11	**	11	1514.00	5.14	1.00	16.18	63.0	
12	**	12	501.00	5.16	1.00	18.02	63.0	
13	**	1	154.00	16.12	1.00	19.95	60.0	
14	**	2	145.00	16.61	1.00	21.83	61.0	
15	**	3	21172.63	1.99	0.48	23.18	63.0	
16	**	4	23651.76	1.99	0.43	24.47	60.0	
17	**	5	52715.55	1.84	0.23	25.57	64.0	
18	**	6	58188.57	1.98	0.18	26.61	64.0	
19	**	7	108464.00	1.72	0.12	27.61	64.0	
20	**	8	29123.90	1.66	0.11	28.55	64.0	
21	**	9	1397.00	5.35	1.00	30.43	64.0	
22	**	10	1532.00	5.11	1.00	32.30	62.0	
23	**	11	40.00	31.62	1.00	34.18	61.0	
24	**	12	56.00	26.73	1.00	36.02	77.0	
25	**	1	27408.00	1.97	0.38	37.30	77.0	
26	**	2	28148.72	1.91	0.39	38.61	80.0	
27	**	3	86688.41	1.83	0.14	39.67	77.0	
28	**	4	84768.12	1.85	0.14	40.72	78.0	
29	**	5	193952.00	1.28	0.12	41.74	87.0	
30	**	6	163079.66	1.47	0.11	42.68	80.0	

TABLE-2

Expt. # : 2

Date/Time : 03/05/99 ; 12-30 P.M.

Tube #	Radioactivity for 200 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.65]	$\mu$ Ci/ml (A <sub>1</sub> ) on counting [dpm/444000]	$\mu$ Ci/ml (A <sub>0</sub> ) after 12 h incubation [A <sub>1</sub> e <sup>-λt</sup> ]
1	See the attached				
2	Met				
3		25260 <del>16260</del>	38861 <del>25015</del>	0.0875	0
4		57214	88021	0.1982	
5		135755	208854	0.4704	
6					
7					
8		22411	34479	0.0776	
9		55451	85310	0.1921	
10		118793	182759	0.4116	

11)

12)

13

27778      42735      0.0962

14

85728      131889      0.2970

15

178515      274639      0.6185



TABLE-3

Expt. #: 2

Date/Time: 03/05/99; 10:00 a.m.

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [uCi/ml x 10 <sup>6</sup> Cells/ml]
1				
2				
3	480, 495, 478	484	1937333	0.04516
4	450, 477, 458	461	1846666	0.1073
5	575, 589, 567	577	2308000	0.2038
6				
7				
8	435, 449, 429	437	1750666	0.0434
9	445, 455, 462	454	1816000	0.1057
10	540, 559, 547	548	2194666	0.1875

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13 560, 550, 542 550 2202666 0.0436

14. 610, 625, 609 614 2458666 0.1208

15 675, 685, 687 682 2729333 0.2266

160

TABLE-4

Expt #: 2

Date: 03/12/99

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1.2	160	155	168	} 158.83	-
2.2	152	157	161		-
3.2	17	22	27	22	0.138
4.4	23	30	38	0.33	0.0019
5.4	3	2	4	0.03	0.00018
6.2	156	142	150	} 147.5	
7.2	143	149	145		
8.2	18	25	30	24.33	0.165
9.4	14	19	25	0.19-	0.0013
10.4	3	4	5	0.04	0.00027

11.2	160	167	172	} 160.66	
12.2	151	149	165		
13.2	20	24	28	24	0.147
14.4	23	27	35	0.28	0.0017
15.4	3	3	2	0.023	0.00016