

### V79 COLONY FORMING ASSAY

Experiment Name :  $^3\text{HTdR}$  +0 or 10% DMSO (cluster, 100% labeling);

Exp. # : 2;

Experiment performed by : A. Bishayee

Date: 01/10/00;

1. Set the rocker-roller at 37°C incubator with 5% CO<sub>2</sub>, set the Coulter Counter, wash cells (from two 150 cm<sup>2</sup> flasks, 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 ~4,000,000 cells/ml in MEMB [Actual count : 4,010,666 cells/ml]
3. Transfer 1 ml of cell suspension into 14 14-ml tubes (Falcon plastic test tube, 17x100 mm) labeled 1-14 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: 01/10/00; 3-00 pm
5. Prepare MEMB containing radioactivity in hood  
 $120 \mu\text{l } ^3\text{HTdR}$  (Stock :  $(\mu\text{Ci}/\mu\text{l on } 10/20)$  ) + 4.88 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: 01/10/00; 7-00 pm

Tube #	<sup>3</sup> HTdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ <sup>3</sup> HTdR (ml) [24uCi/ml I]
1	0	1.0	1.0	0
2	0	1.0	1.0	0
3	0.5	1.0	0.96	0.04
4	2	1.0	0.834	0.166
5	4	1.0	0.67	0.33
6	8	1.0	0.34	0.66
7	12	1.0	0	1
8	0	1.0	1.0	0
9	0	1.0	1.0	0
10	0.5	1.0	0.96	0.04
11	2	1.0	0.834	0.166
12	4	1.0	0.67	0.33
13	8	1.0	0.34	0.66
10 <sup>14</sup>	12	1.0	0	1

7. Return test tubes to roller for 12 h.

Date/Time: 01/10/00 12:75 7-30 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: 01/11/00, 9-15 am from each tube

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

Tubes 1-7: 0% DMSO

3

Tubes 8-14: 10% DMSO

17. Decant supernatant, click tubes, vortex, resuspend in 8 ml of MEMA with or without 10% DMSO
18. Centrifuge tubes for 5 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 with or without 10% DMSO, 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: 01/11/00; 11-15 a.m.*
23. Transfer 30 ul supernatant *in three sets* of 6 ml scintillation vials containing 6 ml liquid scintillation cocktail (Ecolume, ICN) from 150 ul supernatant removed earlier (Step 10) and count them for radioactivity *in triplicate into*  
*Date/Time: 01/11/00; 12:00*
24. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the contents by using pasteur pipets to 14 corresponding 14-ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-14 both on cap and wall) containing 10 ml wash MEMA in each  
*Date/Time: 01/14/00; 10-00 a.m.*
25. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in corresponding 14 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 *wash*
  - 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. *wash*
28. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA *and maintain at room temp.*
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 µl 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 µl of cell suspension (in triplicate) to 6 ml scintillation vial containing 6 ml cocktail.

37. Incubate petridishes for 1 week

38. Count vials for radioactivity

Date/Time : 01/14/00, 2:00 PM.

39. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol.

Stain colonies with 0.5% crystal violet

40. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.

30 µl medium

USER: 6 ID:H3 HOWELL      PRESET TIME: 1.00      TUE 11 JAN 2000 11:53  
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N      RS232:N  
1 ACC:N GCF: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	16.00	50.00	1.00	2.34	91.0	
2	**	2	10.00	63.25	1.00	4.73	90.0	
3	**	3	8.00	70.71	1.00	6.66	92.0	
4	**	4	11.00	60.30	1.00	8.68	91.0	
5	**	5	7.00	75.59	1.00	10.86	92.0	
6	**	6	15.00	51.64	1.00	12.94	92.0	
7	**	7	6717.00	2.44	1.00	15.23	90.0	
8	**	8	6616.00	2.46	1.00	17.41	92.0	
9	**	9	6636.00	2.46	1.00	19.39	92.0	
10	**	10	29256.41	1.87	0.39	20.90	92.0	
11	**	11	28671.05	1.92	0.38	22.71	93.0	
12	**	12	26633.33	1.89	0.42	24.45	91.0	
13	**	13	55670.00	1.90	0.20	25.62	92.0	
14	**	14	53976.74	1.86	0.22	26.85	92.0	
15	**	15	60155.00	1.82	0.20	28.02	93.0	
16	**	16	113984.00	1.68	0.12	29.53	93.0	
17	**	17	108041.67	1.60	0.14	31.22	92.0	
18	**	18	115797.10	1.58	0.14	32.28	93.0	
19	**	19	155796.47	1.51	0.11	33.74	90.0	
20	**	20	178014.50	1.28	0.14	35.39	92.0	
21	**	21	174860.00	1.51	0.10	36.68	91.0	
22	**	22	10.00	63.25	1.00	38.65	93.0	
23	**	23	6.00	81.65	1.00	40.67	93.0	
24	**	24	4.00	100.0	1.00	42.94	92.0	
25	**	25	13.00	55.47	1.00	45.07	91.0	
26	**	26	9.00	66.67	1.00	47.04	92.0	
27	**	27	7.00	75.59	1.00	49.02	94.0	
28	**	28	8.00	70.71	1.00	51.04	92.0	
29	**	29	11.00	60.30	1.00	53.27	91.0	
30	**	30	8.00	70.71	1.00	55.66	92.0	
31	**	31	6588.00	2.46	1.00	57.69	94.0	
32	**	32	6903.00	2.41	1.00	59.67	92.0	
33	**	33	6747.00	2.43	1.00	61.76	93.0	
34	**	34	25552.50	1.98	0.40	63.12	91.0	
35	**	35	58382.86	1.98	0.17	64.37	92.0	
36	**	36	58242.11	1.90	0.19	65.47	91.0	
37	**	37	102606.66	1.61	0.15	66.65	92.0	
38	**	38	107619.63	1.51	0.16	67.84	93.0	
39	**	39	110969.32	1.49	0.16	69.03	93.0	
40	**	40	153251.44	1.22	0.17	70.30	94.0	
41	**	41	168805.31	1.45	0.11	71.24	93.0	
42	**	42	177086.66	1.23	0.15	72.38	93.0	

TABLE-1

Expt. # : 2

Date/Time : 01/11/00; 11-53 am

Tube #	Medium count for 30 ul (cpm)	Avg. cpm	dpm [cpm/0.58]	$\mu$ Ci/ml (A) on counting [dpm/66600]	$\mu$ Ci/ml (A <sub>0</sub> ) on addition [A <sub>0</sub> /e <sup>-λt</sup> ]
1					
2					
3		6656	11476	0.1723	
4		28186	48596	0.7297	
5		56600	97586	1.465	
6		112607	194150	2.915	
7		169556	292339	4.389	
8					
9					
10		6746	11631	0.1746	

11	25552	44055	0.6615
12	58312	100537	1.509
13	10706	184594	2.772
14	166380	286863	4.307

**	1	1	5.00	89.44	1.00	68.26	111.0
**	2	1	12.00	57.74	1.00	70.18	109.0
**	3	1	14.00	53.45	1.00	72.11	110.0
**	4	1	13.00	55.47	1.00	74.13	123.0

200 pl cells  
01/14/00,

PAGE

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#
97	**	5	14.00	53.45	1.00	76.40	109.0
98	**	6	12.00	57.74	1.00	78.49	110.0
99	**	7	4461.00	2.99	1.00	80.37	110.0
100	**	8	4574.00	2.96	1.00	82.60	110.0
101	**	9	4564.00	2.96	1.00	84.73	110.0
102	**	10	48212.17	1.95	0.57	86.38	110.0
103	**	11	48660.87	1.93	0.57	88.03	110.0
104	**	12	48937.39	1.92	0.57	89.98	110.0
105	**	13	42669.09	1.85	0.28	91.32	111.0
106	**	14	43448.00	1.92	0.25	92.53	111.0
107	**	15	42966.66	1.97	0.24	93.69	110.0
108	**	16	77617.14	1.72	0.17	94.94	110.0
109	**	17	78869.57	1.92	0.14	96.00	111.0
110	**	18	78340.91	1.97	0.13	97.56	110.0
111	**	1	421866.66	1.48	0.15	98.75	108.0
112	**	2	118950.92	1.44	0.16	99.94	110.0
113	**	3	133398.23	1.69	0.11	101.34	108.0
114	**	4	15.00	51.64	1.00	103.33	109.0
115	**	5	21.00	43.64	1.00	105.31	108.0
116	**	6	13.00	55.47	1.00	107.33	109.0
117	**	7	3.00	115.5	1.00	109.36	108.0
118	**	8	12.00	57.74	1.00	111.29	111.0
119	**	9	14.00	53.45	1.00	113.27	109.0
120	**	10	4778.00	2.89	1.00	115.50	108.0
121	**	11	4787.00	2.89	1.00	117.83	111.0
122	**	12	4790.00	2.89	1.00	119.97	109.0
123	**	13	16347.97	1.99	0.62	121.59	110.0
124	**	14	16670.77	1.92	0.65	123.42	107.0
125	**	15	16910.57	1.96	0.62	125.31	109.0
126	**	16	33458.46	1.92	0.33	126.50	109.0
127	**	17	33955.88	1.86	0.34	127.76	113.0
128	**	18	34223.33	1.97	0.30	129.03	111.0
129	**	1	67109.09	1.90	0.17	130.52	108.0
130	**	2	69593.33	1.96	0.15	131.63	110.0
131	**	3	69700.00	1.74	0.19	132.74	110.0
132	**	4	93862.86	1.56	0.17	133.99	113.0
133	**	5	98440.00	1.80	0.12	134.99	108.0
134	**	6	100873.95	1.83	0.12	136.44	109.0

TABLE-2

Expt. #: 2

Date/Time: 01/14/00, 3:00pm

Tube #	Radioactivity for 200 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.58]	$\mu\text{Ci/ml (A)}$ on counting [dpm/444000]	$\mu\text{Ci/ml (A}_0)$ after 12 h incubation [ $A_0/e^{-\lambda t}$ ]
1					
2					
3		4533	7815	0.0176	
4		18603	32074	0.0722	
5		43027	74185	0.1671	
6		78099	134653	0.3033	
7		121404	209318	0.4714	
8					
9					
10		4785	8250	0.0186	

11	16642	28693	0.0646
12	33878	58411	0.1316
13	68800	118621	0.2672
14	97725	168491	0.3795



Sent: Thursday, January 06, 2000 9:46 AM

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TABLE-3

Expt. # : 2

Date/Time : 01/14/00

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]	Kbq/cell [pCi/cell x 148]
1	598, 607, 588				
2	575, 566, 559				
3	457, 435, 461	451	1804000	0.0097	1.44
4	522, 535, 542	533	2132000	0.0339	5.01
5	545, 519, 527	530	212333	0.0788	11.66
6	488, 475, 462	475	1900000	0.1596	23.63
7	562, 575, 569	568	2274666	0.2072	30.67
8	513, 522, 530				
9	501, 511, 507				
10	475, 465, 470	470	1880000	0.0099	1.46
11	495, 489, 512	498	1994666	0.0323	4.79
12	445, 439, 452	445	1781333	0.0739	10.93
13	432, 417, 420	423	1692000	0.1579	23.37
14	455, 429, 439	441	<del>2164000</del> 1764000	0.2151	31.84

TABLE-4

Expt # : 2

Date : 01/21/00

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1.2	158	148	152	} 149	
2.2	141	139	156		
3.2	130	138	123	130.33	0.8747
4.2	49	60	54	54.33	0.3646
5.3	97	103	91	9.7	0.0651
6.4	67	58	76	0.67	0.0045
7.4	16	13	11	0.13	0.00089
8.2	132	142	129	} 133.66	
9.2	140	128	131		
10.2	113	120	128	120.33	0.9002

11.2	77	83	90	83.33	0.6234
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12.2	13	16	20	16.33	0.1221
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13.3	43	51	60	5.13	0.0384
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14.4	80	90	71	0.8033	0.0060
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