

## V79 COLONY FORMING ASSAY

Experiment Name :  $^3\text{HTdR}$  (100% labeling, cluster, suspension);  
 Experiment performed by : A. Bishayee

Exp. #: 2;  
 Date: 01/03/00

1. Set the rocker-roller at  $37^\circ\text{C}$  incubator with 5%  $\text{CO}_2$ , set the Coulter Counter, wash cells (from two  $150\text{ cm}^2$  flask, 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 : 4,009,333 cells/ml]
3. Transfer 1 ml of cell suspension into 14 12 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  $37^\circ\text{C}$ , 5%  $\text{CO}_2$  Date/Time: 01/03/00 ; 2-30 pm.
5. Prepare MEMB containing radioactivity in hood  
 $80\ \mu\text{l } ^3\text{HTdR}$  (Stock :  $\mu\text{Ci}/\mu\text{l}$  on 10/20/99 ) + 4.92 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: 01/03/00 ; 7-15 pm.

Tube #	<sup>3</sup> HTdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ <sup>3</sup> HTdR (ml) [16uCi/ml I]
1	0	1.0	1.0	0
2	0	1.0	1.0	0
3	0.5	1.0	0.938	0.062
4	1	1.0	0.875	0.125
5	2	1.0	0.75	0.25
6	4	1.0	0.5	0.5
7	8	1.0	0	1
8	0	1.0	1	0
9	0	1.0	1	0
10	0.5	1.0	0.938	0.062
11	1	1.0	0.875	0.125
12	2	1.0	0.75	0.25
13	4	1.0	0.5	0.5
14	8	1.0	0	1

Take ~~80~~ <sup>1</sup> ~~ml~~ stock +  
4.92 ml MEMB = 5 ml

7. Return test tubes to roller for 12 h . Date/Time: 01/03/00; 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: 01/04/00; 9-10 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 10 ml wash MEMA
16. Centrifuge tubes for 10 min at 2000 rpm, 4°C

17. Decant supernatant, click tubes, vortex
18. Resuspend for tube 8-14 in 2 ml of MEMA and transfer to 10.5°C for 72 h
19. For tube 1-7, 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:** 01/04/00 ; 11-00 a.m.
23. Transfer 30 ul supernatant in three sets of 6 ml scintillation vials containing 6 ml liquid scintillation cocktail (Ecolume) from 150 ul supernatant removed earlier (Step 10) and count them for radioactivity **Date/Time:** 01/04/00; 12-50 pm.
24. After 72 h, for tubes 1-7, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the content to 8 12 ml tubes (Falcon plastic test tube, 17x100 mm, labeled both on cap and wall) containing 10 ml wash MEMA by using pasteur pipet **Date/Time:**
25. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes. For tubes 8-14, add 8 ml wash MEMA
26. Centrifuge all 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 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 20 ml scintillation vial containing 6 ml cocktail (Aquasol) *Ecolume*
37. Incubate petridishes for 1 week

38. Count vials for radioactivity **Date/Time** : 01/10/00; 1-15 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.

30 ul medium

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 TUE 04 JAN 2000 12:50  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N  
 H#: 1 AQC:N BCF:N RCM:N  
 CHANNEL 1-LL: 0 UL: 400 ZSIGMA: 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	ZSIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	14.00	53.45	1.00	1.80	91.0	
2	**	2	10.00	63.25	1.00	3.73	91.0	
3	**	3	12.00	57.74	1.00	5.70	91.0	
4	**	4	4.00	100.0	1.00	7.77	92.0	
5	**	5	8.00	70.71	1.00	9.84	95.0	
6	**	6	8.00	70.71	1.00	11.97	93.0	
7	**	7	7727.00	2.28	1.00	14.34	91.0	
8	**	8	9057.00	2.10	1.00	16.28	94.0	
9	**	9	9062.00	2.10	1.00	18.26	93.0	
10	**	10	18398.18	1.99	0.55	19.78	96.0	
11	**	11	18738.18	1.97	0.55	21.30	94.0	
12	**	12	18304.35	1.95	0.57	22.94	95.0	
13	**	13	37196.36	1.98	0.28	24.28	92.0	
14	**	14	38921.43	1.92	0.28	25.99	94.0	
15	**	15	36270.77	1.84	0.33	27.18	93.0	
16	**	16	70040.00	1.81	0.17	28.43	94.0	
17	**	17	69446.66	1.96	0.15	29.55	95.0	
18	**	18	70047.88	1.74	0.19	30.87	95.0	
19	**	1	137190.19	1.34	0.16	32.12	93.0	
20	**	2	136205.72	1.30	0.17	33.37	93.0	
21	**	3	133384.00	1.55	0.12	34.89	93.0	
22	**	4	10.00	63.25	1.00	37.32	94.0	
23	**	5	11.00	60.30	1.00	39.35	97.0	
24	**	6	13.00	55.47	1.00	41.68	96.0	
25	**	7	9.00	66.67	1.00	43.69	95.0	
26	**	8	18.00	47.14	1.00	45.67	95.0	
27	**	9	31.00	35.92	1.00	47.84	95.0	
28	**	10	7656.00	2.29	1.00	50.27	91.0	
29	**	11	8365.00	2.19	1.00	52.26	94.0	
30	**	12	8338.00	2.19	1.00	54.14	94.0	
31	**	13	15854.69	1.99	0.64	55.71	94.0	
32	**	14	16664.06	1.94	0.64	57.27	94.0	
33	**	15	17432.00	1.92	0.62	58.76	95.0	
34	**	16	34046.15	1.90	0.33	60.15	93.0	
35	**	17	36236.66	1.92	0.30	61.62	94.0	
36	**	18	36496.55	1.94	0.29	63.02	94.0	
37	**	1	59350.00	1.84	0.20	64.23	90.0	
38	**	2	66873.69	1.77	0.19	65.33	92.0	
39	**	3	66319.02	1.92	0.16	66.52	93.0	
40	**	4	132159.42	1.48	0.14	67.58	96.0	
41	**	5	133143.62	1.26	0.19	68.91	97.0	
42	**	6	129352.94	1.61	0.12	70.37	95.0	

TABLE-1

Expt. #: 2

Date/Time: 01/04/00; 12-50 pm

Tube #	Medium count for 30 ul (cpm)	Avg. cpm	dpm [cpm/0.58]	$\mu$ Ci/ml ( $A_0$ ) on counting [dpm/66600]	$\mu$ Ci/ml ( $A_0$ ) on addition [ $A_1/e^{-\lambda t}$ ]
1					
2					
3		8615	14854	0.22	
4		18480	31862	0.48	
5		37462	6490	0.97	
6		69844	120421	1.81	
7		135593	233781	3.51	
8					
9					
10		8119	13999	0.21	

11		16650	28706	0.43	
12		35592	61366	0.92	
13		64180	110656	1.66	
14		131551	226812	3.41	

USER: 6 ID:H3 HOWELL      PRESET TIME: 1.00      MON 10 JAN 2000 13:19  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N      RS232:N  
 H#: 1 AGC:N QCF: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

SAM	FOS	CH	CPM	ZSIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	9.00	66.67	1.00	1.78	111.0	
2	**	2	14.00	53.45	1.00	3.81	111.0	
3	**	3	4.00	100.0	1.00	5.78	113.0	
4	**	4	9.00	66.67	1.00	7.91	112.0	
5	**	5	10.00	63.25	1.00	9.79	112.0	
6	**	6	9.00	66.67	1.00	11.72	114.0	
7	**	7	2423.00	4.06	1.00	13.63	114.0	
8	**	8	2766.00	3.80	1.00	15.61	114.0	
9	**	9	2639.00	3.89	1.00	17.63	113.0	
10	**	10	3300.00	3.48	1.00	19.81	115.0	
11	**	11	3753.00	3.26	1.00	21.69	120.0	
12	**	12	5120.00	3.58	1.00	23.77	118.0	
13	**	13	20137.04	1.92	0.54	25.22	121.0	
14	**	14	12247.27	1.99	0.82	27.12	119.0	
15	**	15	11715.61	1.99	0.87	29.01	119.0	
16	**	16	35103.45	1.98	0.29	30.22	114.0	
17	**	17	33790.00	1.99	0.30	31.48	115.0	
18	**	18	5.00	89.44	1.00	33.46	88.0	
19	**	1	62148.57	1.92	0.17	34.76	112.0	
20	**	2	45541.66	1.83	0.24	36.12	112.0	
21	**	3	61173.68	1.86	0.19	37.22	114.0	
22	**	4	11.00	60.30	1.00	39.14	114.0	
23	**	5	9.00	66.67	1.00	41.12	115.0	
24	**	6	9.00	66.67	1.00	43.09	113.0	
25	**	7	3.00	115.5	1.00	45.22	113.0	
26	**	8	10.00	63.25	1.00	47.60	112.0	
27	**	9	9.00	66.67	1.00	49.57	113.0	
28	**	10	4645.00	2.93	1.00	51.56	113.0	
29	**	11	4655.00	2.93	1.00	53.49	114.0	
30	**	12	3980.00	3.17	1.00	55.61	112.0	
31	**	13	10195.00	1.98	1.00	57.58	114.0	
32	**	14	9795.00	2.02	1.00	59.47	113.0	
33	**	15	9022.00	2.11	1.00	61.50	112.0	
34	**	16	18937.39	1.92	0.57	62.94	115.0	
35	**	17	19259.26	1.96	0.54	64.60	113.0	
36	**	18	14801.67	1.99	0.60	66.17	113.0	
37	**	1	36513.79	1.94	0.29	67.43	106.0	
38	**	2	31815.87	2.00	0.31	68.77	113.0	
39	**	3	33393.84	1.92	0.33	69.96	116.0	
40	**	4	64030.68	1.96	0.16	71.14	115.0	
41	**	5	63395.75	1.60	0.19	72.46	113.0	
42	**	6	62147.37	1.84	0.19	73.57	113.0	

	Coulter Count	Avg. Cell Count	Vol. containing $10^6$ cells (ml)
			01/07/00
{ 1	525, 540, 523	529	0.472
{ 2	560, 574, 550	561	0.445
3	251, 249, 248	249	1.00 (~ 0.8 plates)
4	<del>408</del> , 396, 446, 424, 430   433		0.577
5.	309, 292, 312	359	0.695
6.	396, 373, 396	388	0.644
7.	452, 432, 429	437	0.571
{ 8.	423, 443, 437	434	0.576
{ 9	383, 367, 385	378	0.661
10.	375, 379, 408	387	0.645
11.	401, 398, 399	399	0.626
12	345, 365, 340	350	0.714
13	395, 367, 385	382	0.654
14	436, 449, 458	447	0.558



TABLE-2

Expt. # : 2 ✓

Date/Time : 01/10/00; 1-15 pm.

Tube #	Radioactivity for 200 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.58]	$\mu\text{Ci/ml (A}_0\text{)}$ on counting [dpm/444000]	$\mu\text{Ci/ml (A}_t\text{)}$ after 12 h incubation [ $A_t/e^{-\lambda t}$ ]
1					
2					
3		2609	4498	0.01013	$\times 10^{-1}$
4		3391	5846	0.1316	$\times 10^{-1}$
5		20137	34718	0.7819	$\times 10^{-1}$
6		34446	59390	1.337	$\times 10^{-1}$
7		61660	106311	2.394	$\times 10^{-1}$
8					
9					
10		4426	7632	0.1718	$\times 10^{-1}$

11	9670	16673	$0.3755 \times 10^{-1}$
12	19098	32927	$0.7416 \times 10^{-1}$
13.	33907	58460	$1.316 \times 10^{-1}$
14.	63088	108773	$2.449 \times 10^{-1}$

TABLE-3

Expt. # :

Date/Time :

mbq/cell  
[pci/cell  
37]

Kb/cell  
[pci/cell  
x  
148]

~~3.76~~  
0.376  
0.281  
2.14  
20.14  
3.18  
31.87  
5.07  
50.67

0.440  
0.440  
0.870  
0.870  
1.96  
19.59  
3.18  
31.86  
5.07  
50.67

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	See the attached sheet 329			
2		561		0.1017
3		249		0.1017 x 10 <sup>-1</sup>
4		433		0.0759 x 10 <sup>-1</sup>
5		359		0.5444 x 10 <sup>-1</sup>
6		388		0.8614 x 10 <sup>-1</sup>
7		437		1.369 x 10 <sup>-1</sup>
8		434		
9		378		
10		387		0.1108 x 10 <sup>-1</sup>

11. 399 0.2352 x 10<sup>-1</sup>

12. 350 0.5297 x 10<sup>-1</sup>

13. 382 0.8612 x 10<sup>-1</sup>

14. 447 1.369 x 10<sup>-1</sup>

15.05  
15.05 x 10<sup>-1</sup>  
11.24 x 10<sup>-1</sup>  
80.5 x 10<sup>-1</sup>  
127.49 x 10<sup>-1</sup>  
202.69 x 10<sup>-1</sup>

TABLE-4

Expt # :

Date :

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony <i>for x.2</i>	SF
1.2	157	162	149	} 161.16	
2.2	168	172	159		
3.2	106	99	93	99.3	0.6163
4.2	98	87	77	87.3	0.5419
5.3	79	80	92	8.36	0.0519
6.3	37	32	28	3.23	0.0200
7.4	39	48	57	0.48	0.0029
8.2	142	138	132	} 131.83	
9.2	131	129	179		
10.2	36	44	51	43.6	0.3312

SE (%)

4  
7  
5  
10

11.2	18	26	37	27	0.2048
12.3	40	47	34	4.03	0.0306
13.4	132	139	118	1.29	0.0098
14.4	20	16	13	0.16	0.0012