

## V79 COLONY FORMING ASSAY

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

Exp.# 2

Experiment performed by : A. Bishayee

Date: 02/17/00

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 : 3,993,600 cells/ml]
3. Transfer 1 ml of cell suspension into 10 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: 02/17/00, 4:00 pm
5. Prepare MEMB containing radioactivity in hood  
 $48\mu\text{l } ^3\text{HTdR}$  (Stock :  $\mu\text{Ci}/\mu\text{l}$  on 1/31/00) + 12 ml MEMB
6. After 3-4 h, remove test tubes from roller and add MEMB with radioactivity according to Table below. Date/Time: 02/17/00, 7:30 pm

Tube #	$^3\text{HTdR}$ uCi/ml	Cells in MEMB (ml)	MEMB+ $^3\text{HTdR}$ [4uCi/ml] (ml)
1	2	1.0	1
2	2	1.0	1
3	2	1.0	1
4	2	1.0	1
5	2	1.0	1
6	2	1.0	1
7	2	1.0	1

02/17/00	7-15 pm	0 W
02/18/00	9-00 a.m	14 h
02/19/00	2-00 pm	44 h
02/20/00	2-00 pm	68 h
02/21/00	2-00 pm	92 h
02/22/00	2-00 pm	116 h
02/23/00	2-00 pm	140 h
02/24/00	2-00 pm	164 h
02/25/00	2-00 pm	188 h

8	2	1.0	1
9	2	1.0	1
10	2	1.0	1

7. Return test tubes to roller for 12 h. Date/Time: 02/17/00; 7-15 P.M.
8. Next day, while test tubes are in roller label 10 gamma-tubes (12 X 75 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: 02/18/00; 9-00 A.M.
10. Remove buckets from centrifuge and carefully remove 150 µl of supernatant from each tube and place in pre-labeled 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 2 ml of wash MEMA for tube #1, syringe three times, transfer 100 ul for cell count and 200 ul (in triplicate) for radioactivity count.
- ~~18. Centrifuge all other tubes (2-10) for 5 min at 2000 rpm, 4°C~~
19. Transfer the cell suspension <sup>from tube 2-10</sup> 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. Take tube #2, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the contents by using pasteur pipets to 14-ml tubes (Falcon plastic test tube, 17x100 mm) containing 10 ml wash MEMA
23. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in corresponding 14 ml tubes
24. Wash cells three times by centrifuging the tubes for 10 min at 2000 rpm, 4°C resuspend in 2 ml of wash MEMA for tube #2, syringe three times, transfer 100 ul for cell count and 200 ul (in triplicate) for radioactivity count.
25. Transfer tubes 3-10 at 10°C Date/Time: 02/18/00; 11-30 A.M.
26. Transfer 30 ul supernatant from from 150 ul supernatant removed earlier (Step 10) in triplicate in 6 ml scintillation vials containing 6 ml liquid scintillation cocktail (Ecolume, ICN) and count

them for radioactivity

**Date/Time:**

- 02/19/00  
2000m
- 02/20/00  
2000m
- 02/21/00  
2000m
27. After 24 or 48, carefully remove the supernatant from the top of the tubes 3 or 4 respectively, wash cells three times by centrifuging the tubes for 10 min at 2000 rpm, 4°C resuspend in 2 ml of wash MEMA, syringe three times, transfer 100 ul for cell count and 200 ul (in triplicate) for radioactivity count.
  28. After 72 h, for tubes 5-10, 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
  34. Determine cell concentration by transferring 100 µl to Coulter cup
  34. For tube #5, transfer 200 ul in triplicate for radioactivity count
  35. For tube #6 and 7, plate 1,000,000, 500,000, 250,000 and 250,000 cells in 75 cm<sup>2</sup> flask containing 10 ml MEMA.
  36. Following every 24 h, take 2 flasks with same no. of cells plated in descending order, trypsinize, resuspend in 2 or 1 ml of wash MEMA, and perform cell as well as radioactivity count.

Time of measuring uptake of radioactivity

02/22/00 : 2-00 pm

02/23/00 : 2-00 pm

USER: 6 ID:H3 HOWELL      PRESET TIME: 1.00      FRI 18 FEB 2000 13:14  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N      RS232:N  
 HPL 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	17870.00	1.93	0.60	1.44	89.0	
2	**	2	18730.97	1.94	0.56	3.02	92.0	
3	**	3	20001.91	1.95	0.52	4.62	92.0	
4	**	4	17611.38	1.92	0.62	6.51	91.0	
5	**	5	20003.70	1.92	0.54	8.17	92.0	
6	**	6	19129.63	1.97	0.54	9.62	92.0	
7	**	7	17782.30	2.00	0.56	11.20	91.0	
8	**	8	18429.57	1.94	0.57	12.65	91.0	
9	**	9	19258.18	1.94	0.55	14.18	90.0	
10	**	10	16612.50	1.94	0.64	15.93	92.0	
11	**	11	19638.10	1.97	0.52	17.53	93.0	
12	**	12	18712.96	1.99	0.54	18.98	93.0	
13	**	13	17722.12	2.00	0.56	20.57	91.0	
14	**	14	19499.03	2.00	0.51	22.10	92.0	
15	**	15	19035.85	1.99	0.53	24.05	92.0	
16	**	16	17593.04	1.99	0.57	25.99	91.0	
17	**	17	17099.19	1.95	0.62	27.63	92.0	
18	**	18	19433.01	2.00	0.51	29.17	91.0	
19	**	19	17543.33	1.95	0.60	30.79	92.0	
20	**	20	19084.96	1.93	0.56	32.37	92.0	
21	**	21	19690.74	1.94	0.54	34.02	93.0	
22	**	22	18868.47	1.95	0.56	35.81	91.0	
23	**	23	20078.00	2.00	0.50	37.27	92.0	
24	**	24	20029.63	1.92	0.54	38.73	93.0	
25	**	25	18108.33	1.92	0.60	40.31	90.0	
26	**	26	19563.81	1.97	0.52	41.71	93.0	
27	**	27	19301.85	1.96	0.54	43.37	92.0	
28	**	28	18230.63	1.99	0.56	45.14	93.0	
29	**	29	19181.48	1.97	0.54	46.60	93.0	
30	**	30	19510.91	1.93	0.55	48.12	93.0	
31	**	31	42360.00	1.94	0.25	49.34	111.0	
32	**	32	41355.17	1.83	0.29	50.76	111.0	
33	**	33	41898.18	1.86	0.28	52.09	111.0	
34	**	34	35165.52	1.98	0.29	53.30	110.0	
35	**	35	35496.55	1.97	0.29	54.51	111.0	
36	**	36	36143.33	1.92	0.30	55.77	111.0	
37	**	37	45592.46	1.82	0.26	57.11	110.0	
38	**	38	47132.00	1.84	0.25	58.32	110.0	
39	**	39	46036.36	1.99	0.22	59.87	109.0	

} Tube #5 following  
 3 day; Expt #1

Expt #2

USER: 6 ID:H3 HOWELL      PRESET TIME: 1.00      WED 23 FEB 2000 20:59  
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N      RS232:N  
: 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	1514.00	5.14	1.00	1.79	112.0	
2	**	2	1658.00	4.91	1.00	3.72	113.0	
3	**	3	1670.00	4.89	1.00	5.69	113.0	
4	**	4	1705.00	4.84	1.00	7.67	112.0	
5	**	5	1659.00	4.91	1.00	10.04	112.0	
6	**	6	1649.00	4.93	1.00	11.98	113.0	

Expt #1

USER: 6 ID:H3 HOWELL      PRESET TIME: 1.00      THU 24 FEB 2000 16:28  
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: 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	719.00	7.46	1.00	2.15	112.0	
2	**	2	81773.00	7.19	1.00	4.12	113.0	
3	**	3	750.00	7.30	1.00	6.11	115.0	
4	**	4	738.00	7.36	1.00	8.13	113.0	
5	**	5	82775.00	7.18	1.00	10.16	115.0	
6	**	6	744.00	7.33	1.00	12.04	116.0	

*Expt #2*

PAGE: 1

USER: 6 ID: H3 HOWELL      PRESET TIME: 1.00      FRI 25 FEB 2000 15:46  
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR: N      RS232: N  
H#: 1 AGC: N DCF: 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	210.00	13.80	1.00	2.19	112.0	
2	**	2	185.00	14.70	1.00	4.22	114.0	
3	**	3	195.00	14.32	1.00	6.24	113.0	
4	**	4	201.00	14.11	1.00	8.23	115.0	
5	**	5	180.00	14.91	1.00	10.21	116.0	
6	**	6	170.00	15.34	1.00	12.48	114.0	



TABLE-1

Expt. # : 2

Date/Time : 02/18/00; 1-15 pm.

Tube #	Medium count for 30 ul (cpm)	Avg. cpm	dpm [cpm/0.58]	$\mu$ Ci/ml ( $A_1$ ) on counting [dpm/66600]	$\mu$ Ci/ml ( $A_0$ ) on addition [ $A_1/e^{-\lambda t}$ ]
1		18867	32529	0.4884	
2		18914	32610	0.4896	
3		18489	31878	0.4786	
4		18320	31597	0.4743	
5		18752	32331	0.4855	
6		18041	31106	0.4671	
7		18772	32366	0.4859	
8		19658	33893	0.5089	
9		18990	32742	0.4916	
10		18973	32713	0.4912	

TABLE-2

Expt. # : ✓

Date/Time :

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	42360, 41355, 41898	41871	72191	0.1626	
2	35165, 35496, 3643	35601	61381	0.1382	
3	37103, 37926, 36963	37330	64363	0.1449	
4	43736, 43698, 42659	43364	74766	0.1684	
5	37685, 35910, 35703	36432	62814	0.1414	
6.1	4128, 3777, 3964	3956	6821	0.0153	
6.2	3969, 3800, 3812	3860	6655	0.0149	
7.1	1514, 1658, 1670	1614	2782	0.0063	
7.2	1705, 1659, 1649	1671	2881	0.0065	
8.1	719, 773, 750	748	1290.2	0.0029	

8.2 738, 775, 744 752 1297 0.0029

9.1 210, 195, 195 196 339 0.00076

9.2 201, 180, 170 183 316 0.00071

Cells were resuspended in 2 ml for tubes 1-5, 8 and 9 and in 1 ml for tubes 6, and 7.

TABLE-3

Expt. # : 2

Date/Time :

<u>n</u>	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]	mBq/cell [pCi/cell x 37]
14h	1	4745, 4924, 4732	4800	1920133	0.0847	3.13
14h	2	3712, 3811, 3790	3771	1508400	0.0916	3.39 <sup>3.26</sup>
44h	3	4012, 3922, 3939	3957	1583066	0.0915	3.39
68h	4	<del>4814, 4927, 4993</del> 43736, 45648, 4659	4910	1964133	0.0857	3.17
92h	5	<del>4110, 4219, 4271</del> 3392, 3414, 3401	<del>4202</del> 3404	<del>1681055</del> 1361600	0.0841	3.11
116h	6.1	2493, 2337, 2338	2389	955733	0.0160	0.59
	6.2	2417, 2491, 2364	2424	969600	0.0153	0.57 <sup>0.58</sup>
140h	7.1	5322, 5286, 5218	5275	2110133	0.0029	0.109
	7.2	5198, 5239, 5203	5213	2085333	0.0031	0.115 <sup>0.112</sup>
164h	8.1	4120, 4132, 4107	4119	1647866	0.0017	0.065
	8.2	4217, 4107, 4198	4174	1669600	0.0017	0.064 <sup>0.065</sup>
188h	9.1	13006, 13153, 13122	13093	5237200	0.00015	0.0054
	9.2	12895, 12798, 12957	12883	5153333	0.00014	0.0051