V79 COLONY FORMING ASSAY

Experiment Name : ^{3}HTdR toxicity (cluster, 100% labeling); **Exp. # :** 2;
Investigator: A. Bishayee **Date:** 11/30/98

1. Set the rocker-roller at 37°C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 150 cm^2 flusk, 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 transfering 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 : 3,729,333 cells/ml)
3. Transfer 1 ml of cell suspension into ten 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_2

   Date/Time: 11/30/98; 4-00 p.m.
5. Prepare MEMB containing radioactivity in hood

   14 µl ^{3}HTdR (Stock : 1 µCi/µl on 11/12/98) + 3 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:** 11/30/98; 7-00 p.m

Tube #	^{3}HTdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{3}HTdR (ml) [4uCi/ml]
1	0	1.0	1.0	0
2	0	1.0	1.0	0
3	0.01	1.0	0.995	0.005
4	0.05	1.0	0.975	0.025
5	0.1	1.0	0.950	0.050
6	0.2	1.0	0.900	0.100
7	0.5	1.0	0.750	0.250
8	0.75	1.0	0.625	0.375
9	1	1.0	0.500	0.500
10	2	1.0	0	1

4 µCi
1000 µl →

7. Return test tubes to roller for 12 h. **Date/Time:** 11/30/98; 7-20 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:** 12/1/98; 9-30 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, resuspend in 7 ml of MEMA
18. Centrifuge tubes for 10 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, 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:** 12/1/98; 11-30 a.m.
23. 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:** 12/1/98; 2-20 p.m.
24. 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: 12/09/98; 10-00 a.m.
25. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 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
    - 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 transfering 100 µl to Coulter cup
34. Vortex tube, transfer 0.5 ml into dilusion 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 100 µl of cell suspension (in triplicate) to 20 ml scintillation vial containing 6 ml cocktail (Aquasol)
37. Incubate petridishes for 1 week
38. Count vials for radioactivity **Date/Time :** 12/04/98; 12-00 Noon
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.

Expt #2

11/30/98

Initial Cell count = 712, 930, 955

Avg. Cell count = 932

Cell conc. = 932 x 4000

= 3,729,333 cells/ml

3HTdR toxicity,
Expt #2

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 TUE 01 DEC 1998 14:18
[redacted]IPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
[redacted] 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

30 ul medium

SAM	POS	CH		CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**- 1	1		21.00	43.64	1.00	1.60	59.0	
2	**- 2	1	1M	15.00	51.64	1.00	3.33	58.0	
3	**- 3	1		21.00	43.64	1.00	5.02	58.0	
4	**- 4	1		26.00	39.22	1.00	6.80	57.0	
5	**- 5	1	2M	19.00	45.88	1.00	8.63	58.0	
6	**- 6	1		25.00	40.00	1.00	10.37	59.0	
7	**- 7	1		275.00	12.06	1.00	12.04	58.0	
8	**- 8	1	3M	277.00	12.02	1.00	13.73	59.0	
9	**- 9	1		241.00	12.88	1.00	15.41	58.0	
10	**-10	1		1158.00	5.88	1.00	17.14	57.0	
11	**-11	1	4M	1245.00	5.67	1.00	18.87	57.0	
12	**-12	1		1138.00	5.93	1.00	20.61	59.0	
13	**- 1	1		2201.00	4.26	1.00	22.40	58.0	
14	**- 2	1	5M	2526.00	3.98	1.00	24.08	58.0	
15	**- 3	1		2396.00	4.09	1.00	25.77	57.0	
16	**- 4	1		4449.00	3.00	1.00	27.40	58.0	
17	**- 5	1	6M	4651.00	2.93	1.00	29.08	58.0	
18	**- 6	1		4720.00	2.91	1.00	30.82	59.0	
19	**- 7	1		11487.78	1.97	0.90	32.39	57.0	
20	**- 8	1	7M	11030.53	1.95	0.95	34.02	55.0	
21	**- 9	1		12460.00	1.94	0.85	35.56	59.0	
22	**-10	1		16551.22	1.98	0.62	37.00	56.0	
23	**-11	1	8M	18069.03	1.98	0.56	38.39	58.0	
24	**-12	1		18125.22	1.96	0.57	39.84	59.0	
25	**- 1	1		21256.84	1.99	0.48	41.10	58.0	
26	**- 2	1	9M	22846.67	1.97	0.45	42.22	58.0	
27	**- 3	1		23144.44	1.96	0.45	43.33	57.0	
28	**- 4	1		45946.66	1.97	0.23	44.17	58.0	
29	**- 5	1		45471.70	1.82	0.26	45.26	60.0	
30	**- 6	1	10M	44537.78	2.00	0.23	46.20	59.0	

TABLE-1

Expt. # : 2 **Date/Time :** 12/01/98; 2-20 p.m.

Tube #	Medium count for 30 ul (cpm)	Avg. cpm	dpm [cpm/0.65]	μ Ci/ml (A_t) on counting [dpm/~~22200~~ 66600]	μ Ci/ml (A_o) on addition [$A_t/e^{-\lambda t}$]
1	See the attached				
2	Sheet				
3		264.3	406.6	0.0061	
4		1180.3	1815.8	0.027	
5		2374.3	3652.8	0.0548	
6		4606.6	7087.1	0.1064	
7		11659	17936.9	0.2693	
8		17581.6	27048.7	0.4061	
9		22415.3	34485.1	0.5177	
10		45318	69720	1.046	

3H-Tdr Toxicity

Expt # 2

USER: & ID:HS HOWELL PRESET TIME: 1.00 FRI 04 DEC 1998 12:10
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
[redacted]. 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:Q 1.00000
HALF LIFE(DAYS):N

200 µl cells

SAM	POS	CH		CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**- 1	1	1C	25.00	40.00	1.00	1.61	80.0	
2	**- 2	1		23.00	41.70	1.00	3.44	78.0	
3	**- 3	1		18.00	47.14	1.00	5.18	80.0	
4	**- 4	1	2C	19.00	45.88	1.00	6.86	78.0	
5	**- 5	1		26.00	39.22	1.00	8.54	80.0	
6	**- 6	1		21.00	43.64	1.00	10.18	79.0	
7	**- 7	1		221.00	13.45	1.00	11.86	78.0	
8	**- 8	1	3C	176.00	15.08	1.00	13.54	78.0	
9	**- 9	1		227.00	13.27	1.00	15.23	79.0	
10	**-10	1		796.00	7.09	1.00	16.96	80.0	
11	**-11	1	4C	754.00	7.28	1.00	18.63	81.0	
12	**-12	1		723.00	7.44	1.00	20.62	81.0	
13	**- 1	1		2011.00	4.46	1.00	22.41	79.0	
14	**- 2	1	5C	2130.00	4.33	1.00	24.04	81.0	
15	**- 3	1		1848.00	4.65	1.00	25.77	75.0	
16	**- 4	1		3495.00	3.38	1.00	27.49	73.0	
17	**- 5	1	6C	3954.00	3.18	1.00	29.22	80.0	
18	**- 6	1		4109.00	3.12	1.00	30.86	80.0	
[redacted]	**- 7	1		10060.00	1.99	1.00	32.59	79.0	
[redacted]	**- 8	1	9C	10084.00	1.99	1.00	34.38	80.0	
21	**- 9	1		11034.74	1.95	0.95	36.01	82.0	
22	**-10	1		13151.25	1.95	0.80	37.48	80.0	
23	**-11	1	8C	13058.06	1.99	0.77	38.87	80.0	
24	**-12	1		13009.03	1.99	0.77	40.37	81.0	
25	**- 1	1		17881.67	1.93	0.60	41.70	82.0	
26	**- 2	1	9C	18927.27	1.96	0.55	42.92	81.0	
27	**- 3	1		19440.00	1.98	0.52	44.17	79.0	
28	**- 4	1		41508.00	1.96	0.25	45.09	80.0	
29	**- 5	1		39072.73	1.93	0.28	46.08	80.0	
30	**- 6	1	10C	40088.00	2.00	0.25	47.10	81.0	

TABLE-2

Expt. # : 2 **Date/Time :** 12/04/98; 12:00 noon

Tube #	Radioactivity for 200 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.65]	μCi/ml (A_t) on counting [dpm/444000]	μCi/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1	See the attached sheet				
2					
3		208	320	0.00072	
4		757.6	1165.6	0.00262	
5		1996.3	3071.2	0.0069	
6		3852.6	5927.1	0.0133	
7		10392.6	15988.7	0.036	
8		13072.6	20111.7	0.0452	
9		~~18749.3~~ ~~12082~~	~~28845~~ ~~18584~~	0.0649 ~~0.2497~~	
10		40222.6	61881.0	0.1393	

TABLE-3

Expt. # : 2 **Date/Time :** 12/04/98 ; 1000 am

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	252, 262, 270	261		
2	245, 221, 259	241		
3	276, 236, 249	253.6	1014666	0.0007
4	302, 279, 271	284	1136000	0.0023
5	284, 294, 291	289	1158666	0.0059
6	404, 416, 426	415	1661333	0.008
7	299, 295, 336	310	1240000	0.029
8	244, 244, 237	239	958666	0.0471
9	231, 225, 228	228	912000	0.0711
10	279, 288, 300	289	1156000	0.1205

TABLE-4

Expt # : 2 **Date :** 12/11/98

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1·2	120	111	99	116·5	
2·2	131	121	117		
3·2	104	114	96	104·6	0·8984
4·2	93	100	86	93	0·7982
5·2	70	78	63	70·3	0·6037
6·2	58	68	49	58·3	0·5007
7·2	29	22	19	23·3	0·2002
8·3	104	115	94	10·4	0·0895
9·4	116	126	107	1·16	0·0099
10·4	238	29	18	0·23	0·002