V79 COLONY FORMING ASSAY

Experiment Name : ^{3}HTdR toxicity (cluster, 50% labeling); **Exp. #** : 2;
Investigator: A. Bishayee **Date:** 01/11/99

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 ~2,000,000 cells/ml in MEMB [Actual count : 2168000 cells/ml)
3. Transfer 1 ml of cell suspension into 20 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:** 01/11/99; 2-30 p.m.
5. Prepare MEMB containing radioactivity in hood
   30 µl ^{3}HTdR (Stock : 1 µCi/µl on 11/12/98) + 3 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:** 7-15 p.m / 01/11/99

Tube #	^{3}HTdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{3}HTdR (ml) [8uCi/ml]
1	0	1.0	1.0	0
2	0	1.0	1.0	0
3	0.02	1.0	0.995	0.005
4	0.1	1.0	0.975	0.025
5	0.2	1.0	0.950	0.050
6	0.4	1.0	0.900	0.100
7	1	1.0	0.750	0.250
8	1.5	1.0	0.625	0.375
9	2	1.0	0.500	0.500
10	4	1.0	0	1

10⁵x

7. Return test tubes to roller for 12 h . **Date/Time:** 01/11/99; 7-30 p.m.

for lindane Mutn
2 tube – Medium control
2 " – Lindane "
3 " Lindane + 3H — 1, 3 & 6 µCi/ml
3 "

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:** 01/12/99; 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
20. Add 10 ml of wash MEMA to second set of tubes containing 2,000,000 cells, and follow steps 11-17, suspend in 7 ml of MEMA and transfer cells to the corresponding tubes containing 2,000,000 cells in step 19
21. Centrifug tubes for 10 min at 2000 rpm, 4°C
22. Decant supernatant, click tubes, vortex
23. Transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips
24. Again add 200 ul ice cold MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
25. Centrifuge tubes for 5 min at 1000 rpm, 4°C
26. Transfer tubes at 10°C for 72 h. **Date/Time:** 01/19/99; 11-30 a.m.
27. 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:** 01/12/99; 11-00 a.m.
28. 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: 01/15/99; 12-00 Noon
29. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes
30. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***

31. 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.
32. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
33. Centrifuge tubes for 10 min at 2000 rpm, 4°C
34. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
35. Centrifuge tubes for 10 min at 2000 rpm, 4°C
36. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
37. Determine cell concentration by transfering 100 µl to Coulter cup
38. 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.
39. 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.
40. Transfer 200 µl of cell suspension (in triplicate) to 20 ml scintillation vial containing 6 ml cocktail (Aquasol)
41. Incubate petridishes for 1 week
42. Count vials for radioactivity **Date/Time :** 01/15/99, 2-00 pm.
43. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
44. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.

Expt #2

01/11/99

Initial cell count = 926, 1013, 885

Avg. cell count = 941

Cell conc = 941 x 4000 = 3765333

we need 11 ml of 2,000,000 cells/ml = 22000000

Vol required = $\frac{22000000}{3765333}$ = 5.8 ml

Take 6 ml cells + 5 ml MEMB = 11 ml

After dilution =

Final count = 551, 526, 549

Avg count = 542

Cell conc = 542 x 4000

= 2,168,000 Cells/ml

F-451

3HTOR Conicity (50% Labeling)

Expt #2

PAGE: 1

ER: 6 ID:H3 HOWELL PRESET TIME: 1.00 TUE 12 JAN 1999 11:03
MPLF REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
[redacted] AGC:N BCF:N RCM:N
[redacted]EL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
TA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:Q 1.00000
LF LIFE(DAYS):N

M	POS	CH		CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**- 1	1	1M	18.00	47.14	1.00	1.60	59.0	
2	**- 2	1		22.00	42.64	1.00	3.23	58.0	
3	**- 3	1	2M	7.00	75.59	1.00	4.87	61.0	
4	**- 4	1		6.00	81.65	1.00	6.55	64.0	
5	**- 5	1	3M	560.00	8.45	1.00	8.13	62.0	
6	**- 6	1		660.00	7.78	1.00	9.87	63.0	
7	**- 7	1	4M	2608.00	3.92	1.00	11.55	62.0	
8	**- 8	1		2908.00	3.71	1.00	13.23	62.0	
9	**- 9	1	5M	5264.00	2.76	1.00	14.87	61.0	
0	**-10	1		5888.00	2.61	1.00	16.55	63.0	
1	**-11	1	6M	11046.49	1.98	0.93	18.11	63.0	
2	**-12	1		11814.12	2.00	0.85	19.64	62.0	
3	**- 1	1	7M	27400.00	1.97	0.38	20.79	62.0	
4	**- 2	1		29414.29	1.97	0.35	21.81	63.0	
5	**- 3	1	8M	41429.09	1.87	0.28	22.80	62.0	
6	**- 4	1		47092.00	1.84	0.25	23.72	63.0	
7	**- 5	1	9M	59428.57	1.96	0.17	24.61	62.0	
8	**- 6	1		64200.00	1.77	0.20	25.37	65.0	
9	**- 7	1	10M	112288.00	1.69	0.12	26.12	63.0	
[redacted]	**- 8	1		122419.99	1.48	0.15	26.95	64.0	

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TABLE-1

Expt. # : 2 **Date/Time :** 01/12/99 11:00 a.m.

Tube #	Medium count for 30 ul (cpm)	Avg. cpm	dpm [cpm/0.65]	μ Ci/ml (A_t) on counting [dpm/66600]	μ Ci/ml (A_o) on addition [$A_t/e^{-\lambda t}$]
1	See the attached				
2	sheet				
3		610	938	0.014	
4		2758	4243	0.063	
5		5576	8578	0.128	
6		11430	17584	0.264	
7		28407	43703	0.656	
8		44260	68093	1.022	
9		61814	95098	1.427	
10		117353	180543	2.710	

F451

Expt #2

200µl cells

SER: 6 ID:H3 HOWELL PRESET TIME: 1.00 FRI 15 JAN 1999 14:04
[redacted]E REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
[redacted] ADC:N QCF:N RCM:N
HANNEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
ATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:0 1.000000
ALF LIFE(DAYS):N

AM	POS	CH		CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**- 1	1	1c	12.00	57.74	1.00	1.55	78.0	
2	**- 2	1		7.00	75.59	1.00	3.18	77.0	
3	**- 3	1	2c	8.00	70.71	1.00	4.87	75.0	
4	**- 4	1		6.00	81.65	1.00	6.75	73.0	
5	**- 5	1	3c	470.00	9.23	1.00	8.38	77.0	
6	**- 6	1		412.00	9.85	1.00	10.06	78.0	
7	**- 7	1	4c	2051.00	4.42	1.00	11.74	78.0	
8	**- 8	1		2169.00	4.29	1.00	13.43	80.0	
9	**- 9	1	5c	4138.00	3.11	1.00	15.11	78.0	
10	**-10	1		4257.00	3.07	1.00	16.74	81.0	
11	**-11	1	6c	9961.00	2.00	1.00	18.42	82.0	
12	**-12	1		9867.00	2.01	1.00	20.07	84.0	
13	**- 1	1	7c	15902.22	1.93	0.68	21.42	72.0	
14	**- 2	1		17480.00	1.99	0.57	22.62	79.0	
15	**- 3	1	8c	31507.69	1.98	0.33	23.56	87.0	
16	**- 4	1		29914.29	1.95	0.35	24.48	78.0	
17	**- 5	1	9c	43036.00	1.93	0.25	25.40	80.0	
18	**- 6	1		45120.00	1.88	0.25	26.32	78.0	
19	**- 7	1	10c	100693.33	1.63	0.15	27.13	81.0	
20	**- 8	1		105832.00	1.74	0.12	27.87	85.0	

[redacted]

TABLE-2

Expt. # : 2 **Date/Time :** 01/15/99; 2-00 P.M

Tube #	Radioactivity for 200 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.65]	μ Ci/ml (A_t) on counting [dpm/444000]	μ Ci/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1	See the				
2	attached sheet				
3		441	678.4	0.00152	
4		2110	3246	0.00731	
5		4197	6457	0.01454	
6		9914	15252	0.0344	
7		16691	25678	0.05783	
8		30710	47246	0.1064	
9		45078	69350	0.15619	
10		103262	158865	0.3578	

TABLE-3

Expt. # : 2 **Date/Time :** 01/15/99 ; 11-00 a.m.

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [uCi/ml x 10^6 / Cells/ml]	Kbq/cell
1	488, 482, 478	482	1930666	–	
2	510, 472, 463	481	1926666	–	
3	517, 505, 506	509	2037333	0.00075	0.111
4	468, 458, 455	460	1841333	0.00397	0.587
5	528, 527, 524	526	2105333	0.00691	1.022
6	622, 648, 609	626	2505333	0.01373	2.032
7	~~345~~, 442, 422, 432	432	1728000	0.03347	4.953
8	467, 438, 438	447	1790666	0.05941	8.792
9	481, 452, 522	485	1940000	0.08051	11.91
10	487, 516, 482	495	1980000	0.18071	26.74

TABLE-4

Expt # : 2 **Date :** 1/22/99

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1·2	145	139	121	} 131·66	–
2·2	129	119	137	}	–
3·2	130	135	124	129·66	0·9848
4·2	125	115	109	116·33	0·8835
5·2	95	101	107	101	0·7671
6·2	85	92	78	85	0·6456
7·2	59	69	78	68·6	0·5215
8·2	49	58	67	58	0·4405
9·2	38	44	33	38	0·2911
10·3	100	95	106	10·33	0·0784