V79 COLONY FORMING ASSAY

Experiment Name : ^{137}Cs and ^{3}HTdR toxicity (western blot); 100% Label **Exp. # :** 1;

Investigator: A. Bishayee **Date:** 04/05/99

1. Set the rocker-roller at 37^{0}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 : 3,968,000 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:** 04/05/99; 3-30 P.m.
5. After 3-4 h, remove tubes # 1 and 2 from the roller, irradiate tube # 2 using Mark I irradiator (10 Gy), wash cells in both tubes with 8 ml MEMB, suspend pellet in 2 ml PBS, transfer 1 ml cell suspension into Eppendorf tube, wash, remove the supernatant, and disperse the pellet by vortexing, store at -20°C.
6. Prepare MEMB containing radioactivity in hood

   8 µl ^{3}HTdR (Stock : 1 µCi/µl on 3/8/99) + 2 ml MEMB
7. Remove tubes 2-10 from roller and add MEMB with or without radioactivity according to Table below. **Date/Time:** 04/05/99; 7-00 P.m.

Tube #	^{3}HTdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB + ^{3}HTdR (ml) 4uCi/ml	MEMA (ml)	
1	0	1.0	1	-	-	0Gy — For acute irradiation (10 Gy) study
2	0	1.0	1	-	-	10Gy
3	0	1.0	1	0	-	0 µci/ml
4	0.75	1.0	0.625	0.375	-	0.75 "
5	0	1.0	1	0	0.4	0 " — For ^{3}HTdR Study
6	0.75	1.0	0.625	0.375	0.4	0.75 "
7	0	1.0	1	0	0.4	0
8	0.75	1.0	0.625	0.375	0.4	0.75 "
9	0	1.0	1	0	0.4	
10	0.75	1.0	0.625	0.375	0.4	0.75

8. Return test tubes to roller for 12 h . **Date/Time:** 04/06/99; 9-15 a.m.
9. Next day, while test tubes are in roller label 10 gamma-tubes (13 X 100 mm VWR glass test tube)
10. After ~12 h incubation period, remove tubes and centrifuge at 2000 rpm at 4°C for 10 min (***precooled centrifuge***) . **Date/Time:** 04/06/99; 9-00 a.m
11. Remove buckets from centrifuge and carefully remove 150 µl of supernatant and place in prelabeled gamma-tube.
12. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
13. Centrifuge tubes for 10 min at 2000 rpm, 4°C
14. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
15. Centrifuge tubes for 10 min at 2000 rpm, 4°C
16. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
17. Centrifuge tubes for 10 min at 2000 rpm, 4°C
18. Decant supernatant, click tubes, vortex, resuspend in 2 ml PBS (tubes 3 and 4) or 0.4 ml MEMA (tubes 5-10)
19. Transfer 1 ml suspension from tubes 3 and 4 to Eppendorf tube and follow step 5.
20. Transfer the cell suspension from tubes 5-10 in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips
21. Centrifuge tubes for 5 min at 1000 rpm, 4°C
22. Transfer tubes at 10°C for 72 h. **Date/Time:** 04/06/99; 11-10 a.m.
23. Following 24, 48 or 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:**
24. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes
25. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***
26. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
27. Centrifuge tubes for 10 min at 2000 rpm, 4°C
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 2 ml PBS and follow step 5.

Expt #1 04/05/99

Cell count

Initial cell count = ~~666, 665~~, 990, 987, 999

Avg cell count = 992

Cell conc. = 3,968,000 cells/ml

Irradiation

i) Take the tube containg 2 ml of cell suspension

ii) Place the tube onto a plastic box (Rexin pipet box)

iii) Irradiate the tube at Mark I irradiator

Total Dose (Rad)	Dose rate (Rad/min)	Time (min)	Atten	Turn Table position
1000	387.66	2.58	X-2	3

30 µl medium

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 WED 07 APR 1999 10:56
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:0 1.00000
HALF LIFE(DAYS):N

SAM	POS	CH		CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**- 1	1	3M	17.00	48.51	1.00	1.75	57.0	
2	**- 2	1		9.00	66.67	1.00	3.62	55.0	
3	**- 3	1	4M	39905.45	1.91	0.28	4.77	57.0	
4	**- 4	1		41494.54	1.87	0.28	5.91	56.0	
5	**- 5	1	5M	10.00	63.25	1.00	7.83	57.0	
6	**- 6	1		16.00	50.00	1.00	9.76	56.0	
7	**- 7	1	6M	30690.67	1.86	0.38	10.99	56.0	
8	**- 8	1		34338.46	1.89	0.33	12.18	59.0	
9	**- 9	1	7M	18.00	47.14	1.00	14.07	58.0	
10	**-10	1		7.00	75.59	1.00	15.93	57.0	
11	**-11	1	8M	31761.76	1.92	0.34	17.19	57.0	
12	**-12	1		34418.46	1.89	0.33	18.38	57.0	
13	**- 1	1	9M	14.00	53.45	1.00	20.36	57.0	
14	**- 2	1		9.00	66.67	1.00	22.29	58.0	
15	**- 3	1	10M	32227.69	1.95	0.33	23.47	56.0	
1A	**- 4	1		35203.18	1.90	0.31	24.61	56.0	

TABLE-1

Expt. # : 1 **Date/Time :** 04/07/99; 11-00 a.m

Tube #	Medium count for 30 ul (cpm)	Avg. cpm	dpm [cpm/0.65] E=0.65;Y=1	µCi/ml (A_t) on counting [dpm/66600]	µCi/ml (A_o) on addition [$A_t/e^{-\lambda t}$]
1					
2					
3					
4		40699	62614	0·9402	
5					
6		32514	50021	0·7512	
7					
8		33089	50906	0·7643	
9					
10		33715	51869	0·7788	

200µl cells

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 MON 12 APR 1999 10:41
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
[illegible]: 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:0 1.00000
HALF LIFE(DAYS):N

SAM	POS	CH		CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**- 1	1	MEHA →	13.00	55.47	1.00	1.78	60.0	
2	**- 2	1	PBS →	9.00	66.67	1.00	3.72	53.0	
3	**- 3	1	3c	17.00	48.51	1.00	5.60	60.0	
4	**- 4	1		4.00	100.0	1.00	7.48	60.0	
5	**- 5	1	4c	15717.04	1.94	0.68	9.03	61.0	
6	**- 6	1		15148.15	1.98	0.68	10.58	60.0	
7	**- 7	1	5c	12.00	57.74	1.00	12.47	61.0	
8	**- 8	1		9.00	66.67	1.00	14.23	64.0	
9	**- 9	1	6c	6500.00	2.48	1.00	16.11	68.0	
10	**-10	1		6841.00	2.42	1.00	17.99	71.0	
11	**-11	1	7c	10.00	63.25	1.00	19.92	66.0	
12	**-12	1		9.00	66.67	1.00	21.80	67.0	
13	**- 1	1	8c	7834.00	2.26	1.00	23.73	64.0	
14	**- 2	1		7971.00	2.24	1.00	25.62	68.0	
15	**- 3	1	9c	9.00	66.67	1.00	27.45	71.0	
16	**- 4	1		8.00	70.71	1.00	29.27	66.0	
17	**- 5	1	10c	7684.00	2.28	1.00	31.16	67.0	
18	**- 6	1		7349.00	2.33	1.00	32.99	68.0	

TABLE-2

Expt. # : 1 **Date/Time :** 04/12/99; 10-45 a.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					
2					
3					
4		15432	23742	0·0534	
5					
6		6670	10262	0·0231	
7					
8		7902	12157	0·0273	
9					
10		7516	11563	0·0260	

TABLE-3

Expt. # : **Date/Time :**

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]	nCi/cluster pCi/Cell × 4000
1					
2					
3	712, 725, 730				
4	622, 635, 627	628	2512000	0·0213	
5	678, 665, 665				
6	572, 541, 535	549	2197333	0·0105	42
7	545, 562, 569				
8	512, 523, 533	522	2090666	0·0131	52·4
9	507, 511, 531				
10	545, 521, 510	525	2101333	0·0124	49·4