V79 COLONY FORMING ASSAY

Experiment Name : ^{3}HTdR +10%DMSO (cluster, 100% labeling); **Exp. # :** 1;
Experiment performed by : A. Bishayee **Date:** 11/11/99

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 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 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 : 5,061,333 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:** 11/11/99; 2-00 p.m
5. Prepare MEMB containing radioactivity in hood
   70 µl ^{3}HTdR (Stock : 1 µCi/µl on 10/20/99) +3.5 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:** 11/11/99; 7-20 p.m.

Tube #	^{3}HTdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{3}HTdR (ml) [20uCi/ml]
1	0	1.0	1.0	0
2	0	1.0	1.0	0
3	0.1	1.0	0.99	0.01
4	0.5	1.0	0.95	0.05
5	1	1.0	0.9	0.1
6	2	1.0	0.8	0.2
7	4	1.0	0.6	0.4
8	6	1.0	0.4	0.6
9	8	1.0	0.2	0.8
10	10	1.0	0	1

7. Return test tubes to roller for 12 h . **Date/Time:** 11/11/99, 7-30 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:** 11/12/99, 9-00 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 with 10% DMSO
18. Centrifuge tubes for 5 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 with 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:** 11/12/99, 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:** 11/12/99, 12-00 a.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:** 11/15/99, 9-30 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 200 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 :** 11/15/99, 11-30 a.m.
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.

11/11/99

Initial count = ~~1229~~, 1257, 1274, 1265

Avg count = 1265

Cell conc. = 5,061,333

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 FRI 12 NOV 1999 12:01
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
H[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:0 1.00000
HALF LIFE(DAYS):N

SAM	POS	CH		CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**- 1	1		7.00	75.59	1.00	1.85	87.0	
2	**- 2	1	1M	14.00	53.45	1.00	3.93	91.0	
3	**- 3	1		6.00	81.65	1.00	6.05	88.0	
4	**- 4	1		7.00	75.59	1.00	8.33	90.0	
5	**- 5	1	2M	12.00	57.74	1.00	10.31	88.0	
6	**- 6	1		13.00	55.47	1.00	12.33	88.0	
7	**- 7	1		646.00	7.87	1.00	14.71	88.0	
8	**- 8	1	3M	670.00	7.73	1.00	17.14	90.0	
9	**- 9	1		660.00	7.78	1.00	19.12	90.0	
10	**-10	1		2763.00	3.80	1.00	21.11	91.0	
11	**-11	1	4M	2801.00	3.78	1.00	23.14	90.0	
12	**-12	1		2715.00	3.84	1.00	25.16	91.0	
13	**-13	1		5292.00	2.75	1.00	27.18	93.0	
14	**-14	1	5M	5812.00	2.62	1.00	29.32	90.0	
15	**-15	1		5893.00	2.61	1.00	31.30	92.0	
16	**-16	1		11548.57	1.99	0.88	33.26	91.0	
17	**-17	1	6M	12387.80	1.98	0.82	35.41	91.0	
18	**-18	1		12912.26	2.00	0.77	37.26	91.0	
[redacted]	**- 1	1		23754.84	1.90	0.47	38.79	92.0	
20	**- 2	1	7M	25291.76	1.93	0.43	40.59	90.0	
21	**- 3	1		25970.37	1.95	0.41	42.22	87.0	
22	**- 4	1		34760.00	1.96	0.30	43.68	89.0	
23	**- 5	1	8M	39030.00	1.85	0.30	44.95	92.0	
24	**- 6	1		35628.57	1.89	0.31	46.28	91.0	
25	**- 7	1		48478.26	1.89	0.23	47.94	92.0	
26	**- 8	1	9M	50966.66	1.81	0.24	49.30	92.0	
27	**- 9	1		48566.66	1.85	0.24	50.46	92.0	
28	**-10	1		57165.71	2.00	0.17	51.70	94.0	
29	**-11	1	10M	61615.00	1.80	0.20	52.87	90.0	
30	**-12	1		62340.43	1.85	0.19	54.17	90.0	
31	**-13	1		12.00	57.74	1.00	56.16	96.0	
32	**-14	1	1C	8.00	70.71	1.00	58.08	87.0	
33	**-15	1		8.00	70.71	1.00	60.06	89.0	
34	**-16	1		11.00	60.30	1.00	62.43	96.0	
35	**-17	1	nc	3.00	115.5	1.00	64.77	90.0	
36	**-18	1		13.00	55.47	1.00	66.77	89.0	
37	**- 1	1		239.00	16.96	1.00	68.91	88.0	
38	**- 2	1	3c	167.00	15.48	1.00	70.93	91.0	
39	**- 3	1		169.00	15.38	1.00	73.05	90.0	
40	**- 4	1		719.00	7.46	1.00	75.07	91.0	
41	**- 5	1	9c	899.00	6.67	1.00	76.99	95.0	
42	**- 6	1		811.00	7.02	1.00	79.16	90.0	
43	**- 7	1		1298.00	5.55	1.00	81.53	91.0	
[redacted]	**- 8	1	3c	1645.00	4.93	1.00	83.52	93.0	
[redacted]	**- 9	1		1495.00	5.17	1.00	85.50	90.0	
46	**-10	1		2592.00	3.93	1.00	87.58	96.0	

30 µl medium

100 µl cells after 12h labeling

SAM	POS	CH	CPM	2SIG%	TIME		
[redacted]	**-11	1	3321.00	3.47	1.00	89.70	92.0
48	**-12	1	2801.00	3.78	1.00	91.68	93.0
49	**-13	1	6831.00	2.62	1.00	93.67	90.0
50	**-14	1	6627.00	2.46	1.00	95.74	91.0
51	**-15	1	6441.00	2.49	1.00	98.08	91.0
52	**-16	1	7599.00	2.29	1.00	100.35	91.0
53	**-17	1	9091.00	2.10	1.00	102.33	94.0
54	**-18	1	9362.00	2.07	1.00	104.36	92.0
55	**- 1	1	10237.00	1.98	1.00	106.49	89.0
56	**- 2	1	12965.82	1.98	0.79	108.21	93.0
57	**- 3	1	11558.47	1.94	0.92	110.15	92.0
58	**- 4	1	12380.61	1.98	0.82	112.35	90.0
59	**- 5	1	15936.92	1.97	0.65	113.98	94.0
60	**- 6	1	16001.60	2.00	0.62	115.68	90.0

B001634

Cells in 2ml MEM

100µl cells ; MS = 50µl

11/12/99

1	589, 608, 570
2	617, 624, 607
3	575, 595, 606
4	512, 514, 536
5	588, 566, 568
6	545, ~~509~~, 529, 520
7	494, 463, ~~444~~ 445
8	504, 480, 474
9	491, 479, 491
10	~~499~~, ~~548~~; 416, 429, 420

11/15/99

1	511, 490, 485	
2	507, 482, 499	
3	573, 548, 566	562.3
4	563, 574, 558	
5	493, 483, 472	
6	456, 440, 465	
7	459, 429, 431	
8	411, 419, 395	
9	385, 399, 379	
10	335, 350, 341	

TABLE-1

Expt. # : 1 **Date/Time :** 11/12/99; 12-00 noon

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					
2					
3		658	1013·3	0·015	
4		2759	4245·6	0·063	
5		5665	8716·4	0·130	
6		12282	18895	0·283	
7		25005	38469	0·577	
8		36472	56111	0·842	
9		49336	75902	1·13	
10		60373	92882	1·39	

12h after labeling

TABLE-2

Expt. # : 1 **Date/Time :** 11/12/99; 1200

Tube #	Radioactivity for 200 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.65]	μ Ci/ml (A_t) on counting [dpm/2220000]	μ Ci/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1					
2					
3		158	243.5	0.00109	
4		809.6	1245.6	0.0056	
5		1479.3	2275.8	0.0102	
6		2904	4468.7	0.0201	
7		6299	9691	0.0436	
8		8684	13360	0.0601	
9		11586	17825	0.0802	
10		14772	22726	0.1023	

12h after labeling

TABLE-3

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

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [$\frac{uCi/ml \times 10^6}{Cells/ml}$]
1		589		
2		616		
3		592	2368000	0·00046
4		520	2082666	0·00268
5		574	2296000	0·00444
6		531	2125333	0·00945
7		467	1869333	0·0233
8		486	1944000	0·0309
9		487	1948000	0·0411
10		421	1686666	0·0606

200µl cells

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 MON 15 NOV 1999 11:41
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
H#: 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	1c	6.00	81.65	1.00	1.85	93.0	
2	**- 2	1		7.00	75.59	1.00	3.78	93.0	
3	**- 3	1		15.00	51.64	1.00	5.86	96.0	
4	**- 4	1	2c	11.00	60.30	1.00	7.83	94.0	
5	**- 5	1		8.00	70.71	1.00	10.07	94.0	
6	**- 6	1		12.00	57.74	1.00	12.54	92.0	
7	**- 7	1	3c	596.00	8.19	1.00	14.67	96.0	
8	**- 8	1		513.00	8.83	1.00	16.99	93.0	
9	**- 9	1		446.00	9.47	1.00	19.46	92.0	
10	**-10	1	4c	2391.00	4.09	1.00	21.68	97.0	
11	**-11	1		2567.00	3.95	1.00	23.72	97.0	
12	**-12	1		2524.00	3.98	1.00	25.99	102.0	
13	**-13	1		~~3473.00~~	3.39	1.00	28.47	93.0	
14	**-14	1	5c	5051.00	2.81	1.00	30.40	98.0	
15	**-15	1		5003.00	2.83	1.00	32.47	96.0	
16	**-16	1	6c	7010.00	2.39	1.00	34.51	92.0	
17	**-17	1		9547.00	2.05	1.00	36.68	98.0	
18	**-18	1		12088.09	1.98	0.84	38.45	100.0	
19	**- 1	1	7c	21314.00	1.94	0.50	39.97	98.0	
20	**- 2	1		15308.27	1.98	0.67	41.66	92.0	
21	**- 3	1		16451.56	1.95	0.64	43.42	93.0	
22	**- 4	1	8c	24387.95	1.99	0.41	45.10	94.0	
23	**- 5	1		24724.44	1.90	0.45	46.52	90.0	
24	**- 6	1		24963.86	1.96	0.41	47.94	89.0	
25	**- 7	1	9c	26746.99	1.90	0.41	49.37	89.0	
26	**- 8	1		62212.77	1.85	0.19	50.68	94.0	→ sample was added twice
27	**- 9	1		7.00	75.59	1.00	53.02	86.0	→ no sample
28	**-10	1	10c	37523.33	1.89	0.30	54.27	90.0	
29	**-11	1		41248.00	1.97	0.25	55.49	92.0	
30	**-12	1		40701.89	1.93	0.26	56.77	91.0	

after 72h at 10·5°C

TABLE-2

Expt. # : 1 **Date/Time :** 11/15/99; 11-45 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		518	797	0·0018	
4		2494	3836	0·0086	
5		5027	7733	0·0174	
6		9548	14689	0·033	
7		17691	27216	0·0612	
8		24691	37986	0·0855	
9		29652	45619	0·1027	
10		39824	61267	0·1379	

after 72h at 10°5°C

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 x10^6 Cells/ml]	
1		495			
2		496			
3		562.3		0.0008	0.118
4		565	2260000	0.0038	0.562
5		482	1930666	0.0091	1.35
6		453	1814666	0.0181	2.68
7		439	1758666	0.0347	5.14
8		408	163333	0.0523	7.74
9		387	1550666	0.0662	9.79
10		342	1368000	0.1008	14.92

KBq/ Cuvier [pci/cell x 148]

11/22/99

1.2	102, 96, 108 }	96.33	
2.2	90, 88, 94 }		
3.2	81, 98, 89	89.33	0.9273
4.2	79, 81, 85	81.66	0.8477
5.2	72, 69, 74	71.66	0.7439
6.2	59, 53, 51	54.33	0.5639
7.3	293, 283, 299	29.16	0.3027
8.3	103, 111, 106	10.66	0.1107
9.3	54, 63, 50	5.56	0.0577
10.4	55, 57, 49	0.52	0.0054

0.78