

## V79 COLONY FORMING ASSAY

Experiment Name :  $^3\text{H}_2\text{O}$  + 10% DMSO; Exp. # : 2; Investigator: A. Bishayee  
 Date: 06/22/98

1. Set the rocker-roller at 37°C incubator, set the Coulter Counter, wash cells (from 75 cm<sup>2</sup> flask, subcultured 1:2, 24h before) with PBS, trypsinize cells, resuspend in 7 ml MEMB, 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 ~4,00,000 cells/ml in MEMB (final volume 11 ml) [Actual count : 401,333 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. Roll the tubes for 3-4 h at 37°C, 5% CO<sub>2</sub> Date/Time: 06/22/98; 3-45 p.m.
5. Obtain  $^3\text{H}_2\text{O}$  from refrigerator (25 mCi/ml) NEN Catalog # NET001C
6. After 3-4 h, remove test tubes from roller and add MEMB and/or  $^3\text{H}_2\text{O}$  according to Table below. Date/Time: 06/22/98; 7-45 p.m.

Tube #	$^3\text{H}_2\text{O}$ mCi/ml	Cells in MEMB (ml)	MEMB (ul)	$^3\text{H}_2\text{O}$ (ul) [25 mCi/ml]	DMSO (ul)	MEMB (ul)	
1	0	1.0	800	0	200	0	
2	0	1.0	800	0	200	0	
3	0.25	1.0	780	20	200	0	
4	0.75	1.0	740	60	200	0	
5	1.25	1.0	700	100	200	0	
6	0	1.0	800	0	0	200	
7	0	1.0	800	0	0	200	
8	0.25	1.0	780	20	0	200	
9	0.75	1.0	740	60	0	200	
10	1.25	1.0	700	100	0	200	

7. Return test tubes to roller for 12 h, increase the elevation angle of the roller.

Date/Time: 06/22/98; 8-00 p.m.

... ..

... ..

1.2 ... ..

2.2 ... ..

3.2, 3.3

4.2, 4.3, 4.4

5.2, 5.3, 5.4

6.2

7.2

8.2, 8.3, 8.4

9.2, 9.3, 9.4

10.3, 10.4

	00
25	00
91	00
26	00
27	00

8. While test tubes are in roller, obtain sterile DMSO (100%) from refrigerator, thaw it, move roller to 10.5°C, obtain ice
9. After ~12 h incubation period, remove tubes from incubator, chill on ice
10. Add DMSO (while vortexing) or MEMB according to the Table, vortex, quickly return to ice  
Date/Time : 06/23/98; 9-25 a.m.
11. Transfer tubes to roller at 10.5 °C for 72 h. Date/Time: 06/23/98; 9-30 a.m.
12. After 72 h, remove tubes, place on ice and centrifuge at 2000 rpm at 4°C for 10 min  
(precooled centrifuge) Date/Time: 06/26/98; 1-00 p.m.
13. Transfer 10 ul medium to test tubes
14. Add 8 ml ice-cold wash MEMA, vortex
15. Centrifuge tubes for 10 min at 2000 rpm, 4°C
16. Labeling and preparation of dilution tubes and colony dishes
  - load 48 mm petri dishes with 4 ml MEMA
  - load 30 T-tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 2.2, 2.3, 2.4, X.2, X.3, X.4, etc.
17. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
18. Centrifuge tubes for 10 min at 2000 rpm, 4°C
19. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
20. Centrifuge tubes for 10 min at 2000 rpm, 4°C
21. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
22. Centrifuge tubes for 10 min at 2000 rpm, 4°C
23. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
24. Determine cell concentration by transferring 100 µl to Coulter cup
25. Vortex tube, 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.
26. 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.
27. Transfer 100 µl of cell suspension (in triplicate) to prelabelled vial (C) for each tube
28. Incubate petridishes for 1 week
29. Add 490 ul MEMB in tubes containing 10 ul of medium (step 13), vortex, transfer 10 ul in triplicate into prelabelled vials (M).
30. Add 3 ml liquid scintillation cocktail to vials and count for radioactivity
31. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol.  
Stain colonies with crystal violet
32. Count colonies (50 or more cells). There must be between 25 and 250 colonies for the dish to be a valid data point.

Expt #2

6/22/98

$$\begin{aligned}\text{Initial Cell Count} &= 7404, 7568, 7281, 7404 \\ \text{Avg. Cell Count} &= 7458.6 \\ \text{Cell conc.} &= 7458 \times 400 \\ &= 2983466 \text{ cells/ml}\end{aligned}$$

For dilution

$$\begin{aligned}\text{Vol of cell suspension required} &= \frac{4400000}{2983466} \\ &= 1.47 \text{ ml}\end{aligned}$$

Take 1.47 ml cells + 9.53 ml MEMB = 11 ml

After dilution,

$$\begin{aligned}\text{Final Count} &= 1021, 991, 998 \\ \text{Avg. count} &= 1003.3 \\ \text{Cell Conc.} &= 1003.3 \times 400 \\ &= 401333 \text{ cells/ml}\end{aligned}$$

USER:10 ID:TRITIUM PRESET TIME: 1.00

FRI 26 JUN 1998 17:09

SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N

#: 1 AQC:N QCF:N RCM:N 2 PHASE MONITOR: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

	SAM	PDS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
Back	1	**	1	14.00	53.45	1.00	1.58	50.0	
	2	**	2	18.00	47.14	1.00	3.37	57.0	
1M	3	**	3	30.00	36.51	1.00	5.15	56.0	
	4	**	4	17.00	48.51	1.00	6.88	56.0	
	5	**	5	62.00	25.40	1.00	8.58	55.0	
	6	**	6	47.00	29.17	1.00	10.37	54.0	
2M	7	**	7	67.00	24.43	1.00	12.15	55.0	
	8	**	8	49688.00	1.79	0.25	13.12	56.0	
	9	**	9	45092.00	1.88	0.25	14.09	56.0	
3M	10	**	10	44124.00	1.90	0.25	15.07	54.0	
	11	**	11	129893.33	1.43	0.15	15.94	55.0	
4M	12	**	12	132920.00	1.42	0.15	16.82	56.0	
	13	**	13	137906.66	1.39	0.15	17.69	56.0	
	14	**	14	186491.44	1.11	0.17	18.65	56.0	
5M	15	**	15	191906.66	1.18	0.15	19.54	56.0	
	16	**	16	196793.33	1.16	0.15	20.43	57.0	
	17	**	17	96.00	20.41	1.00	22.17	55.0	
6M	18	**	18	91.00	20.97	1.00	23.90	55.0	
	19	**	1	96.00	20.41	1.00	25.77	56.0	
	20	**	2	49.00	28.57	1.00	27.52	54.0	
7M	21	**	3	49.00	28.57	1.00	29.25	57.0	
	22	**	4	62.00	25.40	1.00	31.03	55.0	
	23	**	5	42472.73	1.85	0.28	32.07	56.0	
8M	24	**	6	44172.00	1.90	0.25	33.06	55.0	
	25	**	7	44264.00	1.90	0.25	34.02	57.0	
	26	**	8	138013.33	1.39	0.15	34.90	55.0	
9M	27	**	9	139526.66	1.38	0.15	35.78	55.0	
	28	**	10	131853.33	1.42	0.15	36.66	55.0	
	29	**	11	227617.14	1.00	0.17	37.62	57.0	
10M	30	**	12	222822.86	1.01	0.17	38.59	57.0	
	31	**	13	207600.00	1.05	0.17	39.56	55.0	
	37	**	1	25.00	40.00	1.00	41.43	79.0	
1c	38	**	2	20.00	44.72	1.00	43.22	78.0	
	39	**	3	19.00	45.88	1.00	45.00	80.0	
	40	**	4	26.00	39.22	1.00	46.73	79.0	
2c	41	**	5	16.00	50.00	1.00	48.52	80.0	
	42	**	6	13.00	55.47	1.00	50.29	79.0	
	43	**	7	1828.00	4.68	1.00	52.02	80.0	
3c	44	**	8	1964.00	4.51	1.00	53.77	77.0	
	45	**	9	1797.00	4.72	1.00	55.50	80.0	
	46	**	10	4870.00	2.87	1.00	57.23	80.0	
4c	47	**	11	5032.00	2.82	1.00	58.98	81.0	
	48	**	12	4922.00	2.85	1.00	60.71	79.0	
	49	**	13	7914.00	2.25	1.00	62.44	79.0	
5c	50	**	14	8124.00	2.22	1.00	64.18	80.0	
	51	**	15	8183.00	2.21	1.00	65.97	79.0	

	SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
	52	**	-16	1	22.00	42.64	1.00	67.77	79.0
6c	53	**	-17	1	28.00	37.80	1.00	69.56	79.0
	54	**	-18	1	24.00	40.82	1.00	71.29	78.0
	55	**	- 1	1	28.00	37.80	1.00	73.10	79.0
7c	56	**	- 2	1	23.00	41.70	1.00	74.83	79.0
	57	**	- 3	1	23.00	41.70	1.00	76.58	80.0
	58	**	- 4	1	1975.00	4.50	1.00	78.36	82.0
8c	59	**	- 5	1	1824.00	4.68	1.00	80.14	82.0
	60	**	- 6	1	2041.00	4.43	1.00	81.88	80.0
	61	**	- 7	1	5768.00	2.63	1.00	83.62	79.0
9c	62	**	- 8	1	5781.00	2.63	1.00	85.36	81.0
	63	**	- 9	1	5491.00	2.70	1.00	87.15	80.0
	64	**	-10	1	8055.00	2.23	1.00	88.93	80.0
10c	65	**	-11	1	8004.00	2.24	1.00	90.73	76.0
	66	**	-12	1	7683.00	2.28	1.00	92.51	79.0

TABLE-1

Expt. # : 2

Date/Time : 06/26/98; 5-15 p.m

Tube #	Medium count for 10 ul of 1:50 dilution (cpm)	Avg. cpm	dpm [cpm/0.52]	$\mu$ Ci/ml (A) on counting [dpm/444]	$\mu$ Ci/ml (A <sub>0</sub> ) <del>on addition</del> ml [A <sub>0</sub> ]
1	4, 16, 3	7.6	14.7	0.03	
2	48, 33, 53	44.6	85.8	0.19	
3	49674, 45078, 44110	46287.3	89014.1	200.48	0.2004
4	129879, 132906, 137892	133559	256844.2	578.47	0.5784
5	186477, 191892, 196779	191716	368684.6	830.3	0.8303
6	82, 77, 82	80.3	154.4	0.34	
7	35, 35, 48	39.3	75.6	0.17	
8	42458, 44158, 44250	43622	83888.4	188.93	0.1889
9	137999, 139512, 131839	136450	262403.8	590.99	0.5909
10	227603, 222808, 207586	219332	421792.9	949.98	0.9499

TABLE-2

Expt. # : 2

Date/Time : 06/29/98; 5-10 p.m

Tube #	Radioactivity for 100 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.52]	$\mu$ Ci/ml (A) on counting [dpm/222000]	$\mu$ Ci/ml (A <sub>0</sub> ) after 12 h incubation [A <sub>0</sub> e <sup>-λt</sup> ]
1	11, 6, 5				
2	12, 2, -1				
3	1814, 1950, 1783	1849	3555.7	0.0160	
4	4856, 5018, 4908	4927.3	9475.6	0.0426	
5	7900, 8110, 8169	8059.6	15499.3	0.0698	
6	8, 14, 10				
7	14, 9, 9				
8	1961, 1810, 2027	1932.6	3716.6	0.0167	
9	5754, 5767, 5477	5666	10896.1	0.0490	
10	8041, 7990, 7669	7900	15192.3	0.0684	



**TABLE-3**

Expt. # : 2

Date/Time : 06/26/98; 2-30 P.M.

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 400]	pCi/cell [uCi/cell x10 <sup>6</sup> Cells/ml]
1	781, 845, 730, 765			
2	695, <del>754</del> , 687, 726			
3	677, 672, 633	660.6	264266	0.0605
4	698, 695, 702	698.3	279333	0.1525
5	<del>893</del> , 833, 865, 865	854.3	341733	0.2042
6	591, 612, 625			
7	752, 770, 787			
8	593, 580, 597	590	236000	0.0707
9	659, 651, 658	656	262400	0.1867
10	659, 618, 636	637	255066	0.2681

0.25

0.75

1.25

**TABLE-4**

Expt. #: 2

Date: 07/03/98

**Colony Counts and Survival Fraction**

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1.2	180	189	176	} 185	
2.2	190	182	193	}	
3.2	144	150	138	144	0.7783
4.2	82	89	73	81.33	0.4396
5.2	39	42	35	38.66	0.2089
6.2	210	204	196	} 205.66	
7.2	215	210	199	}	
8.2	51	61	42	51.33	0.2496
9.3	120	127	113	12	0.0583
10.4	168	161	152	1.6	0.0077

EXPT # 2

DMF = 2.92

NATIONAL  
12-183  
MILWAUKEE

SF

0.1

0.01

Semi-Logarithmic  
3 Cycles x 10 to the inch

