

### V79 COLONY FORMING ASSAY

**Experiment Name :**  $^3\text{H}_2\text{O}$ + 5-12.5 % DMSO; **Exp. # :** 1; **Investigator:** A. Bishayec  
**Date:** 06/29/98

1. Set the rocker-roller at 37°C incubator, set the Coulter Counter, wash cells (from 75 cm<sup>2</sup> flusk, 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 : 472133 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:** 06/29/98; 4-30 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:** 06/29/98; 8-00 p.m.

Tube #	$^3\text{H}_2\text{O}$ Conc. (mCi/ml)	Cells in MEMB (ml)	MEMB (ul)	$^3\text{H}_2\text{O}$ [25 mCi/ml] (ul)	Sterile DMSO (ul)	MEMB (ul)	DMSO Conc. (%)
1	0	1.0	750	0	0	250	0
2	0	1.0	750	0	100	150	5
3	0	1.0	750	0	150	100	7.5
4	0	1.0	750	0	200	50	10
5	0	1.0	750	0	250	0	12.5
6	0.75	1.0	690	60	0	250	0
7	0.75	1.0	690	60	100	150	5
8	0.75	1.0	690	60	150	100	7.5
9	0.75	1.0	690	60	200	50	10
10	0.75	1.0	690	60	250	0	12.5

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

**Date/Time:** 06/29/98; 8-10 p.m.

Handwritten title or heading, possibly "List of items" or similar.

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1.2

2.2

3.2

4.2

5.2

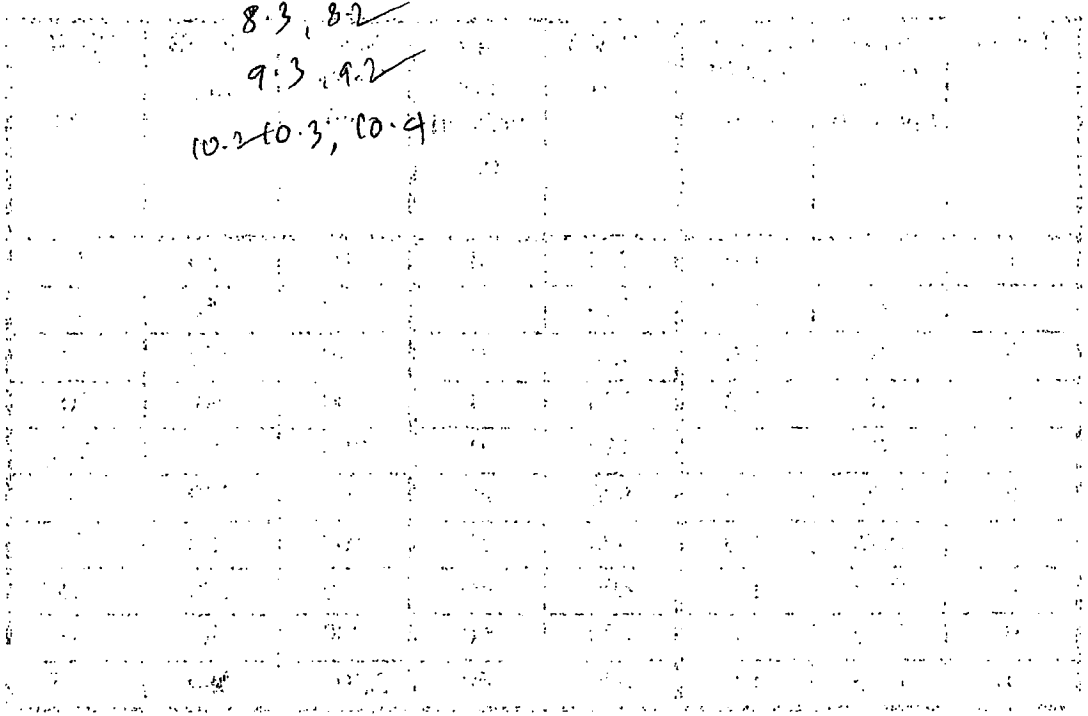
6.3

7.3, 7.2

8.3, 8.2

9.3, 9.2

10.2 to 10.3, 10.4



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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 : 06/30/98; 9-30 a.m.
11. Transfer tubes to roller at 10.5 °C for 72 h. Date/Time: 06/30/98; 9-40 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: 07/03/98; 10-30 a.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 #1

06/29/98

Initial Cell Count = 6955, 6945, 6993  
Avg. Cell count = 6964.3  
Cell conc = 2,785,733 cells/ml

For dilution,

$$\text{Vol of cell suspension required} = \frac{4400000}{2785733}$$

$$= 1.57 \text{ ml}$$

Take 1.57 ml of cells + 9.43 ml MEMB = 11 ml

After dilution,

Final Cell Count = 1155, 1177, 1209  
Avg. cell count = 1180.3  
Cell conc = 4,721,333 cells/ml

# Efficiency Check

USER:10 ID:TRITIUM      PRESET TIME: 1.00      MON 06 JUL 1998 16:30  
 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	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	27.00	38.49	1.00	1.68	52.0	→ Background & aquasol tube
2	**	2	195497.14	1.08	0.17	2.64	54.0	} 10ul 1:50 diluted medium #10
3	**	3	189211.44	1.10	0.17	3.61	56.0	
4	**	4	18.00	47.14	1.00	5.35	95.0	→ Background & cocktail from Rad
5	**	5	156160.00	1.31	0.15	6.23	99.0	} 10ul 1:50 diluted medium tube #10
6	**	6	155426.66	1.31	0.15	7.12	100.0	

Expt# 1

USER:10 ID:TRITIUM PRESET TIME: 1.00 MON 06 JUL 1998 10:54  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N  
 H#: 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	PUS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	18.00	47.14	1.00	1.57	50.0	
2	**	2	27.00	38.49	1.00	3.37	57.0	
3	**	3	25.00	40.00	1.00	5.10	57.0	
4	**	4	20.00	44.72	1.00	6.89	56.0	
5	**	5	28.00	37.80	1.00	8.68	58.0	
6	**	6	29.00	37.14	1.00	10.50	62.0	
7	**	7	23.00	41.70	1.00	12.29	57.0	
8	**	8	26.00	39.22	1.00	14.03	59.0	
9	**	9	26.00	39.22	1.00	15.80	56.0	
10	**	10	22.00	42.64	1.00	17.53	57.0	
11	**	11	22.00	42.64	1.00	19.27	58.0	
12	**	12	32.00	35.36	1.00	21.06	58.0	
13	**	13	23.00	41.70	1.00	22.79	56.0	
14	**	14	35.00	33.81	1.00	24.52	57.0	
15	**	15	32.00	35.36	1.00	26.32	57.0	
16	**	16	30.00	36.51	1.00	28.10	56.0	
17	**	17	169674.28	1.16	0.17	29.06	57.0	
18	**	18	167331.44	1.17	0.17	30.02	57.0	
19	**	19	167440.00	1.55	0.10	31.03	57.0	
20	**	20	189093.33	1.19	0.15	31.92	56.0	
21	**	21	188826.66	1.19	0.15	32.80	56.0	
22	**	22	172885.72	1.15	0.17	33.77	56.0	
23	**	23	162577.14	1.19	0.17	34.72	58.0	
24	**	24	159508.58	1.20	0.17	35.68	57.0	
25	**	25	155840.00	1.31	0.15	36.56	55.0	
26	**	26	170153.33	1.25	0.15	37.44	56.0	
27	**	27	169693.33	1.25	0.15	38.32	56.0	
28	**	28	1467313.33	1.26	0.15	39.22	57.0	
29	**	29	170680.00	1.25	0.15	40.09	56.0	
30	**	30	169570.00	1.54	0.10	41.03	54.0	
31	**	31	161626.66	1.28	0.15	41.91	56.0	
37	**	1	32.00	35.36	1.00	43.83	81.0	
38	**	2	24.00	40.82	1.00	45.62	82.0	
39	**	3	29.00	37.14	1.00	47.35	81.0	
40	**	4	22.00	42.64	1.00	49.18	83.0	
41	**	5	28.00	37.80	1.00	50.97	81.0	
42	**	6	29.00	37.14	1.00	52.76	81.0	
43	**	7	26.00	39.22	1.00	54.54	79.0	
44	**	8	29.00	37.14	1.00	56.27	79.0	
45	**	9	30.00	36.51	1.00	58.07	83.0	
46	**	10	15.00	51.64	1.00	59.90	78.0	
47	**	11	20.00	44.72	1.00	61.69	78.0	
48	**	12	25.00	40.00	1.00	63.42	78.0	
49	**	13	24.00	40.82	1.00	65.21	82.0	
50	**	14	25.00	40.82	1.00	66.94	80.0	
51	**	15	27.00	40.82	1.00	68.67	81.0	

SAM	PUS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
52	**	16	13376.00	2.00	0.75	70.16	82.0	
53	**	17	13659.36	1.94	0.77	71.63	82.0	
54	**	18	13058.06	1.99	0.77	73.20	81.0	
55	**	1	19264.76	1.99	0.52	74.58	79.0	
56	**	2	19634.54	1.92	0.55	75.86	82.0	
57	**	3	19476.19	1.98	0.52	77.15	82.0	
58	**	4	11951.76	1.98	0.85	78.74	80.0	
59	**	5	12243.64	1.99	0.82	80.35	80.0	
60	**	6	12177.65	1.97	0.85	81.93	82.0	
61	**	7	15371.85	1.96	0.68	83.38	79.0	
62	**	8	14948.57	1.96	0.70	84.82	81.0	
63	**	9	15731.85	1.94	0.68	86.28	80.0	
64	**	10	15158.52	1.98	0.68	87.73	81.0	
65	**	11	15618.46	1.98	0.65	89.10	80.0	
66	**	12	15332.86	1.93	0.70	90.53	80.0	

**TABLE-1**

Expt. # : 1

Date/Time : 07/06/98; 10-55 a.m

Tube #	Medium count for 10 ul of <i>1:50 diluted medium</i> (cpm)	Avg. cpm	dpm [cpm/0.52]	$\mu\text{Ci/ml (A)}$ on counting [dpm/444]	$\mu\text{Ci/ml (A}_0)$ on-addition [A/e <sup>-1.7</sup> ]
1	9, 7, 2				
2	10, 11, 5				
3	8, 8, 4				
4	4, 14, 5				
5	17, 14, 12				
6	169656, 167313, 167422	168130	323327	728.2	0.728
7	189075, 188808, 172867	183583	353044	795.1	0.795
8	162559, 159490, 155822	159290	306327	689.9	0.689
9	170135, 169675, 167995	169035	325067	732.1	0.732
10	170662, 169552, 161608	167274	321680	724.5	0.724

TABLE-2

Expt. # : 1

Date/Time : 07/06/98; 10-55 a.m.

Tube #	Radioactivity for 100 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.52]	$\mu$ Ci/ml ( $A_t$ ) on counting [dpm/222000]	$\mu$ Ci/ml ( $A_0$ ) after 12 h incubation [ $A_t/e^{-\lambda t}$ ]
1	14, 6, 11				
2	4, <del>10</del> , 10, 11				
3	8, 11, 12				
4	-3, 2, 7				
5	6, 7, 9				
6	13358, 13641, 13040	13346	25666	0.1156	
7	19246, 19616, 19458	19440	37384	0.1683	
8	11933, 12225, 12159	12105	23280	0.1048	
9	15353, 14430, 15713	15332	29484	0.1328	
10	15140, 15600, 15314	15351	29521	0.1329	



TABLE-3

Expt. # : |

Date/Time : 07/03/98 ; 12-00 noon

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 400]	pCi/cell [uCi/ml x 10 <sup>6</sup> Cells/ml]
1	931, 821, 850, 830			
2	865, 832, 824			
3	829, 790, 791			
4	805, 782, 792			
5	903, 893, 896			
6	805, 725, 719, 749	731	292400	0.3953
7	903, 933, 882	906	362400	0.4644
8	793, 746, 730	756	302533	0.3464
9	807, 822, 816	815	326000	0.4073
10	880, 831, 819, 811	820	328133	0.4050

mCi/ml

0.75



TABLE-4

Expt. #: |

Date: 07/10/98

Colony Counts and Survival Fraction

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony for 3	SF against self control
1.2	185	170	178	177.66	
2.2	190	214	203	202.33	1.13
3.2	170	182	161	171	0.9625
4.2	152	150	149	150.33	0.8461
5.2	124	142	132	132.66	0.7467
6.3	97	86	94	92.33	0.0519
7.2	25	28	28	27	0.1334
8.2	58	62	54	58	0.3391
9.2	72	79	64	71.66	0.4766
10.2	25	26	24	25	0.1884

Conc. of DMSO  
(%)

DMF

5

2.01

7.5

2.54

10

3.06

12.5

1.72

Expt #1

3H<sub>2</sub>O + DMSO

NATIONAL  
12-183  
MADE IN U.S.A.

SF

0.1

0.01

Semi-Logarithmic  
3 Cycles x 10 to 100 inch

