

V79 COLONY FORMING ASSAY

Experiment Name : $^3\text{H}_2\text{O}$ + 5-12.5 % DMSO; Exp. # : 2; Investigator: A. Bishayee

Date: 06/29/98 07/02/98

1. Set the rocker-roller at 37°C incubator, set the Coulter Counter, wash cells (from 75 cm² 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 : 464266 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% CO₂ Date/Time: 07/02/98; 4-00 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: 07/02/98; 8-15 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: 07/02/98; 8-30 p.m.

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 : 07/03/98; 10-00 a.m.
11. Transfer tubes to roller at 10.5 °C for 72 h. Date/Time: 07/03/98; 10-15 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: 07/06/98; 12-45 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

07/02/98

Initial Cell Count = 7263, 7372, 7301
Cell conc = 2924800 Cells/ml
Total volume = 10 ml
Total # of Cells/flask = 29248000
= 2.9 million

For dilution,

$$\text{vol. of cell suspension required} = \frac{4400000}{29248000} = 1.5 \text{ ml}$$

Take 1.5 ml cell suspension + 9.5 ml MEMB = 11 ml

After dilution,

Final cell count = 1193, 1117, 1122
Cell conc = 4,64,266 Cells/ml

USER:10 ID:TRITTIUM PRESET TIME: 1.00 TUE 07 JUL 1998 10:14
 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	16.00	50.00	1.00	1.57	51.0	
2	**	2	26.00	39.22	1.00	3.41	57.0	
3	**	3	32.00	35.36	1.00	5.13	56.0	

Expt #2

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
4	**	4	30.00	36.51	1.00	6.92	57.0	
5	**	5	75.00	23.09	1.00	8.65	56.0	
6	**	6	44.00	30.15	1.00	10.39	56.0	
7	**	7	45.00	29.81	1.00	12.18	57.0	
8	**	8	24.00	40.82	1.00	13.95	55.0	
9	**	9	26.00	39.22	1.00	15.68	57.0	
10	**	10	27.00	38.49	1.00	17.42	56.0	
11	**	11	40.00	31.62	1.00	19.16	55.0	
12	**	12	32.00	35.36	1.00	20.89	56.0	
13	**	13	28.00	37.80	1.00	22.62	56.0	
14	**	14	56.00	26.73	1.00	24.41	58.0	
15	**	15	35.00	33.81	1.00	26.18	57.0	
16	**	16	38.00	32.44	1.00	27.92	55.0	
17	**	17	177580.00	1.23	0.15	28.80	57.0	
18	**	18	170560.00	1.25	0.15	29.67	57.0	
19	**	1	171565.72	1.15	0.17	30.72	55.0	
20	**	2	170622.86	1.16	0.17	31.68	56.0	
21	**	3	178826.66	1.22	0.15	32.56	56.0	
22	**	4	178640.00	1.22	0.15	33.44	56.0	
23	**	5	163093.33	1.28	0.15	34.32	58.0	
24	**	6	165148.58	1.18	0.17	35.29	57.0	
25	**	7	161386.66	1.29	0.15	36.17	56.0	
26	**	8	175885.72	1.14	0.17	37.13	57.0	
27	**	9	177613.33	1.23	0.15	38.01	57.0	
28	**	10	173057.14	1.15	0.17	38.97	56.0	
29	**	11	170700.00	1.25	0.15	39.85	55.0	
30	**	12	172211.44	1.15	0.17	40.82	55.0	
31	**	13	180702.86	1.12	0.17	41.79	56.0	
37	**	1	18.00	47.14	1.00	43.67	51.0	
38	**	2	47.00	29.17	1.00	45.40	80.0	
39	**	3	24.00	40.82	1.00	47.18	78.0	
40	**	4	35.00	33.81	1.00	49.02	80.0	
41	**	5	67.00	24.43	1.00	50.74	80.0	
42	**	6	46.00	29.49	1.00	52.47	79.0	
43	**	7	46.00	29.49	1.00	54.25	80.0	
44	**	8	55.00	26.97	1.00	55.97	80.0	
45	**	9	43.00	30.50	1.00	57.76	80.0	
46	**	10	38.00	32.44	1.00	59.59	80.0	
47	**	11	34.00	34.30	1.00	61.37	80.0	
48	**	12	53.00	27.47	1.00	63.21	83.0	
49	**	13	50.00	28.28	1.00	64.93	78.0	
50	**	14	46.00	29.17	1.00	66.72	79.0	
51	**	15	31.00	35.36	1.00	68.50	78.0	

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
52	**	16	35.00	33.81	1.00	70.28	79.0	
53	**	17	6075.00	2.57	1.00	72.03	81.0	
54	**	18	6309.00	2.52	1.00	73.77	78.0	
55	**	1	6224.00	2.54	1.00	75.60	80.0	
56	**	2	6421.00	2.50	1.00	77.33	80.0	
57	**	3	6305.00	2.52	1.00	79.13	79.0	
58	**	4	6554.00	2.47	1.00	80.91	80.0	
59	**	5	6216.00	2.54	1.00	82.65	79.0	
60	**	6	6452.00	2.49	1.00	84.44	80.0	
61	**	7	6422.00	2.50	1.00	86.23	80.0	
62	**	8	6827.00	2.42	1.00	87.96	79.0	
63	**	9	6836.00	2.42	1.00	89.80	80.0	
64	**	10	6370.10*	1.94	0.77	91.37	99.0	
65	**	11	6585.00	2.46	1.00	93.15	79.0	
66	**	12	6848.00	2.42	1.00	94.93	80.0	
67	**	13	7192.00	2.36	1.00	96.73	81.0	

6853.5

* Sample was added twice by mistake

TABLE-1

Expt. # : 2

Date/Time : 07/07/98; 10-15 a.m.
WV

Tube #	Medium count for 10 ul of 1:50 diluted (cpm) <i>medium</i>	Avg. cpm	dpm [cpm/0.52]	μ Ci/ml (A) on counting [dpm/444]	μ Ci/ml (A ₀) on addition [A/e ^{-0.693T}]
1	10, 16, 14				
2	59, 28, 29				
3	8, 10, 11				
4	24, 16, 12				
5	40, 19, 22				
6	177564, 170544, 171549	173219	333113	750.2	0.750
7	170606, 178810, 178624	176013	338487	762.3	0.762
8	163077, 165132, 161370	163193	313832	706.8	0.706
9	175869, 177597, 173041	175502	33750	760.1	0.760
10	170684, 172195, 180686	174521	335618	755.8	0.755

TABLE-2

Expt. # : 2

Date/Time : 07/07/98; 10-15 a.m

Tube #	Radioactivity for 100 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.52]	μ Ci/ml (A _i) on counting [dpm/222000]	μ Ci/ml (A _o) after 12 h incubation [A _i /e ^{-λt}]
1	29, 6, 17				
2	49, 28, 28				
3	37, 25, 20				
4	16, 35, 32				
5	28, 13, 17				
6	6057, 6291, 6206	6184	11893.5	0.0535	
7	6403, 6287, 6536	6408	12324.3	0.0555	
8	6198, 6434, 6404	6345	12202.5	0.0549	
9	6809, 6818, 6835	6820	13116.6	0.0590	
10	6567, 6830, 7174	6857	13186.5	0.0593	

TABLE-3

Expt. # : 2

Date/Time : 07/06/99; 2-30 P.M.

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 400]	pCi/cell [uCi/ml x 10 ⁶ Cells/ml]
1	746, 740, 752			
2	753, 782, 798			
3	791, 796, 790			
4	749, 752, 761			
5	785, 729, 713			
6	767, 777, 785	776.3	310533	0.0172
7	682, 685, 687	684.6	273866	0.02026
8	731 , 691, 654, 667	670.6	268266	0.02046
9	751 , 677, 648, 659	661.3	264533	0.02230
10	674, 654, 634	654	261600	0.02266

mc/re

0.75

✓

TABLE-4

Expt. #: 2

Date: 07/13/98

Colony Counts and Survival Fraction

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony <i>to 8.2</i>	SF against <i>ref control</i>
1.2	170	162	181	171	
2.2	156	168	169	164.33	0.9609
3.2	159	145	154	152.66	0.8927
4.2	136	131	142	136.33	0.7972
5.2	128	123	118	123	0.7192
6.3	109	106	118	11.1	0.0649
7.2	38	32	37	35.66	0.2170
8.2	49	56	44	49.66	0.3252
9.2	67	60	53	60	0.4401
10.2	25	24	25	24.66	0.2004

% of DMSO

DMF

5

1.83

7.5

2.31

10

3.36

12.5

1.69

EXPT # 2

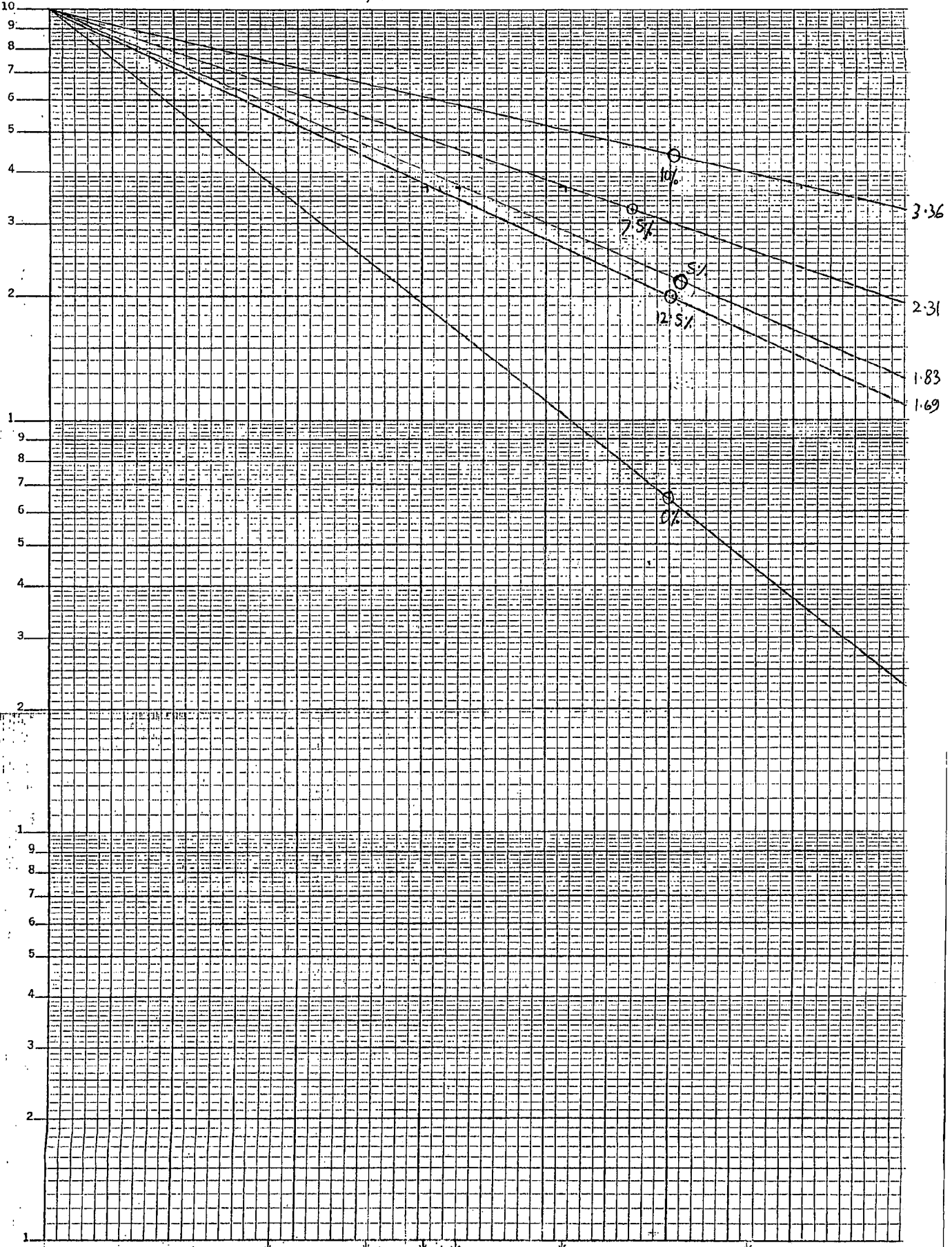
3H₂O + DMSO

1.0
NATIONAL
12-183
MADE IN U.S.A.

SF

0.1

0.01



Semi-Logarithmic
3 Cycles x 10 to (10) inch

0.3

0.6

0.9
mCi/me