

## V79 COLONY FORMING ASSAY

Experiment No.  $^3\text{H}_2\text{O}$  + DMSO Exp. 1

Investigator: Wenhui Li

Date:

1. Trypsinize cells, resuspend in MEMB, cell count
2. Dilute to 400,000 cells/ml in MEMB (final volume 11 ml) *(actually:  $\frac{540000 \text{ cells/ml}}{1350 \times 400} \text{ cells/ml}$ )*
3. Transfer 1 ml of cell suspension into ten 12 ml tubes (T-tube) labeled 1-10
4. Roll for 3-4 h at 37°C, 5% CO<sub>2</sub> **Date/Time:** 3:45 pm, 08/20/96 ~ 7:00 pm.
5. Obtain  $^3\text{H}_2\text{O}$  from refrigerator (25 mCi/ml) NEN cat No. 3247-024
6. After 3-4 h, remove T-tubes from roller and add MEMB +  $^3\text{H}_2\text{O}$  (total added volume 1 ml) containing radioactivity according to Table below. **Date/Time:**

Tube #	$^3\text{H}_2\text{O}$ ( $\mu\text{l}$ )	MEMB ( $\mu\text{l}$ )	DMSO ( $\mu\text{l}$ )	Coulter1	Coulter2	Coulter3	Avg Coulter	Number of Cells
1	0	1000	100	1234	1187	1101	1174	469600
2	0	1000	100	1434	1341	1298	1358	543052
3	20 (0.5mCi)	980	100	1061	1054	1080	1065	425000
4	40 (1 mCi)	960	100	1219	1239	1192	1217	486667
5	80 (2 mCi)	920	100	1237	1245	1236	1239	495733
6	0	1000	0	1179	1132	1121	1144	457600
7	0	1000	0	1303	1239	1303	1282	512667
8	20 (0.5mCi)	980	0	1304	1192	1211	1236	494267
9	40 (1 mCi)	960	0	1166	1182	1121	1156	462533
10	80 (2 mCi)	920	0	1226	1159	1227	1204	481600

7. Return T-tubes to roller for 12 h. **Date/Time:** 7:30 pm, 08/20/96 ~ 7:30 am, 08/21/96

8. While T-tubes are rolling label the following:

Definitions: gamma-tube = 12 X 75 mm test tube

vial = 7 ml scintillation vial

1. Experiments with gamma-emitters

- for each dose point label 6 gamma-tubes 3 C's, 3 M's

2. Experiments with beta or alpha-emitters

- for each dose point label 1 gamma-tubes M's

- for each dose point label 6 vials 3 C's, 3 M's

9. After incubation period, remove tubes, chill on ice. **Before adding DMSO, move roller to 10°C. Date/Time:**
10. Add DMSO according to table while vortexing. Quickly return to ice. **Date/Time:**
11. Transfer tubes to roller at 10°C for 72 h. **Date/Time:** 8:30 AM, 08/21/86 ~
12. After incubation period, remove tubes and place on ice, vortex and transfer 100 µl to gamma-tube labeled (M)   
 *centrifuge 200 10 min 2000 rpm 4°C*
13. ~~Add 8 ml ice cold wash MEMA, vortex~~ Transfer 100 µl supernatant to gamma tube (M)
14. ~~Centrifuge T-tubes for 10 min at 2000 rpm, 4°C. Date/Time:~~
15. Decant supernatant from T-tubes, click tubes, vortex, resuspend in 10-ml wash MEMA
16. Centrifuge T-tubes for 10 min at 2000 rpm, 4°C
17. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
18. Centrifuge T-tubes for 10 min at 2000 rpm, 4°C
19. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
20. Centrifuge T-tubes for 10 min at 2000 rpm, 4°C
21. Decant supernatant, click tubes, vortex, resuspend in 2 ml <sup>Wash</sup> ice cold MEMA
22. Labeling and preparation of dilution tubes and colony flasks
  - load 78 25 cm<sup>2</sup> flasks or 60 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.
23. Syringe 5X, determine cell concentration - transfer 100 µl to Coulter cup *to count cells*
24. Vortex T-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.
25. Transfer 1 ml from dilution tubes into flasks or dishes labeled X.2, X.3, X.4 (in triplicate). Only X.2 should be seeded for control T-tubes.
26. Transfer 10 µl of liquid from M gamma tubes into vials labeled M (in triplicate)
27. Transfer 100 µl of cell suspension to vials labeled C (in triplicate)
28. Incubate flasks for 1 wk
29. Wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with crystal violet or trypan blue.
30. Count colonies (50 or more cells). There must be between 25 and 250 colonies for the flask to be a valid data point.
31. Add 2 ml liquid scintillation cocktail to vials and count for radioactivity

**Colony Counts and Survival Fraction**

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1.2	136	118	105	119.7	101.7
2.2	78	96	95	89.7	
3.2	68	78	55	67	0.64
4.2	73	57	53	61	0.58
5.2	28	37	40		0.33
6.2	182	166	203	183.6	217.3
7.2	235	269	249	251	
8.2	88	95	81	88	0.40
9.2	56	62	66	61.3	0.28
10.3	120	98			6.05

DMSO colonies much healthier

$^3\text{H}_2\text{O} + \text{DMSO}$

1.2	136, 118, 105
2.2	78, 96, 95
3.2	68, 78, 55
4.2	73, 57, 53
5.2	28, 37, 40
6.2	182, 166, 203
7.2	235, 269, 249
8.2	88, 95, 81
9.2	56, 62, 66
10.3	120, 98

Exp. No. \_\_\_\_\_

**kBq/ml Analysis**

Tube.dilution	M	M	M	Avg M	kBq/ml

USER:10 ID:WIPE TEST PRESET TIME: 1.00  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N  
 H#: 1 AQC:N QCF:N RCM:Y 2 PHASE MONITOR:N  
 RCM-TIME: 0.10 INT:999.95

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CHANNEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0  
 CHANNEL 2-LL: 0 UL: 670 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0  
 CHANNEL 3-LL: 0 UL:1000 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	CPM1	CPM2	CPM3	TIME	AVG H#	RCM%	ERR
1	15.00	19.00	28.00	1.00	21.0	5.67	
2	11.00	19.00	31.00	1.00	44.0	26.11	
3	9.00	13.00	21.00	1.00	37.0	22.46	
4	21.00	27.00	40.00	1.00	42.0	4.34	
5	16.00	21.00	33.00	1.00	39.0	6.84	
6	17.00	24.00	32.00	1.00	40.0	6.37	
7	15.00	21.00	25.00	1.00	44.0	8.59	
8	15.00	25.00	28.00	1.00	52.0	24.20	
9	15.00	22.00	25.00	1.00	48.0	8.58	
10	17.00	20.00	28.00	1.00	52.0	3.81	
11	10.00	16.00	28.00	1.00	38.0	4.44	
12	15.00	22.00	37.00	1.00	50.0	6.10	
13	9.00	14.00	25.00	1.00	21.0	2.82	
14	7.00	14.00	21.00	1.00	21.0	0.90	

$^3\text{H}_2\text{O} + \text{DMSO}$

USER: 9 ID:TRITIUM PRESET TIME: 1.00 TUE 27 AUG 1996 09:56  
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N  
AQC:Y 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	CPM1	TIME	AVG H#	ERR
1 IC	43.00	1.00	71.0	
2 IC	72.00	1.00	69.0	
3 IC	86.00	1.00	59.0	
4 2C	81.00	1.00	67.0	
5 2C	115.00	1.00	66.0	
6 2C	53.00	1.00	62.0	
7 3C	1464.00	1.00	73.0	
8 3C	1554.00	1.00	74.0	
9 3C	1546.00	1.00	73.0	
10 4C	1477.00	1.00	68.0	
11 4C	1712.00	1.00	66.0	
12 4C	1414.00	1.00	62.0	
13 5C	3011.00	1.00	72.0	
14 5C	3122.00	1.00	71.0	
15 5C	3342.00	1.00	72.0	
16 6C	172.00	1.00	73.0	
17 6C	257.00	1.00	63.0	
18 6C	541.00	1.00	56.0	
19 7C	231.00	1.00	69.0	
20 7C	438.00	1.00	62.0	
21 7C	192.00	1.00	58.0	
22 8C	1149.00	1.00	67.0	
23 8C	1221.00	1.00	64.0	
24 8C	963.00	1.00	59.0	
25 9C	2151.00	1.00	71.0	
26 9C	2442.00	1.00	73.0	
27 9C	2394.00	1.00	63.0	
28 10C	3974.00	1.00	68.0	
29 10C	4404.00	1.00	64.0	
30 10C	4483.00	1.00	63.0	
31 1M	411.00	1.00	42.0	
32 1M	513.00	1.00	42.0	
33 1M	520.00	1.00	43.0	
34 2M	889.00	1.00	42.0	
35 2M	1575.00	1.00	43.0	
36 2M	1110.00	1.00	42.0	
3M37	2499048.00	0.12	44.0	
3M38	2311288.00	0.12	43.0	
3M39	2591624.00	0.12	43.0	
4M40	4011672.00	0.12	44.0	
4M41	4503824.00	0.12	43.0	
4M42	4497296.00	0.12	43.0	
5M43	9172752.00	0.12	43.0	
5M44	8900840.00	0.10	42.0	
5M45	8558440.00	0.10	44.0	
5M46	2496384.00	0.12	43.0	

1-5 DMSO  
6-10 no DMSO

100%R

10%R