

V79 COLONY FORMING ASSAY

Experiment No. $^3\text{H}_2\text{O}$ + DMSO Exp. 2

Investigator:

R. Howell

Date:

1. Trypsinize cells, resuspend in MEMB, cell count
2. Dilute to 400,000 cells/ml in MEMB (final volume 11 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% CO₂ **Date/Time:** 3:30pm
5. Obtain $^3\text{H}_2\text{O}$ from refrigerator (25 mCi/ml) NEN cat No. NET-001C
6. After 3-4 h, remove T-tubes from roller and add MEMB + $^3\text{H}_2\text{O}$ (total added volume $\frac{1}{10}$ ml) containing radioactivity according to Table below. **Date/Time:** 7:30pm 1/23/97

900 μl .
~~1500~~

Tube #	$^3\text{H}_2\text{O}$ (μl)	MEMB (μl)	MEM B	DMSO (μl)	Coulter1	Coulter2	Coulter3	Avg Coulter
1	0	900	0	100	1310	1315	1236	
2	0	900	0	100	1413	1358	1396	
3	20 (0.5mCi)	880	0	100	1215	1230	1285	
4	60 (1.5mCi)	840	0	100	1430	1307	1137	
5	100 (2.5mCi)	800	0	100	1177	1167	1061	
6	0	900	100	0	1583	1591	1405	
7	0	900	100	0	1549	1458	1418	
8	20 (0.5mCi)	880	100	0	1205	1150	1115	
9	60 (1.5mCi)	840	100	0	1344	1239	1148	
10	100 (2.5mCi)	800	100	0	1311	1494	1420	

7. Return T-tubes to roller for 12 h. **Date/Time:** 9:00am 1/24/97

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