



8. While test tubes are in roller, obtain sterile MEA (2 mg/ml or 2ug/ul) from refrigerator, move roller to 10.5°C, obtain ice
9. After ~12 h incubation period, remove tubes from incubator, chill on ice
10. Add MEA and/or MEMB according to the Table, vortex, quickly return to ice  
Date/Time : 07/21/98; 9-00 a.m.
11. Transfer tubes to roller at 10.5 °C for 72 h. Date/Time: 07/21/98; 9-15 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:
13. Transfer 10 ul medium to test tubes containing 490 ul MEMB (1: 50 dilution)
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. Incubate petridishes for 1 week
28. Transfer 100 µl of cell suspension (in triplicate) to prelabelled vial (C) containing 3 ml liquid scintillation cocktail for each tube
29. Transfer 10 ul (in triplicate) from tubes in step 13 to prelabelled vial (M) containing 3 ml liquid scintillation cocktail for each tube
30. Count vials in steps 28 and 29 for radioactivity in Beckman Scintillation Counter
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

Expt#1

07/20/98

Initial Cell Count = 9374, 9064, 9142, 9120

Avg. Cell count = 9108.6

Cell Conc. = 3,643,466 Cells/ml

For dilution,

$$\begin{aligned} \text{Vol. of Cell Suspension taken} &= \frac{4400000}{3643466} \\ &= 1.2 \end{aligned}$$

# Take 1.2 ml cell suspension + 9.8 ml MEMB = 11 ml

After dilution,

Final count = 1151, 1064, 1014, 1017

Avg. Count = ~~1064~~ 1031.6

Cell Conc. = 412,666 Cells/ml

Exp #1

USER:10 ID:TRITIUM      PRESET TIME: 1.00      FRI 24 JUL 1998 14:47  
 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
1	**	1	21.00	43.64	1.00	1.62	52.0	
2	**	2	16.00	50.00	1.00	3.36	54.0	
3	**	3	15.00	51.64	1.00	5.08	52.0	
4	**	4	39.00	32.03	1.00	6.87	56.0	
5	**	5	47.00	29.17	1.00	8.70	56.0	
6	**	6	36.00	33.33	1.00	10.49	56.0	
7	**	7	37.00	32.88	1.00	12.23	57.0	
8	**	8	37.00	32.88	1.00	14.01	57.0	
9	**	9	30.00	36.51	1.00	15.74	56.0	
10	**	10	39.00	32.03	1.00	17.53	55.0	
11	**	11	40.00	31.62	1.00	19.26	57.0	
12	**	12	33.00	34.82	1.00	21.00	55.0	
13	**	13	42.00	30.86	1.00	22.73	56.0	
14	**	14	51.00	28.01	1.00	24.52	56.0	
15	**	15	37.00	32.88	1.00	26.30	55.0	
16	**	16	32.00	35.36	1.00	28.07	57.0	
17	**	17	51.00	28.01	1.00	29.81	56.0	
18	**	18	39.00	32.03	1.00	31.59	56.0	
19	**	1	167820.00	1.26	0.15	32.56	56.0	
20	**	2	115879.99	1.52	0.15	33.42	54.0	
21	**	3	169302.86	1.16	0.17	34.39	57.0	
22	**	4	164462.86	1.18	0.17	35.36	57.0	
23	**	5	169166.66	1.26	0.15	36.23	54.0	
24	**	6	167874.28	1.17	0.17	37.20	56.0	
25	**	7	168413.33	1.26	0.15	38.08	56.0	
26	**	8	165533.33	1.27	0.15	38.96	57.0	
27	**	9	171171.44	1.16	0.17	39.92	57.0	
28	**	10	128620.00	1.76	0.10	40.85	54.0	
29	**	11	122193.33	1.48	0.15	41.73	53.0	
30	**	12	164000.00	1.18	0.17	42.68	56.0	
31	**	13	137266.66	1.39	0.15	43.57	55.0	
32	**	14	143948.58	1.26	0.17	44.52	54.0	
33	**	15	20979.99	1.48	0.15	45.40	55.0	
37	**	1	123.00	18.03	1.00	47.30	84.0	
38	**	2	123.00	18.03	1.00	49.02	82.0	
39	**	3	146.00	16.55	1.00	50.76	82.0	
40	**	4	126.00	17.82	1.00	52.49	82.0	
41	**	5	118.00	18.41	1.00	54.28	84.0	
42	**	6	135.00	17.21	1.00	56.01	85.0	
43	**	7	118.00	18.41	1.00	57.74	82.0	
44	**	8	146.00	16.55	1.00	59.48	82.0	
45	**	9	162.00	15.71	1.00	61.26	84.0	
46	**	10	62.00	25.40	1.00	62.99	80.0	
47	**	11	63.00	25.20	1.00	64.77	84.0	
48	**	12	59.00	26.04	1.00	66.51	86.0	
49	**	13	66.00	24.62	1.00	68.24	84.0	

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR	
50	**	14	1	50	69.00	24.08	1.00	70.03	83.0
51	**	15	1		86.00	21.57	1.00	71.82	83.0
52	**	16	1		7711.00	2.28	1.00	73.60	81.0
53	**	17	1	60	7799.00	2.26	1.00	75.33	82.0
54	**	18	1		7079.00	2.38	1.00	77.13	76.0
55	**	1	1		7557.00	2.30	1.00	78.94	82.0
56	**	2	1	70	7563.00	2.30	1.00	80.73	79.0
57	**	3	1		7550.00	2.30	1.00	82.53	81.0
58	**	4	1		10482.05	1.98	0.97	84.28	82.0
59	**	5	1	80	10930.81	1.99	0.93	86.00	82.0
60	**	6	1		10712.82	1.96	0.97	87.76	80.0
61	**	7	1		8214.00	2.21	1.00	89.54	79.0
62	**	8	1	90	8584.00	2.16	1.00	91.38	79.0
63	**	9	1		8462.00	2.17	1.00	93.17	80.0
64	**	10	1		8167.00	2.21	1.00	94.90	82.0
65	**	11	1	100	7771.00	2.27	1.00	96.69	80.0
66	**	12	1		8075.00	2.23	1.00	98.48	82.0

TABLE-1

Expt. # : 1

Date/Time : 07/24/98; 2-45 p.m.

Tube #	Medium count for 10 ul (cpm)	Avg. cpm	dpm [cpm/0.52]	$\mu$ Ci/ml (A <sub>c</sub> ) on counting [dpm/444]	$\mu$ Ci/ml (A <sub>o</sub> ) <del>on addition</del> [A <sub>c</sub> /e <sup>-λt</sup> ]
1	22, 30, 19				
2	20, 20, 13				
3	22, 23, 16				
4	25, 34, 20				
5	15, 34, 22				
6	167803, 115862, 169285	150983	290352	653.9	0.6539
7	164445, 169149, 167857	167150	321442	723.9	0.7239
8	168396, 165516, 171154	168355	323760	729.1	0.7291
9	128603, 122176, 163983	138254	265873	598.8	0.5988
10	137249, 143931, 120962	134047	257783	580.5	0.5805

TABLE-2

Expt. # : 1

Date/Time : 07/24/98; 2-45 p.m.

Tube #	Radioactivity for 100 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.52]	$\mu\text{Ci/ml (A}_1)$ on counting [dpm/222000]	$\mu\text{Ci/ml (A}_2)$ after 12 h incubation [ $A_1/e^{-\lambda t}$ ]
1	106, 106, 129				
2	109, 101, 118				
3	101, 129, 145				
4	45, 46, 42				
5	49, 52, 69				
6	7694, 7782, 7062	7512.6	14447	0.06507	
7	7540, 7546, 7533	7539.6	14499	0.06531	
8	10465, 10913, 10695	10691	20559	0.09261	
9	8197, 8567, 8445	8403	16159	0.07279	
10	7754, 7754, 8058	7855	15105	0.06804	

TABLE-3

Expt. # : 1

Date/Time : 07/24/98; 2-45 p.m

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	734, 740, 724	732.6	293066	
2	863, 851, 861	858.3	343333	
3	1000, 1064, 1032	1032	412800	
4	825, 857, 867	849	339866	
5	850, 829, 834	837	335066	
6	764, 771, 779	771	308533	0.2109
7	965, 959, 974	966	386400	0.1690
8	487, 499, 495	493	197466	0.4689
9	815, 834, 831	826	330666	0.2201
10	448, 454, 421	441	176400	0.3857



TABLE-4

Expt. #: 1

Date: 07/31/98

Colony Counts and Survival Fraction

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony for X-2	SF
1:2	212	198	225	211.66	
2:2	196	177	168	180.33	0.8519
3:2	170	178	163	170.33	0.8047
4:2	125	131	120	125.33	0.5921
5:2	110	117	114	113.66	0.5370
6:3	116	122	110	116	0.0548
7:3	218	219	214	217.3	0.1203
8:2	52	60	44	52	0.3052
9:2	43	50	36	43	0.3430
10:2	27	25	23	25	0.2199

MEA conc  
( $\mu\text{g}/\text{ml}$ )

DMF

50

1.52

100

2.78

150

2.50

200

1.80



Exp #1

3H<sub>2</sub>O + 50-200mg/ml MEA

SF

0.1

0.01

Semi-Logarithmic  
3 Cycles x 10 to the inch

