V79 COLONY FORMING ASSAY

Experiment Name : ^3H_2O + 100 ug/ml MEA; **Exp. # :** 2; **Investigator:** A. Bishayee
Date: 07/30/98

1. Set the rocker-roller at 37°C incubator, set the Coulter Counter, wash V-79 cells (from 75 cm^2 Falcon flask, subcultured 1:2, 24h before) with PBS, trypsinize (with Trypsin-EDTA, Life Tech. Cat # 25300-054) cells, resuspend in 7 ml MEMB (Minimum Essential Medium, MEM-S, Life Tech., Cat. # 11380-037 with 10 % Fetal Bovine Serum, Grand Island Biol. Co., Cat. # 240-1555., 10 % Penicillin-Streptomycin, Life Tech., Cat. # 15070-014 and 10 % L-glutamine, Life Tech., Cat # 25030-081), pass five times through 3 cc syringe with 21 gauge needle, perform cell count by transfering 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 : 418,133 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_2 **Date/Time:** 07/30/98; 3-30 p.m.
5. Obtain ^3H_2O from refrigerator (25 mCi/ml) NEN Catalog # NET001C
6. After 3-4 h, remove test tubes from roller and add MEMB and/or ^3H_2O according to Table below. **Date/Time:** 07/30/98; 7-30 p.m.

Tube #	^3H_2O mCi/ml	Cells in MEMB (ml)	MEMB (ul)	^3H_2O (ul) [25 mCi/ml]	Sterile MEA [20ug/ul] (ul)	MEMB (ul)	MEA Conc. (ug/ml)
1	0	1.0	990	0	10	0	100
2	0	1.0	990	0	10	0	100
3	0.25	1.0	970	20	10	0	100
4	0.75	1.0	930	60	10	0	100
5	1.25	1.0	890	100	10	0	100
6	0	1.0	990	0	0	10	0
7	0	1.0	990	0	0	10	0
8	0.25	1.0	970	20	0	10	0
9	0.75	1.0	930	60	0	10	0
10	1.25	1.0	890	100	0	10	0

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

Date/Time: 07/30/98; 7-45 p.m.

8. While test tubes are in roller, obtain sterile MEA 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 or MEMB according to the Table, vortex, quickly return to ice

Date/Time : 07/31/98; 8-50 a.m.

11. Transfer tubes to roller at 10.5 °C for 72 h. **Date/Time:** 07/31/98; 9-10 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:** 08/03/98; 10-00 a.m
13. Transfer 10 ul medium to test tubes
14. Add 8 ml ice-cold wash MEMA (1L contains 9.4 g of powdered Minimum Essential Medium, Life Tech., Cat. # 11700-069, 100 ml Calf Serum , Life Tech., Cat. # 16170-086, 10 ml Penicillin-Streptomycin and 10 ml L-glutamine), vortex
15. Centrifuge tubes for 10 min at 2000 rpm, 4°C
16. Labeling and preparation of dilution tubes and colony dishes
    - load 48 60x25 mm petri dishes with 4 ml MEMA (as above in Wash MEMA except Fetal Bovine Serum in place of Calf Serum)
    - load 30 T-tubes with 4.5 ml wash 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 transfering 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 0.5% 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

07/30/98

Initial Cell count = 7247, 7133, 7323
Avg. Count = 7234
Cell Conc. = 2893733 cells/ml

For dilution,
Vol. of cell suspension required = $\frac{4400000}{2893733}$
= 1.52 ml

Take 1.5 ml cells + 9.5 ml MEMβ = 11 ml

After dilution

Final count = 1076, 1041, 1029, 1066
Avg. Count = 1045
Cell conc. = 418,133 Cells/ml

PAGE: 1

USER:10 ID:TRITIUM PRESET TIME: 1.00 MON 03 AUG 1998 15:43
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	POS	CH		CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**- 1	1	back →	18.00	47.14	1.00	1.62	50.0	
2	**- 2	1		19.00	45.88	1.00	3.36	59.0	
3	**- 3	1	1M	20.00	44.72	1.00	5.15	58.0	
4	**- 4	1		22.00	42.64	1.00	6.88	56.0	
5	**- 5	1		27.00	38.49	1.00	8.61	57.0	
6	**- 6	1	2M	33.00	34.82	1.00	10.35	58.0	
7	**- 7	1		31.00	35.92	1.00	12.14	58.0	
8	**- 8	1		53755.00	1.93	0.20	13.06	58.0	

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9	**- 9	1	3M	53111.11	1.83	0.23	14.05	58.0
10	**-10	1		54660.00	1.91	0.20	14.98	59.0
11	**-11	1		166873.33	1.26	0.15	15.86	57.0
12	**-12	1	4M	168073.33	1.26	0.15	16.74	57.0
13	**-13	1		165626.66	1.27	0.15	17.63	59.0
14	**-14	1		258046.66	1.02	0.15	18.53	58.0
15	**-15	1	5M	262371.44	0.93	0.17	19.49	56.0
16	**-16	1		262233.31	1.01	0.15	20.39	56.0
17	**-17	1		31.00	35.92	1.00	22.12	57.0
18	**-18	1	6M	24.00	40.82	1.00	23.91	54.0
19	**- 1	1		34.00	34.30	1.00	25.77	61.0
20	**- 2	1		40.00	31.62	1.00	27.57	57.0
21	**- 3	1	7M	27.00	38.49	1.00	29.36	56.0
22	**- 4	1		25.00	40.00	1.00	31.10	55.0
23	**- 5	1		54700.00	1.91	0.20	32.02	57.0
24	**- 6	1	8M	53310.00	1.94	0.20	32.95	55.0
25	**- 7	1		56222.22	1.78	0.23	33.94	56.0
26	**- 8	1		161234.28	1.19	0.17	34.91	61.0
27	**- 9	1	9M	162262.86	1.19	0.17	35.87	57.0
28	**-10	1		167485.72	1.17	0.17	36.82	56.0
29	**-11	1		278353.31	0.98	0.15	37.72	57.0
30	**-12	1	10M	266446.66	1.00	0.15	38.62	56.0
31	**-13	1		278240.00	0.98	0.15	39.51	57.0
32	**-14	1		32.00	35.36	1.00	41.30	78.0
33	**-15	1	1C	19.00	45.88	1.00	43.03	79.0
34	**-16	1		33.00	34.82	1.00	44.82	80.0
35	**-17	1		35.00	33.81	1.00	46.61	80.0
36	**-18	1	2C	31.00	35.92	1.00	48.34	82.0
37	**- 1	1		34.00	34.30	1.00	50.17	81.0
38	**- 2	1		1126.00	5.96	1.00	51.95	79.0
39	**- 3	1	3C	1051.00	6.17	1.00	53.73	80.0
40	**- 4	1		1104.00	6.02	1.00	55.52	81.0
41	**- 5	1		3143.00	3.57	1.00	57.26	81.0
42	**- 6	1	4C	3163.00	3.56	1.00	59.00	80.0
43	**- 7	1		3160.00	3.56	1.00	60.78	80.0
44	**- 8	1		5528.00	2.69	1.00	62.57	80.0
45	**- 9	1	5C	5442.00	2.71	1.00	64.37	78.0
46	**-10	1		5288.00	2.75	1.00	66.10	82.0

SAM	POS	CH		CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
47	**-11	1		39.00	32.03	1.00	67.84	82.0	
48	**-12	1	6C	40.00	31.62	1.00	69.60	81.0	
49	**-13	1		34.00	34.30	1.00	71.34	81.0	
50	**-14	1		26.00	39.22	1.00	73.08	81.0	
51	**-15	1	7C	34.00	34.30	1.00	74.82	79.0	
52	**-16	1		39.00	32.03	1.00	76.61	84.0	
53	**-17	1		1081.00	6.08	1.00	78.39	84.0	
54	**-18	1	8C	1097.00	6.04	1.00	80.18	79.0	
55	**- 1	1		1066.00	6.13	1.00	81.99	81.0	
56	**- 2	1		3186.00	3.54	1.00	83.78	84.0	
57	**- 3	1	9C	3231.00	3.52	1.00	85.51	81.0	
58	**- 4	1		3215.00	3.53	1.00	87.30	83.0	
59	**- 5	1		5246.00	2.76	1.00	89.13	81.0	
60	**- 6	1	10C	5308.00	2.75	1.00	90.93	82.0	
61	**- 7	1		5673.00	2.66	1.00	92.71	82.0	

TABLE-1

Expt. # : 2 **Date/Time :** 08/03/98; 3-45 p.m.

Tube #	Medium count for 10 ul (cpm)	Avg. cpm	dpm [cpm/0.52]	μ Ci/ml (A_t) on counting [dpm/444]	μCi/ml (A_o) ~~on addition~~ on counting ~~[$A_t e^{\lambda t}$]~~
1	1, 2, 4				
2	9, 15, 13				
3	53737, 53093, 54642	53824	103507	233·1	0·2331
4	166855, 168055, 165608	166839	320844	722·6	0·7226
5	258028, 262353, 262215	260865	501664	1129·8	1·1298
6	13, 6, 16				
7	22, 9, 7				
8	54682, 53292, 56204	54726	105242	237·0	0·2370
9	161216, 162244, 167467	163642	314696	708·7	0·7087
10	278335, 266428, 278222	274328	527554	1188·1	1·1881

TABLE-2

Expt. # : 2 **Date/Time :** 08/03/98; 3-45 P.M

Tube #	Radioactivity for 100 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.52]	μCi/ml (A_t) on counting [dpm/222000]	~~μCi/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]~~
1	14, 1, 15				
2	17, 13, 16				
3	1108, 1033, 1086	1075·6	2068·5	0·0093	
4	3125, 3145, 3142	3137·3	6033·3	0·0271	
5	5510, 5424, 5270	5401·3	10387·1	0·0467	
6	21, 22, 16				
7	8, 16, 21				
8	1063, 1079, 1048	1063·3	2044·8	0·0092	
9	3168, 3213, 3197	3192·6	6139·7	0·0276	
10	5228, 5290, 5655	5391·0	10367·3	0·0466	

TABLE-3

Expt. # : 2 **Date/Time :** 08/03/98; 11-00 a.m

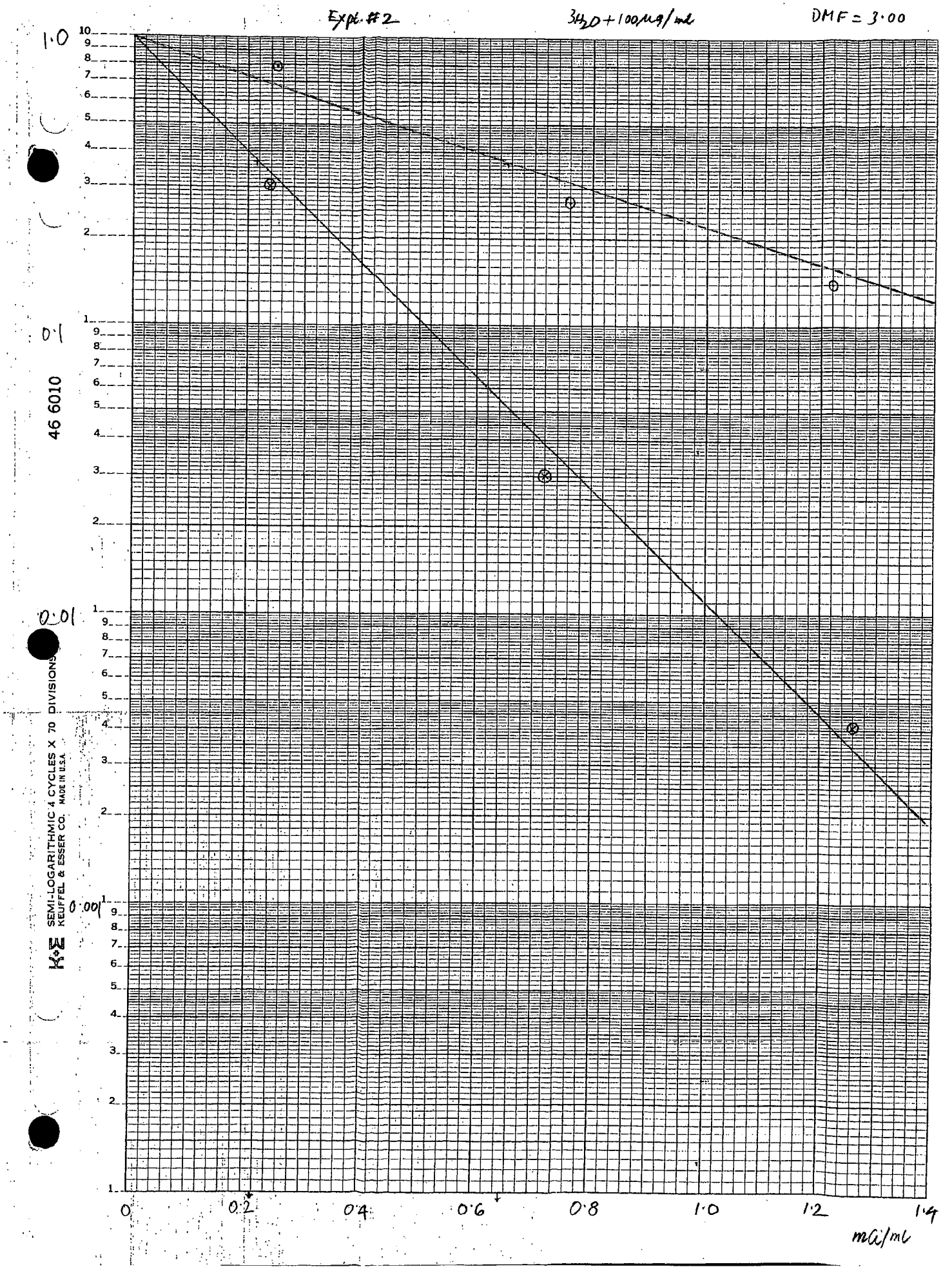
Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 400]	pCi/cell [uCi/ml x10^6 Cells/ml]
1	696, 698, 700	698	279200	
2	668, 650, 645	654.3	261733.	
3	632, 630, 625	629	251600	0.0369
4	575, 561, 549	561.6	224666	0.1206
5	718, 708, 709	711.6	284666	0.1640
6	715, 708, 706	709.6	283866	
7	740, 737, 726	734.3	293733	
8	728, 716, 733	725.6	290266	0.0316
9	695, 693, 688	692	276800	0.0997
10	763, 749, 755	755.6	302266	0.1541

TABLE-4

Expt. #: 2 Date: 08/10/98

Colony Counts and Survival Fraction

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1·2	150	142	139	136.16	
2·2	131	129	126		
3·2	116	101	108	108·33	0·7956
4·2	36	43	31	36·66	0·2692
5·3	185	190	201	19·2	0·1410
6·2	106	105	112	105·5	
7·2	92	119	99		
8·2	40	30	26	32	0·3033
9·3	32	27	37	3·2	0·0303
10·4	40	45	51	0·45	0·0042


Expt. #2
3H2O + 100 µg/ml
DMF = 3.00
1.0
0.1
0.01
0.001
46 6010
K+E SEMI-LOGARITHMIC 4 CYCLES X 70 DIVISIONS
KEUFFEL & ESSER CO. MADE IN U.S.A.
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
mCi/ml