V79 COLONY FORMING ASSAY

Experiment Name : ^3H_2O + 100 ug/ml MEA; **Exp. # :** 1; **Investigator:** A. Bishayee
Date: 07/27/98

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 : 468,000 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:** 07/27/98; 3-45 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:** 07/27/98; 7-45 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	[illegible]0	100
2	0	1.0	990	0	10	[illegible]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	[illegible]0	10	[illegible]00
9	0.75	1.0	930	60	[illegible]0	10	[illegible]00
10	1.25	1.0	890	100	[illegible]0	10	[illegible]00

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

MEM Date/Time: 07/27/98; 8-00 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~~ MEM/and (while vortexing) or MEMB according to the Table, vortex, quickly return to ice

Date/Time : 07/28/98; 9-10 a.m.

11. Transfer tubes to roller at 10.5 °C for 72 h. Date/Time: 07/28/98; 9-20 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: 07/31/98;
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 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.

1ab x1

3 ml
5.4

Expt #1 07/27/98

Initial Cell count = 4996, 4965, 5032
Avg. Cell count = 4997.6
Cell conc. = 1999066 Cells/ml

For dilution,

Vol. of cell suspension taken = $\frac{4400000}{1999066}$

= 2.20

Take 2.2 ml of cells + 8.8 ml MEMB = 11 ml

Final count = 1191, 1202, 1117
Avg. count = 1170
cell conc. = 4,68,000 Cells/ml

$\frac{x}{150+x} = \frac{60}{100}$ x ml Serum

$100x = 60x + 9000$

$40x = 9000$

$x =$

$\frac{x}{150+x} \times$

$150 + x = x$

$1 \quad \frac{x}{150+x} \times 100 = 60$

$\frac{100x}{150+x} = \frac{60}{1}$

$100x = 60x + 9000$

$40x = 225$

100 − 60

75

100 −

150 ml x ml

$\frac{x}{150+x} = \frac{60}{100}$

$\frac{x}{x+100} = \frac{2}{100}$

$100x = 2x + 400$

$98x = 400$

225

$\frac{100x}{x+100} = \frac{0.6}{1}$

$x = 0.6x + 60$

180

$\frac{x}{100+x} = \frac{60}{100}$

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USER:10 ID:TRITIUM PRESET TIME: 1.00 FRI 31 JUL 1998 17:18
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	50.00	28.28	1.00	1.57	50.0	
2	**- 2	1	59.00	26.04	1.00	3.37	57.0	
3	**- 3	1	51.00	28.01	1.00	5.10	56.0	

B013590

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
47	**-11	1	68.00	24.25	1.00	67.99	78.0	
48	**-12	1	67.00	24.43	1.00	69.75	79.0	
49	**-13	1	59.00	26.04	1.00	71.48	78.0	
50	**-14	1	74.00	23.25	1.00	73.26	78.0	
51	**-15	1	67.00	24.43	1.00	75.04	78.0	
52	**-16	1	72.00	23.57	1.00	76.77	79.0	
53	**-17	1	1520.00	5.13	1.00	78.51	79.0	
54	**-18	1	1580.00	5.03	1.00	80.25	79.0	
55	**- 1	1	1596.00	5.01	1.00	82.08	80.0	
56	**- 2	1	3897.00	3.20	1.00	83.81	78.0	
57	**- 3	1	4465.00	2.99	1.00	85.55	79.0	
58	**- 4	1	4372.00	3.02	1.00	87.34	80.0	
59	**- 5	1	7434.00	2.32	1.00	89.08	80.0	
60	**- 6	1	7362.00	2.33	1.00	90.82	79.0	
61	**- 7	1	7072.00	2.38	1.00	92.56	78.0	
62	**- 8	1	2039.00	4.43	1.00	94.36	78.0	
63	**- 9	1	~~22.00~~	~~42.64~~	1.00	96.14	54.0	
64	**-10	1	~~20.00~~	~~44.72~~	1.00	97.88	54.0	

4	**- 4	1	1M	67.00	24.43	1.00	6.89	54.0
5	**- 5	1		70.00	23.90	1.00	8.69	56.0
6	**- 6	1	2M	66.00	24.62	1.00	10.48	56.0
7	**- 7	1		87.00	21.44	1.00	12.21	56.0
8	**- 8	1		58685.00	1.85	0.20	13.13	57.0
9	**- 9	1	3M	58030.00	1.86	0.20	14.05	56.0
10	**-10	1		58428.57	1.98	0.17	15.00	56.0
11	**-11	1		176897.14	1.14	0.17	15.96	56.0
12	**-12	1	4M	176160.00	1.23	0.15	16.83	56.0
13	**-13	1		169633.33	1.25	0.15	17.73	55.0
14	**-14	1		275937.16	0.91	0.17	18.70	56.0
15	**-15	1	5M	281148.56	0.90	0.17	19.68	57.0
16	**-16	1		283976.00	1.06	0.12	20.48	57.0
17	**-17	1		64.00	25.00	1.00	22.22	55.0
18	**-18	1	6M	79.00	22.50	1.00	23.95	56.0
19	**- 1	1		79.00	22.50	1.00	25.82	58.0
20	**- 2	1		60.00	25.82	1.00	27.56	58.0
21	**- 3	1	7M	88.00	21.32	1.00	29.34	57.0
22	**- 4	1		60.00	25.82	1.00	31.12	56.0
23	**- 5	1		55724.44	1.79	0.23	32.11	56.0
24	**- 6	1	8M	55790.00	1.89	0.20	33.03	57.0
25	**- 7	1		54160.00	1.81	0.23	34.02	56.0
26	**- 8	1		156326.66	1.31	0.15	34.90	57.0
27	**- 9	1	9M	174777.14	1.14	0.17	35.87	56.0
28	**-10	1		165840.00	1.27	0.15	36.74	57.0
29	**-11	1		285348.56	0.90	0.17	37.72	55.0
30	**-12	1	10M	289560.00	0.89	0.17	38.70	55.0
31	**-13	1		295693.31	0.95	0.15	39.60	55.0
32	**-14	1		62.00	25.40	1.00	41.38	80.0
33	**-15	1	1C	66.00	24.62	1.00	43.17	81.0
34	**-16	1		64.00	25.00	1.00	44.95	79.0
35	**-17	1		56.00	26.73	1.00	46.73	82.0
36	**-18	1	2C	61.00	25.61	1.00	48.47	84.0
37	**- 1	1		65.00	24.81	1.00	50.33	80.0
38	**- 2	1		1387.00	5.37	1.00	52.12	78.0
39	**- 3	1	3C	1679.00	4.88	1.00	53.85	79.0
40	**- 4	1		51.00	28.01	1.00	55.64	52.0
41	**- 5	1		4504.00	2.98	1.00	57.42	79.0
42	**- 6	1	4C	4607.00	2.95	1.00	59.16	80.0
43	**- 7	1		4668.00	2.93	1.00	60.90	80.0
44	**- 8	1		7733.00	2.27	1.00	62.69	80.0
45	**- 9	1	5C	7724.00	2.28	1.00	64.47	79.0
46	**-10	1		7493.00	2.31	1.00	66.26	78.0

TABLE-1

Expt. # : 1 **Date/Time :** 07/31/98; 5-18 p.m.

Tube #	Medium count for 10 ul (cpm)	Avg. cpm	dpm [cpm/0.52]	µCi/ml (~~A~~) on counting [dpm/444]	~~µ~~Ci/ml (~~X~~) ~~on addition~~ counting ~~[A/e$^{-\lambda t}$]~~
1	~~3, 13, 16~~ 9, 1, 17				
2	20, 16, 37				
3	58635, 57980, 58378	58331	112175	252.6	0.2526
4	176847, 176110, 169583	174180	334961	754.4	0.7544
5	275887, 281098, 283926	280303	539045	1214.0	1.2140
6	14, 29, 29				
7	10, 38, 10				
8	55674, 55740, 54110	55174	106105	238.9	0.2389
9	156276, 174727, 165790	165597	318457	717.2	0.7172
10	285298, 289510, 295643	290150	557981	1256.7	1.2567

TABLE-2

Expt. # : 1 **Date/Time :** 07/31/98; 5-18 pm.

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	12, 16, 14				
2	6, 11, 15				
3	1337, 1629, 1989	1651·6	3176·2	0·0143	
4	4454, 4557, 4618	4543	8736·5	0·0393	
5	7683, 7674, 7443	7600	14615·3	0·0658	
6	18, 17, 9				
7	24, 17, 22				
8	1470, 1530, 1546	1515·3	2914·1	0·0131	
9	3847, 4415, 4322	4194·6	8066·6	0·0363	
10	7384, 7312, 7022	7239·3	13921·7	0·0627	

TABLE-3

Expt. # : 1 **Date/Time :** 07/31/98; 3-00 P.M

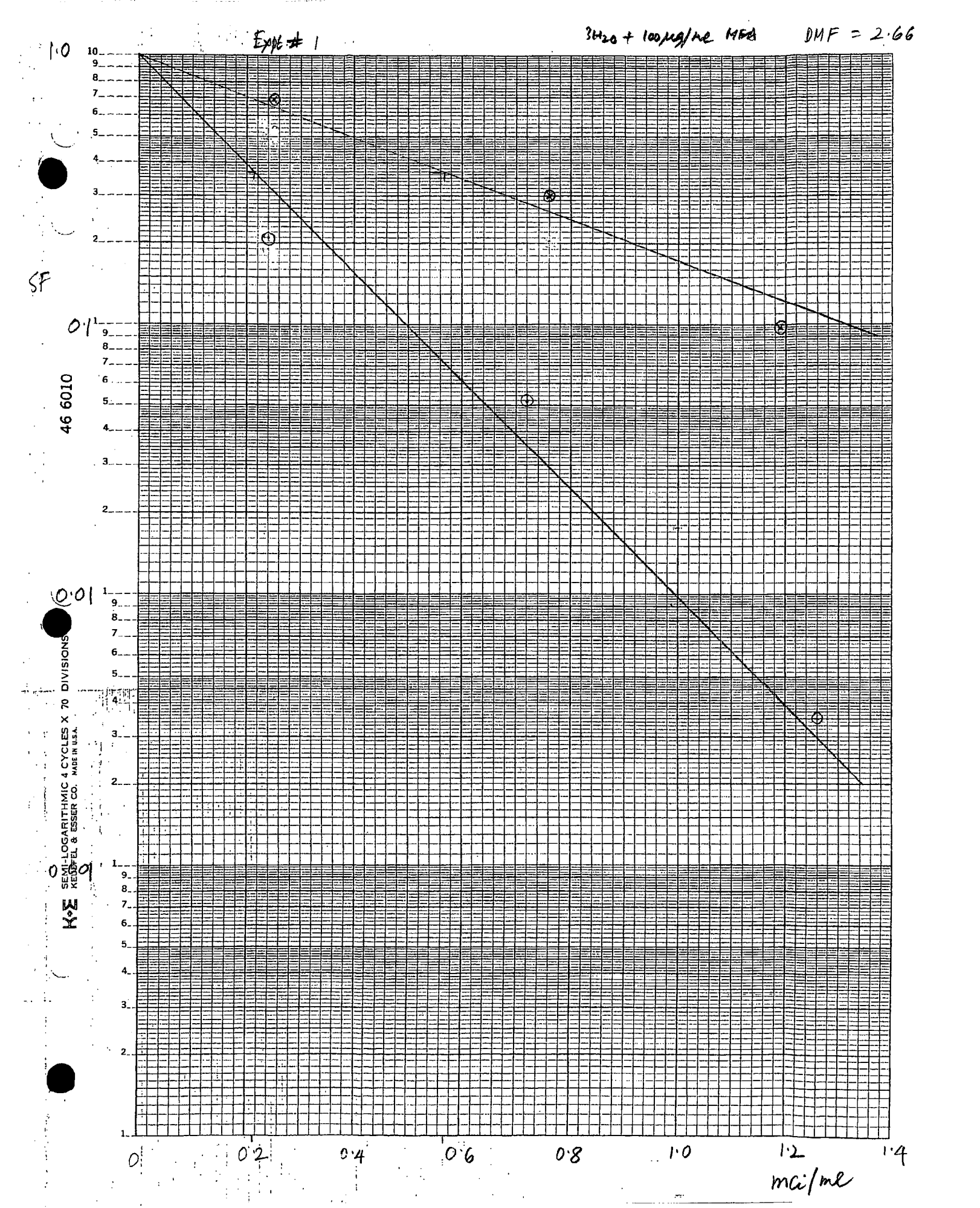
Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 400]	pCi/cell [uCi/ml x 10^6 / Cells/ml]
1	580, 574, 545	566.3	226533	
2	654, 603, 624	627	250800	
3	599, 614, 603	605.3	242133	0.0590
4	585, 597, 541	574.3	229733	0.1710
5	655, 606, 630	630.3	252133	0.2609
6	662, 602, 637	633.6	253466	
7	618, 633, 581	610.6	244266	
8	574, 558, 560	564	225600	0.0580
9	597, 583, 575	585	234000	0.1551
10	615, 593, 619	609	243600	0.2573

TABLE-4

Expt. #: 1 Date: 08/07/98

Colony Counts and Survival Fraction

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony for X.2	SF
1.2	136	142	151	138.83	
2.2	129	136	139		
3.2	109	119	100	109.33	0.7875
4.2	40	38	43	40.33	0.2905
5.3	130	136	143	13.63	0.0982
6.2	120	122	124	122.33	
7.2	129	120	119		
8.2	28	26	24	26	0.2125
9.3	68	64	61	6.43	0.0525
10.4	70	64	67	0.67	0.0054


Expt # 1
^3H_2O + 100 µg/ml MBA
DMF = 2.66
1.0
0.1
0.01
0.001
SF
46 6010
SEMI-LOGARITHMIC 4 CYCLES X 70 DIVISIONS
KEUFFEL & ESSER CO. MADE IN U.S.A.
K&E
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
mCi/ml