V79 COLONY FORMING ASSAY

8x10^5 cells in 2ml, acute

Experiment Name : ^{137}Cs toxicity; **Exp. #** : 1; **Investigator:** A. Bishayee; **Date:** 09/07/98

1. Set the rocker-roller at 37^{0}C incubator with 5% CO_2, set the Coulter Counter, wash cells (from one 75 cm^2 flusk, subcultured 1:2, 24h before) with PBS, trypsinize cells, resuspend in 6 ml MEMB for each flusk, pool, vortex, 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 ~400,000 cells/ml in MEMB (final volume 11 ml) [Actual count : $\frac{389,333}{17\times100}$ cells/ml)
3. Transfer 1 ml of cell suspension into ten 12 ml tubes (Falcon plastic test tube, ~~12x175~~ mm) labeled 1-10 both on cap and wall
4. Roll the tubes for 15 h at 37°C, 5% CO_2 **Date/Time:** 09/07/98; 6-00 p.m.
5. After ~15 h incubation period, remove tubes, add 10 ml wash MEMA, vortex and centrifuge at 2000 rpm at 4°C for 10 min (***precooled centrifuge***) . **Date/Time:** 09/08/98; 10-00 a.m.
6. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
7. Centrifuge tubes for 10 min at 2000 rpm, 4°C
8. Decant supernatant, click tubes, resuspend in 10 ml wash MEMA
9. Centrifuge tubes for 10 min at 2000 rpm, 4°C
10. Deacnt supernatant, click tubes, resuspend in 2 ml cold MEMA
11. Transfer tubes at 10°C for 72 h. **Date/Time:** 09/08/98; 11-00 a.m.
12. After 72 h, place the tubes on the perforated box containing ice (to maintain ~ 10.5°C)
13. The tubes were irradiated using Mark I irradiator (^{137}Cs gamma-ray), one tube at a time, while placing onto a box containing ice as per the Table below

1	1·2
2	2·2
3	3·2, 3·3
4	4·2, 4·3
5	5·2, 5·3
6	6·2, 6·3
7	7·2, 7·3, 7·4
8	8·2, 8·3, 8·4
9	9·2, 9·3, 9·4
10	10·3, 10·4

Tube #	Total Dose (Rad)	Dose rate (Rad/min)	Time (min)	Attenuat.
1	0	0	0	0
2	0	0	0	0
3	200	165	1.21	X-5
4	400	165	2.42	X-5
5	600	165	3.64	X-5
6	800	389	2.06	X-2
7	1000	389	2.57	X-2
8	1200	749.6	1.60	0
9	1600	749.6	2.13	0
10	2000	749.6	2.67	0

14. After irradiation, add 8 ml wash MEMA in each tube
15. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***
16. Labeling and preparation of dilution tubes and colony dishes
    - load 57 60 mm petri dishes with 4 ml MEMA
    - load 39 T-tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2, X.3, X.4, X.5 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 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
20. Determine cell concentration by transfering 100 µl to Coulter cup
21. Vortex tube, transfer 0.5 ml into dilution tube X.4, vortex tube X.4 and transfer 0.5 ml to tube X.3 and vortex tube X.3 and transfer 0.5 ml to tube X.2. Keep tubes on ice.
22. 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.
23. Incubate petridishes for 1 week
24. After 1 week, wash colonies 3 times with normal (1X) saline,and 2 times with methanol. Stain colonies with 0.0 5% crystal violet
25. Count colonies. There must be between 25 and 250 colonies for the flask to be a valid data point.

9/7/98

Initial cell count = 1874, 1991, 1946
Avg. cell count = 1937
Cell conc. = 1937 × 400
= 774800 cells/ml

For dilution,

vol. of cell suspension required = $\frac{4400000}{774800}$ ml
=
= ~~ml~~ 5.67 ml

Take 5.7 ml cellsuspension + 5.3 ml MEM = 11 ml

After dilution,

Final count = 965, 980, 975
Avg. count = 973
Cell conc = 389, 333 cells/ml

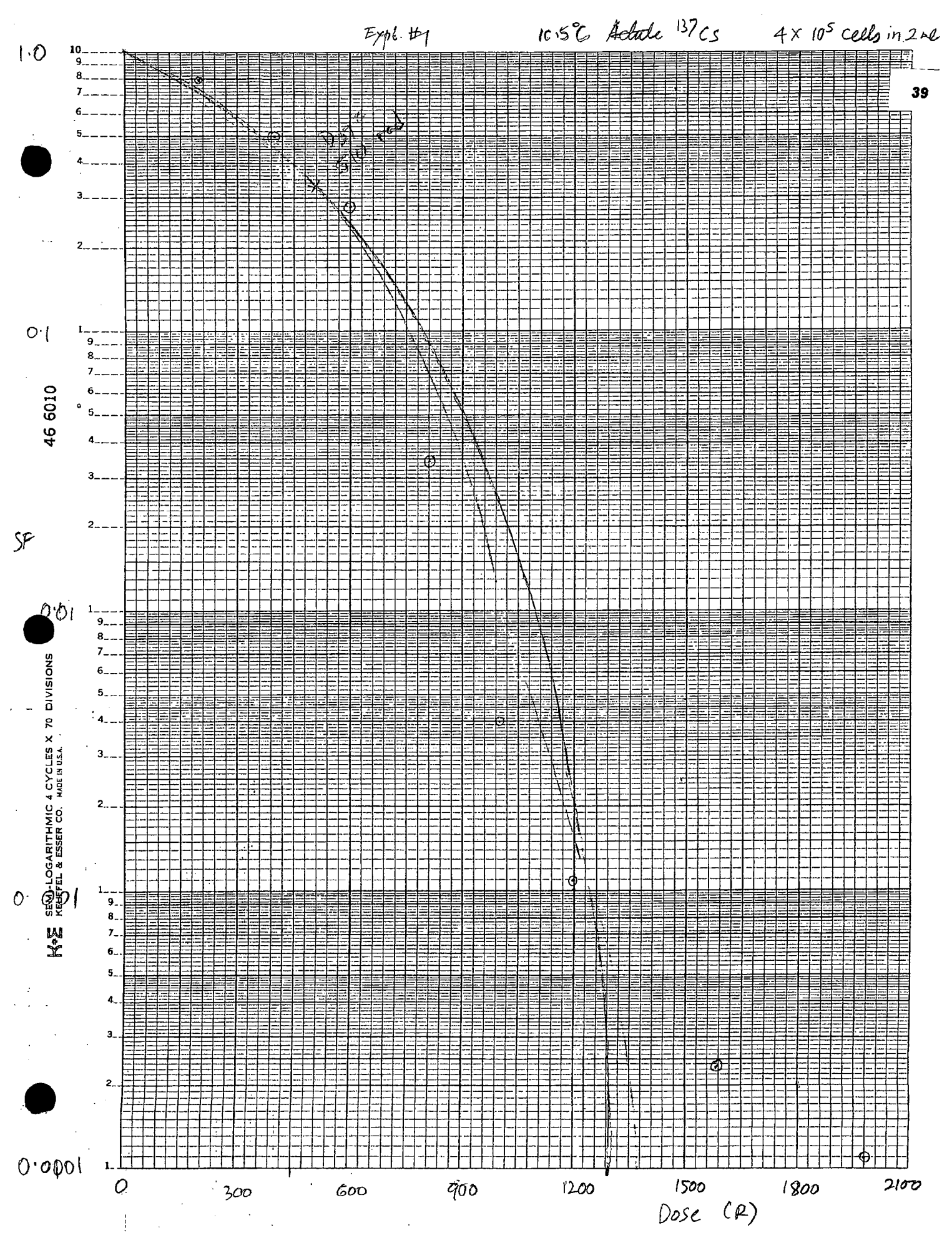
TABLE-4

Expt. #: 1

Date: 09/19/98

Colony Counts and Survival Fraction

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1·2	110	119	125	120·33	
2·2	120	132	116		
3·2	105	96	87	96	0·7977
4·2	70	54	63	62·33	0·5180
5·2	40	28	35	34·33	0·2853
6·3	48	52	44	4·8	0·0398
7·4	58	39	49	0·48	0·004
8·4	15	13	16	0·1466	0·0012
9·4	2	3	4	0·03	0·00024
10·4	1	2	1	0·01	0·00011


Expt. #4
10.5°C Acetate 137Cs
4 × 10^5 cells in 2 ml
39
1.0
0.1
0.01
0.001
0.0001
0
300
600
900
1200
1500
1800
2100
Dose (R)
46 6010
SEMI-LOGARITHMIC 4 CYCLES X 70 DIVISIONS
KEUFFEL & ESSER CO. MADE IN U.S.A.
K+E