V79 COLONY FORMING ASSAY

4x10⁶ cells cluster, acute

Experiment Name : ^{137}Cs toxicity (crossed dose); **Exp. # :** 1; **Investigator:** A. Bishayee
Date: 05/07/98

1. Set the shaker at 37°C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 150 cm^2 flusk, subcultured 1:2, 24h before) with PBS, trypsinize cells, resuspend in 7 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 ~4000,000 cells/ml in MEMB (final volume 11 ml) [Actual count : 4048000 cells/ml)
3. Transfer 1 ml of cell suspension into ten 6 ml tubes (Falcon plastic test tube, 12x175 mm) labeled 1-10 both on cap and wall
4. Roll the tubes for 1h at 37°C, 5% CO_2 **Date/Time:** 05/07/98; 4-00 p.m.
5. After ~1h incubation period, remove tubes, add 2 ml wash MEMA, vortex and centrifuge at 2000 rpm at 4°C for 10 min (***precooled centrifuge***). **Date/Time:** 05/08/98; 9-00 a.m.
6. Decant supernatant, click tubes, vortex, resuspend in 3 ml wash MEMA
7. Centrifuge tubes for 10 min at 2000 rpm, 4°C
8. Decant supernatant, click tubes, resuspend in 200 ul ice cold MEMA,transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using pipet tips
9. Again add 200 ul ice cold MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
10. Centrifuge tubes for 5 min at 1000 rpm, 4°C
11. Transfer tubes at 10°C for 72 h. **Date/Time:** 05/08/98; 11-30 a.m.
12. After 72 h, place the tubes on the perforated plate of Rainin pipet tip box containing ice (to maintain ~ 10.5°C)

Date/Time : 05/11/98; 12-00 noon

13. The tubes were irradiated using Mark I irradiator (^{137}Cs gamma-ray), one tube at a time, while placing onto a separate Rainin pipet tip box containing ice as per the Table below

Tube #	Total Dose (Rad)	Dose rate (Rad/min)	Time (min)	Attenuat.
1	0	0	0	0
2	0	0	0	0
3	~~100~~	101.4	~~0.98~~	X-10
4	~~200~~	101.4	~~1.97~~	X-10
5	~~300~~	101.4	~~2.95~~	X-10
6	~~400~~	101.4	~~3.94~~	X-10
7	~~500~~	~~101.4~~	~~4.93~~	~~X-10~~
8	750	169.6	~~4.42~~	X-5
9	~~1000~~	169.6	5.89	X-5
10	~~1250~~	169.6	7.37	X-5

14. After irradiation, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the content to ten 12 ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA by using pasteur pipet
15. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes
16. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***
17. 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.
18. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
19. Centrifuge tubes for 10 min at 2000 rpm, 4°C
20. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
21. Determine cell concentration by transfering 100 µl to Coulter cup
22. Vortex tube, transfer 0.5 ml into dilution tube X.5, vortex tube X.5 and transfer 0.5 ml to 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.

23. 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.
24. Incubate petridishes for 1 week
25. After 1 week, wash colonies 3 times with normal (1X) saline,and 2 times with methanol. Stain colonies with 0.0 5% crystal violet
26. Count colonies. There must be between 25 and 250 colonies for the flask to be a valid data point.

Expt # 1

05/01/98

Monomeler Select = 50 µl. 80–90% confluent: suspend in 5 ml MEMB for each flask

Initial cell count = 1296, 1347, 1372

Avg cell count = 1338.3

Cell conc. = 1338.3 × 4000

= 5353333 Cells/ml

For dilution

Vol. of cell suspension required = $\frac{44000000}{5353333}$

= 8.2 ml.

Take 8.2 ml of cells + 2.8 ml MEMB = 11 ml

After dilution,

Final Cell count = 1047, 1012, 977

Avg. Cell Count = 1012

Cell. Conc. = 1012 × 4000

= 40, 48, 000 Cells/ml

1.2

2.2

3.2

4.2

5.2, 5.3

6.2, 6.3

7.2, 7.3, 7.4

8.2, 8.3, 8.4

9.2, 9.3, 9.4

~~10.2~~, 10.3, 10.4

05/11/98

Expt #1

Tube #	Coulter count for 100 ml cell suspension (MS = 50 ml)
1	511, 506, 469
2	460, 417, 418
3	432, 452, 463
4	501, 487, 482
5	428, 420, 444
6	422, 407, 427
7	398, 402, 419
8	414, 408, 441
9	403, 405, 413
10	455, 429, 427

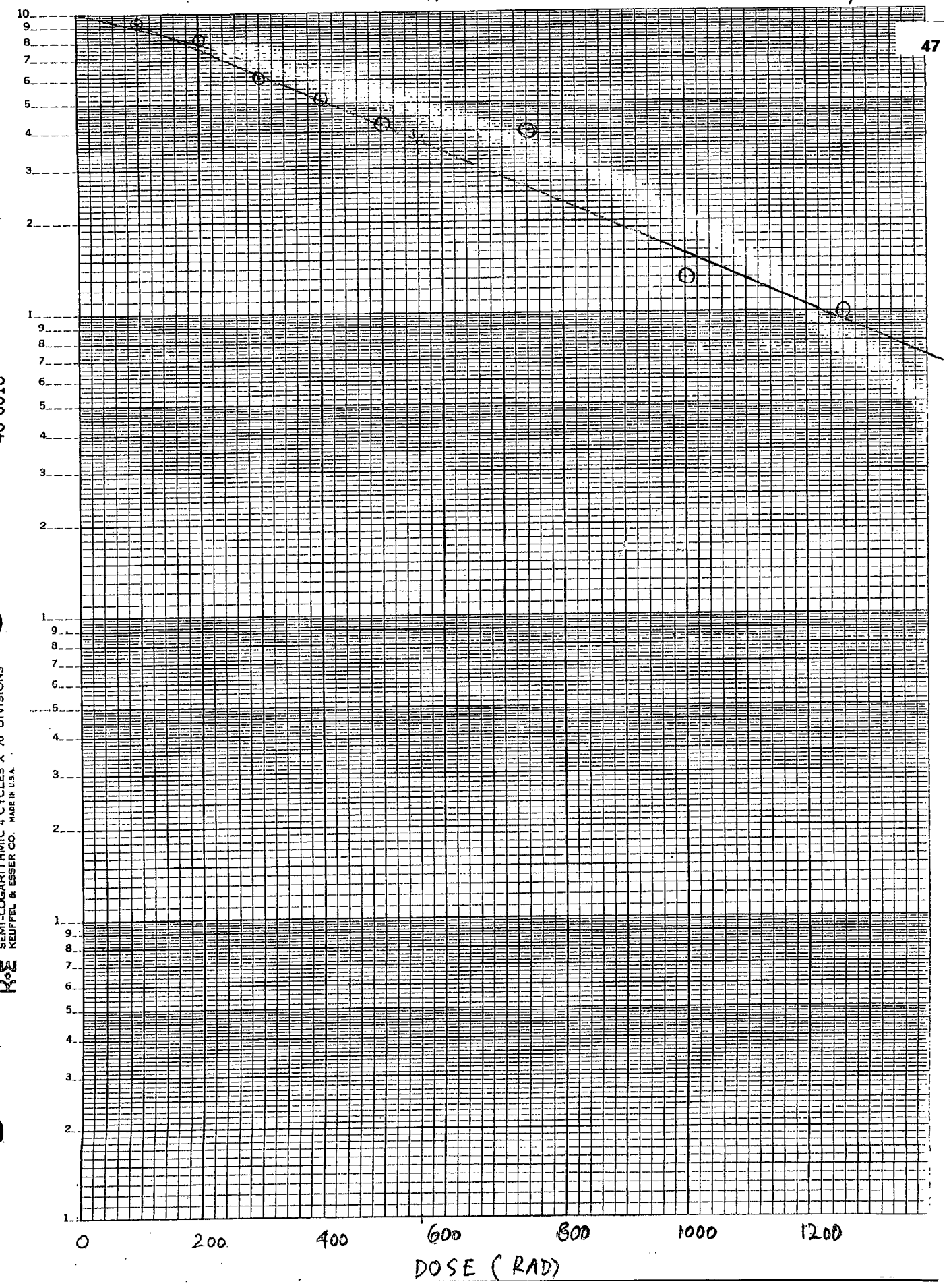
Expt #1

05/18/98

Tube #	# of colonies	Avg. colonies For X·2	SF
1·2	65, 60, 62	64.16	
2·2	59, 67, 72	61.00	
3·2	64, 58, 61	[illegible]	0·0950
4·2	52, 47, 62	53·66	0·83
5·2	42, 40, 38	40	0·62
6·2	41, 31, 32	33.77	0·52
7·2	28, 30, 27	28·33	0·44
8·2	25, 29, 24	26	0·40
9·3	88, 84, 84	8·5	0·13
10·3	65, 62, Coo	6·2	0·097

4×10^6 cells cluster

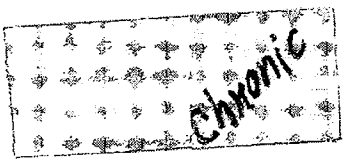
$D_{37} = 560$ R



SF

46 6010

K+E SEMI-LOGARITHMIC 4 CYCLES X 70 DIVISIONS
KEUFFEL & ESSER CO. MADE IN U.S.A.


Chronic