

V79 COLONY FORMING ASSAY

Experiment Name : $^3\text{H}_2\text{O}$ + 10% DMSO; Exp. # : 1; Investigator: A. Bishayee
 Date: 06/18/98

1. Set the rocker-roller at 37°C incubator, set the Coulter Counter, wash cells (from 75 cm² flusk, subcultured 1:2, 24h before) with PBS, trypsinize cells, resuspend in 7 ml MEMB, pass five times through 3 cc syringe with 21 gauge needle, perform cell count by transferring 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 : 507600 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% CO₂ Date/Time: 06/18/98; 4-00 p.m.
5. Obtain $^3\text{H}_2\text{O}$ from refrigerator (25 mCi/ml) NEN Catalog # NET001C
6. After 3-4 h, remove test tubes from roller and add MEMB and/or $^3\text{H}_2\text{O}$ according to Table below. Date/Time: 06/18/98; 7-45 p.m.

Tube #	$^3\text{H}_2\text{O}$ mCi/ml	Cells in MEMB (ml)	MEMB (ul)	$^3\text{H}_2\text{O}$ (ul) [25 mCi/ml]	DMSO (ul)	MEMB (ul)	
1	0	1.0	800	0	200	0	
2	0	1.0	800	0	200	0	
3	0.25	1.0	780	20	200	0	
4	0.75	1.0	740	60	200	0	
5	1.25	1.0	700	100	200	0	
6	0	1.0	800	0	0	200	
7	0	1.0	800	0	0	200	
8	0.25	1.0	780	20	0	200	
9	0.75	1.0	740	60	0	200	
10	1.25	1.0	700	100	0	200	

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

Date/Time: 06/18/98; 8-00 p.m.

$$16 \times 3 = 48$$

1.2

2.2

3.2, 3.3

4.2, 4.3

5.2, 5.3

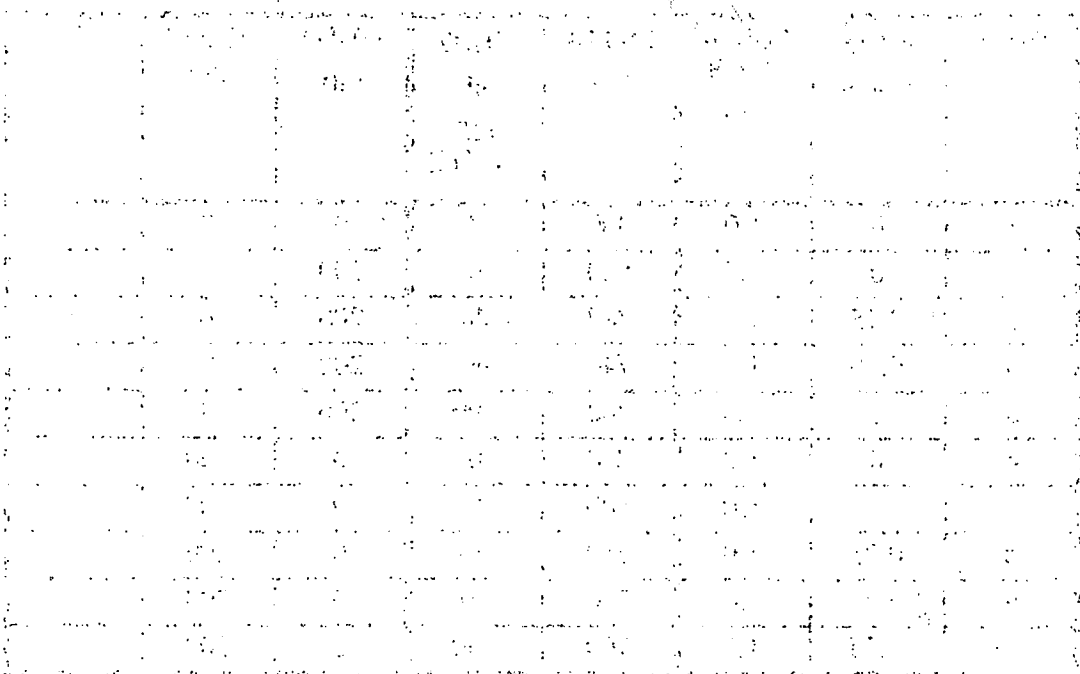
6.2

7.2

8.2, 8.3

9.2, 9.3

10.3, 10.4



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 (while vortexing) or MEMB according to the Table, vortex, quickly return to ice

Date/Time : 06/19/98; 9-10 a.m.

11. Transfer tubes to roller at 10.5 °C for 72 h.

Date/Time: 06/19/98; 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: 06/22/98; 9-30 a.m.

13. Transfer 10 ul medium to test tubes

14. Add 8 ml ice-cold MEMA, vortex

15. Centrifuge tubes for 10 min at 2000 rpm, 4°C

16. Labeling and preparation of dilution tubes and colony dishes

- load 60 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. 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

06/22/98; 1:00 pm

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.

Expt # 1

06/18/78

Initial Cell Count = 9176, 9312, 9188
Avg. Cell Count = 9225.4
Cell Conc. = 9225×400
= 3690133 Cells/ml

For dilution,

$$\text{vol. of Cell suspension taken} = \frac{4400000}{3690133} \\ = \sim 1.2 \text{ ml}$$

Take 1.2 ml Cell + 9.8 ml MEMB = 11 ml

After dilution,

Final Count = ~~995~~, 1212, 1285, 1310
Avg Count = 1269
Cell conc. = 1269×400
= 507600 Cells/ml

USER:10 ID:TRITIUM PRESET TIME: 1.00 MON 22 JUN 1998 15:55
 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	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1M	1	**	1	32.00	35.36	1.00	1.62	62.0
2	**	2	33.00	34.82	1.00	3.37	53.0	
3	**	3	37.00	32.88	1.00	5.15	55.0	
4	**	4	67.00	24.43	1.00	6.93	53.0	
5M	5	**	5	47.00	29.17	1.00	8.65	53.0
6	**	6	56.00	26.73	1.00	10.43	51.0	
7	**	7	15878.46	1.97	0.65	11.81	53.0	
8M	8	**	8	15715.38	1.98	0.65	13.19	51.0
9	**	9	15948.15	1.93	0.68	14.64	51.0	
10	**	10	47533.33	1.93	0.23	15.64	53.0	
11M	11	**	11	50630.00	1.99	0.20	16.57	53.0
12	**	12	49544.00	1.80	0.25	17.64	56.0	
13	**	13	71302.86	1.79	0.17	18.58	55.0	
14M	14	**	14	69234.29	1.82	0.17	19.53	55.0
15	**	15	65617.14	1.87	0.17	20.47	53.0	
16	**	16	58.00	26.26	1.00	22.20	53.0	
17M	17	**	17	73.00	23.41	1.00	23.93	56.0
18	**	18	82.00	22.09	1.00	25.67	55.0	
19	**	1	48.00	28.87	1.00	27.53	53.0	
20M	20	**	2	36.00	33.33	1.00	29.27	53.0
21	**	3	36.00	33.33	1.00	31.10	55.0	
22	**	4	11516.57	1.99	0.88	32.77	55.0	
23M	23	**	5	11766.86	1.97	0.88	34.42	57.0
24	**	6	11551.11	1.96	0.90	36.06	52.0	
25	**	7	45004.00	1.89	0.25	37.02	53.0	
26M	26	**	8	44050.91	1.82	0.28	38.07	53.0
27	**	9	48440.00	1.82	0.25	39.03	55.0	
28	**	10	87217.14	1.62	0.17	39.98	54.0	
29M	29	**	11	87240.00	1.62	0.17	40.93	53.0
30	**	12	81920.00	1.80	0.15	41.81	52.0	
31	**	13	26.00	39.22	1.00	43.54	74.0	
32M	32	**	14	35.00	33.81	1.00	45.32	75.0
33	**	15	31.00	35.92	1.00	47.06	73.0	
34	**	16	31.00	35.92	1.00	48.80	76.0	
35M	35	**	17	26.00	39.22	1.00	50.53	77.0
36	**	18	27.00	38.49	1.00	52.32	75.0	
37	**	1	2937.00	3.69	1.00	54.18	75.0	
38M	38	**	2	3169.00	3.55	1.00	55.97	77.0
39	**	3	3127.00	3.58	1.00	57.69	78.0	
40	**	4	8517.00	2.17	1.00	59.42	75.0	
41M	41	**	5	8897.00	2.12	1.00	61.22	74.0
42	**	6	8772.00	2.14	1.00	63.01	76.0	
43	**	7	15413.85	2.00	0.65	64.38	75.0	
44M	44	**	8	15792.31	1.97	0.65	65.77	75.0
45	**	9	16113.85	1.95	0.65	67.15	76.0	
46	**	10	32.00	35.36	1.00	68.94	76.0	

back > 16

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
47	**	11	23.00	41.70	1.00	70.67	76.0	
48	**	12	31.00	35.92	1.00	72.43	75.0	
49	**	13	33.00	34.82	1.00	74.22	75.0	
50M	50	**	14	35.00	33.81	1.00	75.99	76.0
51	**	15	31.00	35.92	1.00	77.78	76.0	
52	**	16	2279.00	4.19	1.00	79.51	76.0	
53M	53	**	17	2256.00	4.21	1.00	81.24	76.0
54	**	18	1936.00	4.55	1.00	82.98	73.0	
55	**	1	8350.00	2.19	1.00	84.78	75.0	
56M	56	**	2	8134.00	2.22	1.00	86.58	77.0
57	**	3	8313.00	2.19	1.00	88.37	78.0	
58	**	4	15284.29	1.93	0.70	89.80	75.0	
59M	59	**	5	15456.92	2.00	0.65	91.18	74.0
60	**	6	15436.92	2.00	0.65	92.56	79.0	

TABLE-1

Expt. # :

Date/Time :

Tube #	Medium count for 10 ul of 1:50 dilution (cpm)	Avg. cpm	dpm [cpm/0.52]	μ Ci/ml (A) on counting [dpm/22200] 444	μ Ci/ml (A ₀) on addition [A/e ^{-λt}]
1					
2					
3	15878, 15715, 15948	15847	30475	68.63	0.0686
4	47533, 50630, 49544	49235	94683	213.25	0.2132
5	71302, 69234, 65617	68717	132149	297.63	0.2976
6					
7					
8	11516, 11766, 11551	11611	22328	50.29	0.0502
9	45004, 44050, 48440	45831	88137	198.50	0.1985
10	87217, 87240, 81920	85459	164344	370.14	0.3701

$$1 \mu\text{Ci} = 3.7 \times 10^{10} \text{ dps}$$

$$= 2.22 \times 10^{12} \text{ dpm}$$

$$\text{dpm} = \frac{\text{cpm}}{\text{effic} \times \text{yield}} = \frac{\text{cpm}}{0.52 \times 1} = \frac{\text{cpm}}{0.52}$$

$$\mu\text{Ci/ml} = \frac{\text{dpm for 10ul}}{60} \times \frac{100}{37000} \times 50$$

$$= \text{dpm for 10ul} \times 0.00225$$

$$= \frac{\text{dpm for 10ul}}{444}$$

10ul → 500ul
take 10ul

[50 = dilution factor]

8 MBq/ml
28
46

14
28

~~1 dps =~~
 $3.7 \times 10^{10} \text{ dps} = 1 \text{ Ci} = 1000000 = 10^6 \mu\text{Ci}$

$1 \text{ dps} = \frac{10^6}{3.7 \times 10^{10}} \mu\text{Ci}$

$1 \mu\text{Ci} = \frac{3.7 \times 10^{10}}{10^6} \text{ dps} = 3.7 \times 10^4 = 37000 \text{ dps}$

$1 \text{ dps} = \frac{1}{37000} \mu\text{Ci}$

$\frac{\text{dpm}}{60} = \frac{1}{37000} \mu\text{Ci}$

$\frac{\mu\text{Ci}}{37000} = \frac{\text{dpm}}{60}$

$\mu\text{Ci} = \frac{\text{dpm} \times 37000}{60} \times 50 \times 100$
 $= \frac{\text{dpm}}{60}$

2.5 MBq/ml
2.8 MBq/ml
10.9 MBq/ml

$1 \text{ Ci} = 3.7 \times 10^{10} \text{ Bq}$

$10^6 \mu\text{Ci} = 3.7 \times 10^{10} \text{ Bq}$

$1 \mu\text{Ci} = \frac{3.7 \times 10^{10}}{10^6} \text{ Bq}$

$= 3.7 \times 10^4 \text{ Bq}$

$= \frac{3.7 \times 10^4}{10^6} \text{ MBq}$

$= \frac{3.7}{100} = \text{~~0.0037~~ MBq} = 0.037$

$1 \mu\text{Ci} = \text{~~0.0037~~ MBq} = 0.037 \text{ MBq}$

1 K.Rq = 1000 Bq

1 MBq = 1000000 Bq

TABLE-2

Expt. # : 1

Date/Time : 06/22/98; 3-55 P.M.

Tube #	Radioactivity for 300 ul cell suspension (cpm)	Avg. cpm	dpm	μ Ci/ml (A) on counting	μ Ci/ml (A ₀) after 12 h incubation
			[cpm/0.7056] 0.52	[dpm/666000] 222000	[A ₀ e ^{-λt}]
1	107, 119, 115 -3, -2, -1				
2	105, 010, 12				
3	2921, 3153, 3111	3061.6	5887.6	0.0265	
4	8501, 8881, 8756	8712.6	16755.1	0.0754	
5	15397, 15776, 16097	15756.6	30301.2	0.1364	
6	16, 7, 15				
7	17, 19, 15				
8	2263, 2240, 1920	2141	4117.3	0.0185	
9	8334, 8118, 8297	8249.6	15864.7	0.0714	
10	15268, 15440, 15420	15376	29569.2	0.1331	

$$\begin{aligned} \mu\text{Ci/ml} &= \frac{\text{dpm for } 100\mu\text{l}}{60} \times \frac{10^6}{37000} \\ &= \frac{\text{dpm for } 100\mu\text{l}}{222000} \end{aligned}$$

TABLE-3

Expt. # : 1

Date/Time : 06/22/98

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 400]	pCi/cell ^{ml} [uCi/cell x 10 ⁶ Cells/ml]
1	741, 765, 767	757.6	303,066	-
2	778, 775, 814	789	315,600	-
3	805, 823, 811	813	325,200	0.0814
4	809, 783, 825	805.6	322,266	0.2339
5	774, 746, 778	766	306,400	0.4451
6	775, 787, 800	787.3	314,933	-
7	815, 816, 828	819.6	327,866	-
8	816, 823, 787	808.6	323,466	0.0571
9	821, 825, 807	817.6	327,066	0.2183
10	837, 822, 774	811	324,400	0.4102

nci/ml

0.25

0.75

1.25

TABLE-4

Expt. #: 1

Date: 06/29/98

Colony Counts and Survival Fraction

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony for x.2	SF
1.2	228	215	214	} 211.33	
2.2	212	190	209		
3.2	156	163	148	155.66	0.7366
4.2	95	91	80	88.66	0.4195
5.3	246	237	231	20.46	0.0968
6.2	234	226	210	} 222.33	
7.2	223	216	225		
8.2	113	120	106	113	0.5082
9.3	150	162	150	15.4	0.0692
10.4	200	207	193	2	0.0089

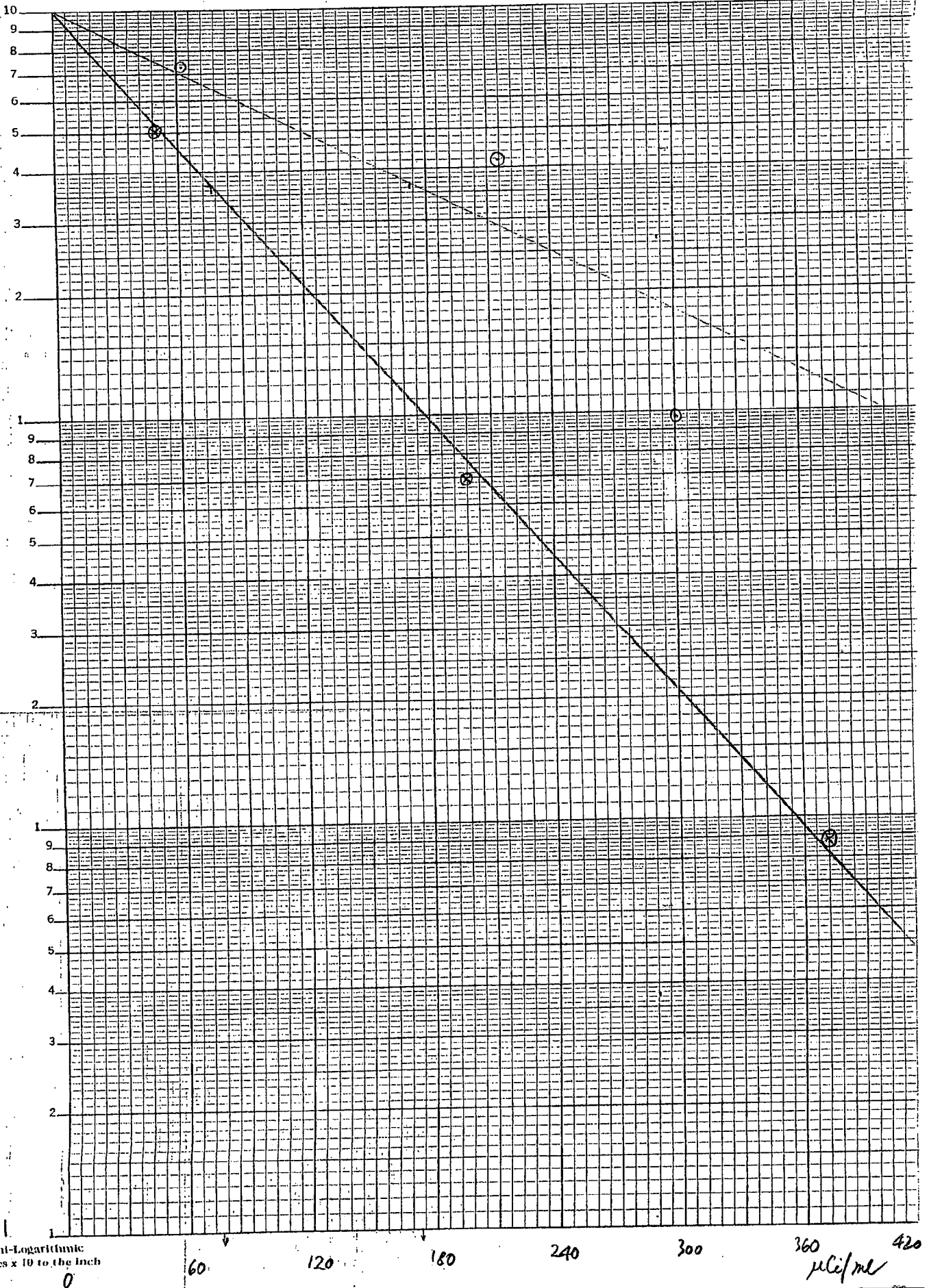
0.1

SF

0.01

0.001

Semi-Logarithmic
3 Cycles x 10 to the Inch
0



Expt #1

DMF = 2.56

NATIONAL
12-183
MADE IN U.S.A.

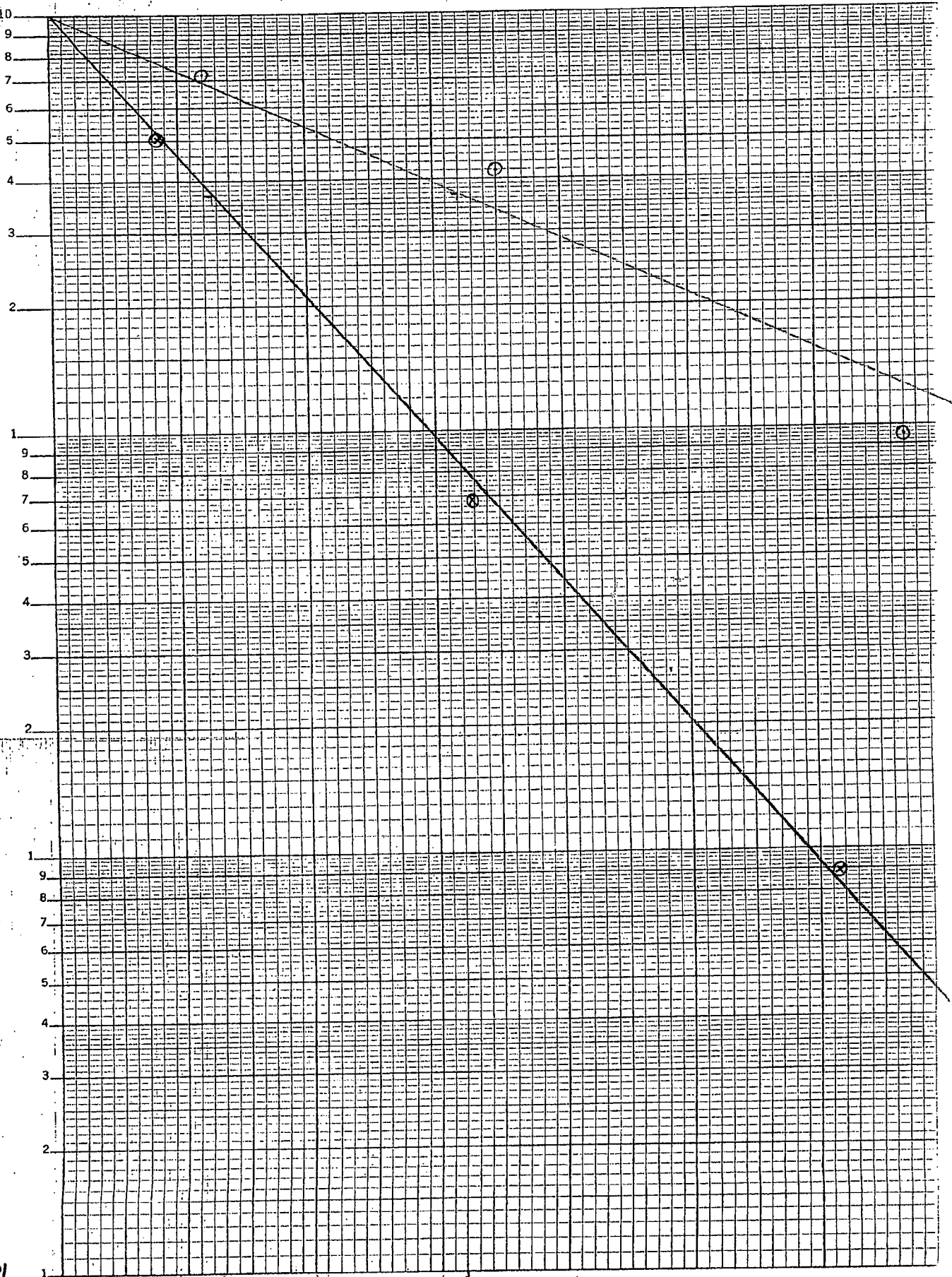
0.1

SF

0.01

0.001

Semi-Logarithmic
3 Cycles x 10 to 10⁶ inch



0.2

0.4 pci/cell