V79 COLONY FORMING ASSAY

Experiment Name : ^{131}IUdR + 5% DMSO; **Exp. # :** 9; **Investigator:** A. Bishayee
Date: 12/04/97

1. Set the rocker-roller at 37^{0}C incubator, set the Coulter Counter, wash cells (from 75 cm^2 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 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 : 4,62,133 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: 12/04/97; 3-30 p.m.(t_1)**
5. Prepare MEMB containing radioactivity in hood
   7.6 µl ^{131}IUdR (prepared on 12/03/97) + 4.992 ml MEMB
6. After 3-4 h, remove test tubes from roller and add MEMB with or without radioactivity according to Table below. **Date/Time: 12/04/97; 7-20 p.m.(t_2)**

Tube #	^{131}IUdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{131}IUdR (ml) [1.2 uCi/ml]	MEMA+ 5% DMSO	MEMA	
1	0	1.0	1.0	0	2.0	0	
2	0	1.0	1.0	0	2.0	0	
3	0.2	1.0	0.67	0.33	2.0	0	
4	0.4	1.0	0.33	0.67	2.0	0	
5	0.6	1.0	0	1.0	2.0	0	
6	0	1.0	1.0	0	0	2.0	
7	0	1.0	1.0	0	0	2.0	
8	0.2	1.O	0.67	0.33	0	2.0	
9	0.4	1.0	0.33	0.67	0	2.0	
10	0.6	1.0	0	1.0	0	2.0	

7. Return test tubes to roller for 12 h, increase the elevation angle of the roller. **Date/Time:**

12/04/97; 7-30 p.m.(t_3)

8. While test tubes are rolling label 40 (4x10) gamma-tubes (13 X 100 mm VWR glass test tube)
9. After ~12 h incubation period, remove tubes and centrifuge at 2000 rpm at 4°C (***precooled centrifuge***). **Date/Time:12/05/97; 9-00 a.m.(t_4)**
10. **During centrifugation, move roller to 10°C and obtain ice**
11. Prepare 11 ml 5% DMSO in MEMA (0.55 ml sterile DMSO + 10.45 ml MEMA), **put on ice**
12. Remove buckets from centrifuge and carefully remove 100 µl of supernatant and place in prelabeled gamma-tube.
13. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
14. Centrifuge tubes for 10 min at 2000 rpm, 4°C
15. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
16. Centrifuge tubes for 10 min at 2000 rpm, 4°C
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 ice cold MEMA + 0 or 5% DMSO as per Table. Keep on ice!
20. Transfer tubes to roller at 10°C for 72 h. **Date/Time: 12/051/97; 12-10 a.m.(t_5)**
21. Transfer 10 ul supernatant in three sets of tubes containing small pieces of tissue paper from 100 ul supernatant removed earlier and count them for radioactivity **Date/Time: 12/05/97; 1-00 p.m.(t_6)**
21. After 72 h, remove tubes and **place on ice**, add 8 ml ***ice cold*** wash MEMA. **Date/Time: 12/08/97; 9-30 a.m.(t_7)**
22. Centrifuge tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***
23. Labeling and preparation of dilution tubes and colony dishes
    - load 57 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.
24. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
25. Centrifuge tubes for 10 min at 2000 rpm, 4°C
26. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
27. Centrifuge tubes for 10 min at 2000 rpm, 4°C
28. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
29. Determine cell concentration by transfering 100 µl to Coulter cup
30. 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.
31. 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.

32. Transfer 300 µl of cell suspension (in triplicate) to gamma tubes for each tube
33. Incubate petridishes for 1 week
34. Count gamma tubes for radioactivity **Date/Time : 12/08/97; 2-30 p.m. (t_7)**
34. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with crystal violet
35. Count colonies (50 or more cells). There must be between 25 and 250 colonies for the flask to be a valid data point.

Expt # 9 12/04/97

Initial cell count = 7415, 7448, 7204

Avg. Cell Count = 7355.6

Cell Conc. = 7355.6 × 400 = 2942266 Cells/ml

For dilution,

Vol. of Original Cell suspension taken = $\frac{4400000}{2942266}$

= 1.49 ml

Take 1.5 ml of Cell suspension + 9.5 ml HEMB = 11 ml

After dilution,

Final Count = 1148, 1118, 1200

Avg. Count = 1155.33

Cell Conc. = 1155.33 × 400 = 4,62,133 Cells/ml

Expt # 9 12/04/97

Stock ^{131}IUdR 0.784 μCi/μl on 12/04/97 at 11-00 a.m.

Prepare 5ml of 1.2 μCi/ml ⇒ 6.0 μCi required

From Stock $0.784\ \mu Ci - 1\ \mu l$

$1 - \frac{1}{0.784}$ "

$6.0 = \frac{6.0}{0.784} = 7.65\ \mu l$

Take 7.65 μl stock ^{131}IUdR, Keep it for 3-4 hr in hood
Add 4.99ml MEMB in hood.

Expt # 9 12/05/94

Tube #	Medium Count (cpm) at 1-00 p.m	Avg. cpm	Avg dpm [cpm / 0.142]
1	2, 4, -2	0	
2	0, 2, 0	0	
3	462, 454, 484	466.6	3286.38
4	881, 975, 1035	963.6	6786.38
5	1393, 1459, 1326	1392.6	9807.51
6	0, 2, 0	0	
7	2, 1, 0	0	
8	387, 360, 336	361	2542.25
9	859, 763, 830, 964	884.3	6227.69
10	1264, 1281, 1263	1270.3	8946.00

$A_0 = \frac{A_t}{e^{-0.693 \times 17 / 193.2}}$

Tube #	μCi/ml upon Counting (A_t) [dpm / 22200.142]	μCi/ml during addition (A_0) [$A_t \times \frac{1}{0.940}$]
1	0	0
2	0	0
3	0.148	0.157 –
4	0.305	0.325 –
5	0.441	0.469 –
6	0	0
7	0	0
8	0.114	0.121 –
9	0.280	0.298 –
10	0.402	0.428 –

5948
5947

Expt # 9

12/08/97

Tube #	Radioactivity (cpm) of 300 µl cell suspension at 2-30 p.m.	Avg. cpm	dpm [Avg. cpm / 0.142]
1	1, −1, 0	0	0
2	0, 0, 0	0	0
3	2639, 2569, 2549	2585.66	18208.92
4	5156, 5172, 5087	5138.33	36185.44
5	8599, 8612, 8629	8613.33	60657.27
6	0, −2, 1	0	0
7	0, 0, 1	0	0
8	2554, 2675, 2607	2612	18394.36
9	5249, 5138, 5260	5215.66	36730.04
10	8627, 8735, 8688	8683.33	61150.23

Tube #	µCi/ml of cells at 2-30 of 12/08	µCi/ml of cells after 12h incubation (÷ 0.7573)
1	0	0
2	0	0
3	0.0273	0.0360
4	0.0543	0.0717
5	0.0910	0.1201
6	0	0
7	0	0
8	0.0276	0.0364
9	0.0551	0.0727
10	0.0918	0.1212

12/08/97
2-30 p.m.

Tube #	Radioactivity (cpm) for 300 ul cell suspension
1	1, -1, 0
2	0, 0
3	2554, 2675, 2560
4	

Expt # 9

Tube #	Coulter Count (for 100 ml cell suspension)	Avg. Count	cells/ml (Avg count × 400)
1	498, 504, 532	511.33	2,04,533.33 ×
2	769, 790, 711	756.66	3,02,666.67 ×
3	799, ~~822~~, 785, 765	783	3,13,200 ✓
4	630, 645, 659	644.66	2,57,866.67 ✓
5	669, 659, 660	662.66	2,65,066.67 ✓
6	765, 745, 730	746.66	2,98,666.67 ×
7	650, 672, 721	681	2,72,400 ×
8	814, 805, 767	795.33	3,18,133.33 ✓
9	732, 719, 674	708.33	2,83,333.33 ✓
10	814, 769, 742	775	3,10,000 ✓

Tube #	pCi/cell → $\frac{\mu Ci/ml}{cells/ml} \times 10^6$ after 12h incubation
1	0
2	0
3	0.1149
4	0.2780
5	0.4530
6	0
7	0
8	0.1144
9	0.2565
10	0.3909

Expt # 9

12/15/97

Dilusion	# of colonies	Avg Colonies (for x.2 dilusion)	SF
1·2	68, 55, 61	61·33 } 62·66	
2·2	66, 61, 65	64·00 }	
3·2	39, 36, 38	37·66	0·6009
4·3	53, 50, 47	5·0	0·0797 *
5·4	100, 96, 98	0·98	0·0156
6·2	62, 68, 77	69·00 } 63·66	
7·2	58, 58, 59	58·33 }	
8·2	30, 35, 37	34·00	0·5340
9·3	46, 48, 44	4·6	0·0722 *
10·4	83, 95, 87	0·88	0·0138

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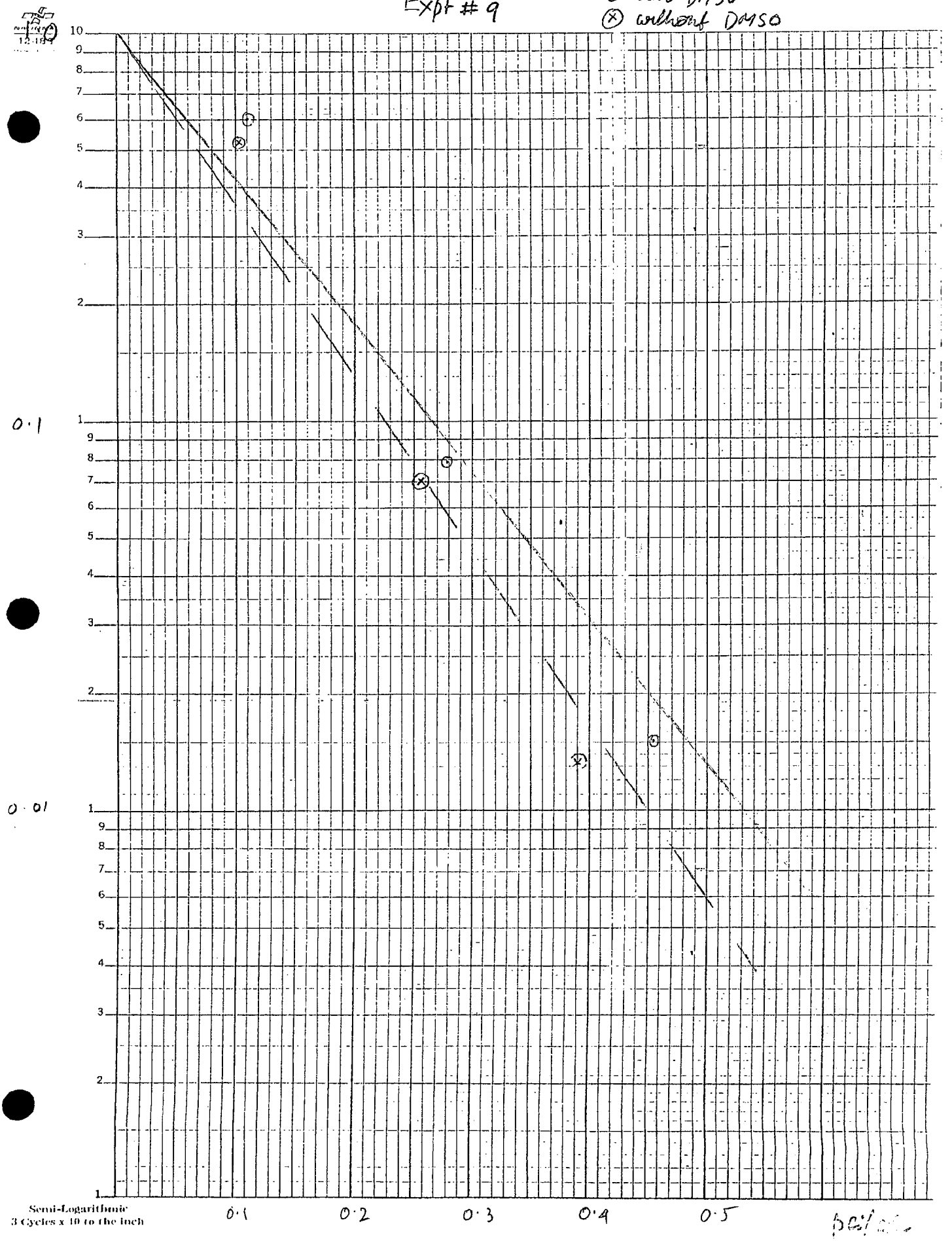
Expt # 9
⊙ with DMSO
⊗ without DMSO
10
9
8
7
6
5
4
3
2
1
0·1
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0·1
0·2
0·3
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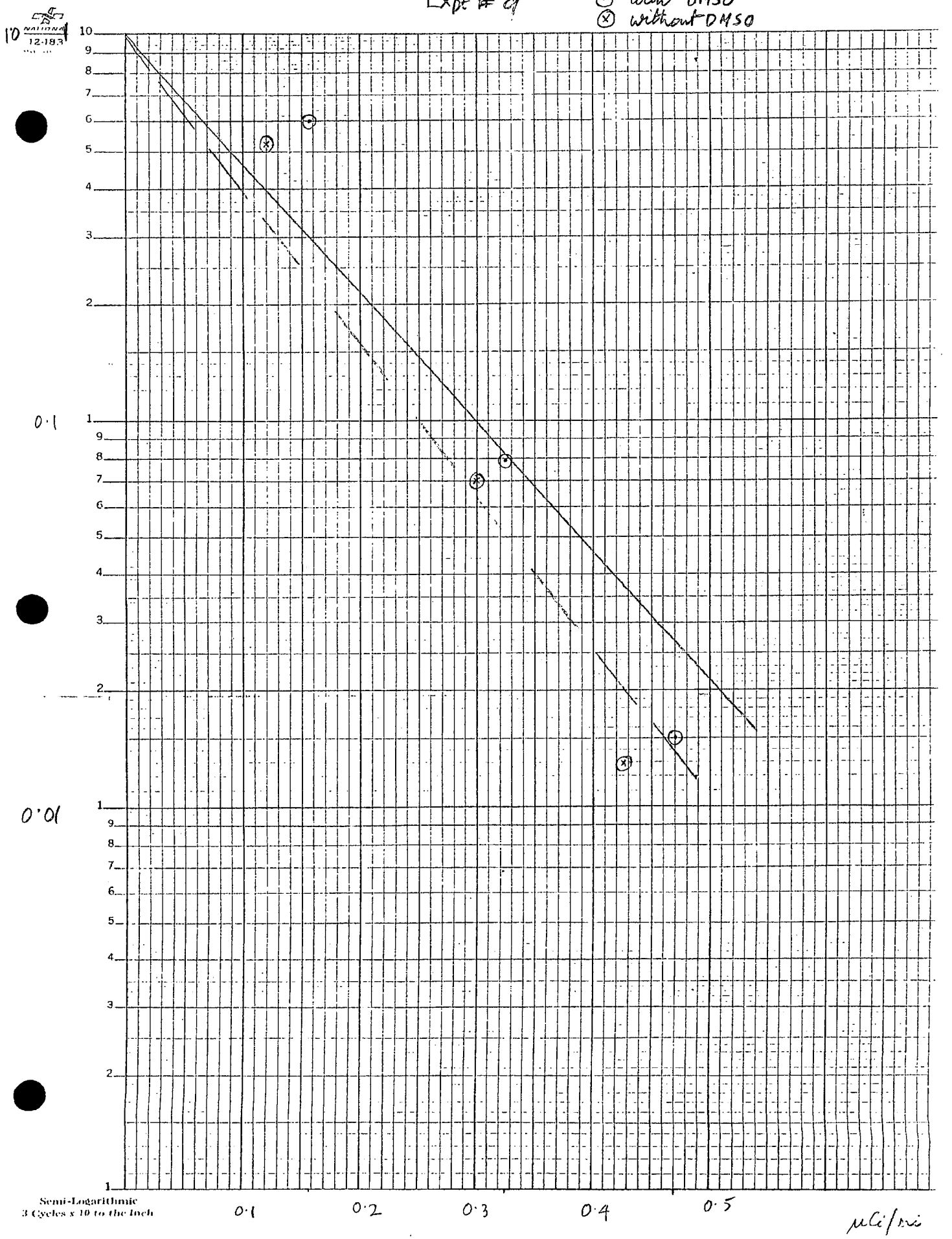
Semi-Logarithmic
3 Cycles x 10 to the inch


Expt # 9
⊙ with DMSO
⊗ without DMSO
10
9
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7
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3
2
0·1
1
0·01
0·1
0·2
0·3
0·4
0·5
μCi/ml
Semi-Logarithmic
3 Cycles x 10 to the Inch

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