

**V79 COLONY FORMING ASSAY**

**Experiment Name :**  $^{131}\text{IUdR}$  + 5% DMSO; **Exp. # :** 8; **Investigator:** A. Bishayee  
**Date:** 11/20/97

1. Set the rocker-roller at 37°C incubator, set the Coulter Counter, wash cells (from 75 cm<sup>2</sup> 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
2. Dilute to ~400,000 cells/ml in MEMB (final volume 11 ml) [Actual count : 4,27,733 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<sub>2</sub> **Date/Time:** 11/20/97; 3-00 p.m.(t<sub>1</sub>)
5. Prepare MEMB containing radioactivity in hood  
 80 µl  $^{131}\text{IUdR}$  (prepared on 10/14/97) + 4.92 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:** 11/20/97; 7-30 p.m.(t<sub>2</sub>)

Tube #	$^{131}\text{IUdR}$ uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ $^{131}\text{IUdR}$ (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	1.0	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:** 11/23/97; 7-45 p.m.(t<sub>3</sub>)

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: 11/21/97; 9-00 a.m.(t<sub>4</sub>)
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: 11/21/97; 10-45 a.m.(t<sub>5</sub>)
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: 11/21/97; 11-00 a.m.(t<sub>6</sub>)
21. After 72 h, remove tubes and place on ice, add 8 ml ice cold wash MEMA. Date/Time: 11/24/97; 9-30 a.m.(t<sub>7</sub>)
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 transferring 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  $\mu$ l 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 : 11/24/97; 2-45 p.m. (t,)**
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.

Exp. #8

(A)

11/20/97

Initial Cell count = 3467, 3520, 3518

Avg. Cell count = 3501.6

Cell conc. =  $3501.6 \times 400 = 1,400,666.7/\text{ml}$

For dilution

$$\begin{aligned} \text{Vol. of original cell suspension taken} &= \frac{4400000}{1400666.7} \\ &= 3.141 \text{ ml} \end{aligned}$$

3.141 ml of Cell Suspension + 7.858 ml MEMB = 11 ml

After dilution,

Final count = 1216, 1150, 1046, 1012

Avg. count = 1069.3

Cell conc. =  $1069.3 \times 400 = 4,27,733 \text{ cells/ml}$

Preparation of MEMB with  $^{131}\text{I}$  Udr

Stock  $^{131}\text{I}$  Udr 1.0  $\mu\text{Ci}/\text{ml}$  on 10/14/97 at 9-30 p.m.  
0.0412  $\mu\text{Ci}/\text{ml}$  11/20/97 at 7-30 p.m.

$$\begin{aligned}
 A_t &= A_0 \times e^{-\lambda t} \\
 &= A_0 \times e^{-\frac{0.693}{8.02} \times 36.91} \\
 &= A_0 \times e^{-3.189} \\
 &= A_0 \times 0.0412
 \end{aligned}$$

$$\begin{aligned}
 t &= 36 \text{ days} + 22 \text{ h} \\
 &= 36 + \frac{22}{24} \\
 &= 36.91 \text{ days}
 \end{aligned}$$

Prepare 5 ml of 1.2  $\mu\text{Ci}/\text{ml} \Rightarrow$  6.0  $\mu\text{Ci}$  needed

$$\begin{aligned}
 \text{Stock} \quad 0.0412 \mu\text{Ci} &- 1 \mu\text{l} \\
 &\quad \quad \quad \quad \quad \quad \frac{1}{0.0412} \\
 6.0 \quad \quad \quad \quad &\quad \quad \quad \frac{6}{0.0412} = 145.6 \mu\text{l} \\
 &\quad \quad \quad \quad \quad \quad = 0.146 \text{ ml}
 \end{aligned}$$

Take 0.146 ml stock  $^{131}\text{I}$  UDR, wait 3 hr at RT  
 Add 4.854 ml MEMB in hood.

1393  
1059  
1320

982

Mark

Expt # 8

⑥

Tube #	Medium Count (Cpm) at 11-00 a.m. of 11/21/97	Avg. Cpm	Avg. dpm
1	0, 2, 0	0	0
2	1, 2, 1	0	0
3	597, 680, 695	657.3	4629.10
4	1412, 1381, 1478	1423.66	10025.82
5	2013, 1457, 2209, 2112	2111.33	14868.54
6	0, -2	0	0
7	0, 1	0	0
8	565, 598, 665,	609.33	4291.07
9	1255, 1359, 1274,	1296	9126.76
10	1898, 1896, 2151	1981.6	13955.39

Tube #	$\mu\text{Ci/ml}$ at 11-00 a.m. of 11/21/97 (66) [ $\frac{\text{dpm} \times 100}{22200 \cdot 142}$ ]	$\mu\text{Ci/ml}$ at 7:30 p.m. 11/20/97 (62) [ $\div 0.9459$ ]
1	0	0
2	0	0
3	0.208	0.219
4	0.451	0.476
5	0.669	0.707
6	0	0
7	0	0
8	0.193	0.204
9	0.411	0.434
10	0.628	0.669

Tube #	Coulter count (for 100 $\mu$ l cell suspension)	Avg. Count	Cells/ml (Avg. count $\times$ 400)
1	568, 555, 533	552	2,20,800
2	570, 512, 500	527.3	2,10,933.3
3	543, 562, 545	550	2,20,000 ✓
4	672, 650, 660	660.6	2,64,266.6 ✓
5	635, 649, 655	646.3	2,58,533.3 ✓
6	557, 549, 572	559.3	2,23,733.3
7	636, 609, 634	626.3	2,50,533.3
8	585, 542, 524	550.3	2,20,133.3 ✓
9	698, 675, 680	684.3	2,73,733.3 ✓
10	498, 512, 475	495.0	1,98,000 ✓

11/24/97

Tube #	Radioactivity (cpm) for 300 $\mu$ l cell suspension at 2-45 p.m. of 11/24/97	Avg. Cpm	dpm (cpm / 0.142)
1	1, 1, 1	0	0
2	3, 1, -1	0	0
3	1330, 1302, 1345	1325.66	9335.63
4	1858, 1828, 1813	1833	12908.45
5	2609, 2667, 2673	2649.66	18659.62
6	-1, 1, 0	0	
7	0, 0, 0	0	
8	1565, 1579, 1533	1559	10978.87
9	2283, 2294, 2330	2302.33	16213.61
10	2724, 2783, 2737	2748	19352.11



dpm  
666080  
↑  
Expt # 8

$$e = \frac{0.693 \times 77.25}{193.2} \text{ (8)}$$

$$= 0.7566$$

Tube #	$\mu\text{Ci/ml}$ at 2-45 pm. of 11/24	$\mu\text{Ci/ml}$ at 9-00 am. of 11/21 ( $\div 0.7566$ )
1	0	0
2	0	0
3	0.0140	0.0185
4	0.0193	0.0256
5	0.0280	0.0370
6	0	0
7	0	0
8	0.0164	0.0217
9	0.0243	0.0321
10	0.0290	0.0384

Tube #	$\text{pci/cell}$ at 2-45 of 11/24	$\frac{\mu\text{Ci/ml}}{\text{cell/ml}} \times 10^6$ $\text{pci/cell}$ ↑ at 9-00 a.m. of 11/21
1		0
2		0
3		0.0840
4		0.0968 ✓
5		0.1431
6		0
7		0
8		0.0985
9		0.1172
10		0.1939

12/1/97

Expt # 8

Division	# of Colonies	Avg. Colonies (for 2.2)	SF
1.2	74, 100, 79	84.3	82.95
2.2	85, 90, 70	81.6	
3.2	38, 32, 44	38	0.4581
4.2	28, 41, 26	31.6	0.3809
5.2	28, 29, 27	28	0.3375
6.2	103, 91, 123	105.6	108.9
7.2	114, 120, 103	112.3	
8.2	26, 25, 24	25	0.2356
9.3	160, 162, 170	16.4	0.1505
10.3	104, 103, 100	10.2	0.0936

Exp # 8

○ with DMSO  
△ without DMSO

AF  
SERIAL  
2-183

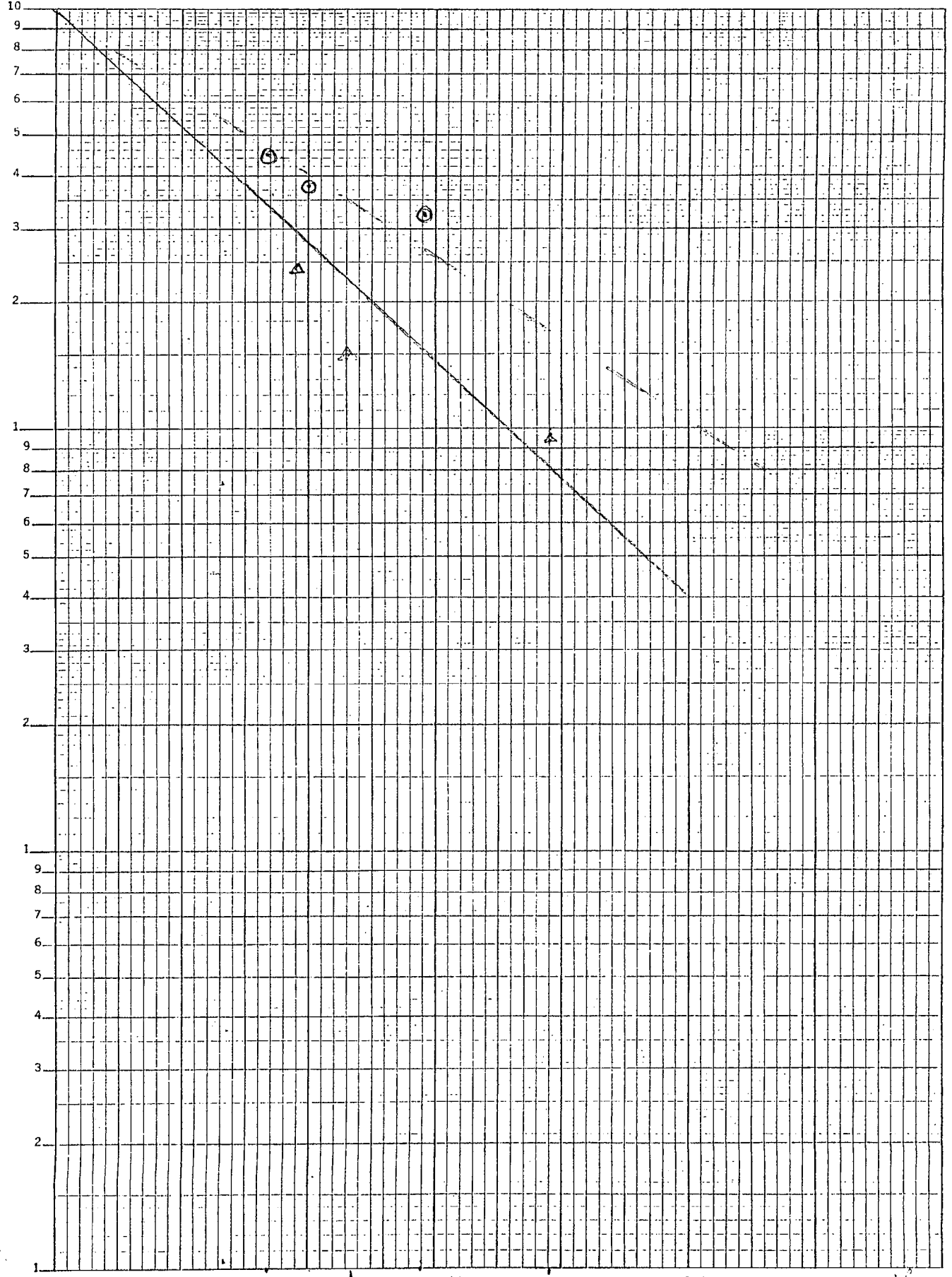
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Survival fraction

0.01

0.001

Semi-Logarithmic  
cycles x 10 to the inch



0.25

0.10

0.15

0.20

0.25

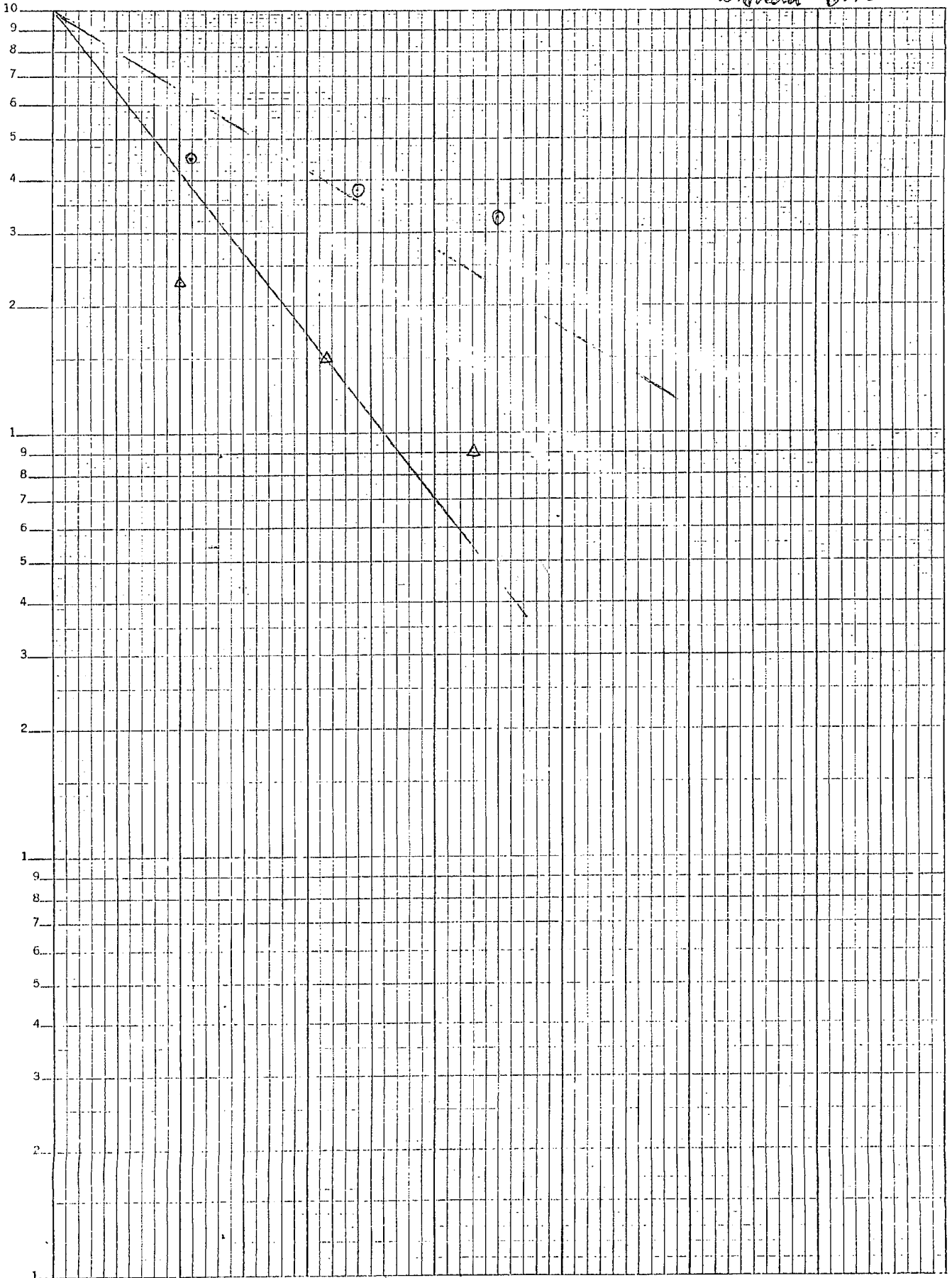
PC

Expt #8

○ with DMSO  
△ without DMSO

3183

Survival Fraction



Semi-Logarithmic  
cycles x 10 to the inch

0.2 0.4 0.6 0.8

μCi/2