V79 COLONY FORMING ASSAY

Experiment Name : ^{131}IUdR + 5% DMSO; **Exp. # :** 7; **Investigator:** A. Bishayee
Date: 11/13/97

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
2. Dilute to ~400,000 cells/ml in MEMB (final volume 11 ml) [Actual count : 4,41,200 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: 11/13/97; 3-10 p.m.(t_1)**
5. Prepare MEMB containing radioactivity in hood
   80 µl ^{131}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: 11/13/97; 6-50 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: 11/13/97; 7-00 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 centriuge)***. **Date/Time:11/14/97; 9-45 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: 11/14/97; 12-30 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: 11/17/97; 2-30 a.m.(t_6)** ✓
21. After 72 h, remove tubes and **place on ice**, add 8 ml ***ice cold*** wash MEMA. **Date/Time: 11/17/97; 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 5 times through 3cc syringe
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 30 gamma tubes
33. Incubate petridishes for 1 week
34. Count gamma tubes for radioactivity **Date/Time : 11/17/97; 3-10 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.

Exp #7

11/13/97

Initial Cell count (100ul) = 3260, 3448, 3456

Avg. Cell count = 3388

Cell Conc. = 3388 × 400 = 1355200 Cells/ml

For dilusion,

Vol. of original Cell Suspension taken = $\frac{4400000}{1355200}$

= 3.246 ml

3.246 ml Cell suspension + 7.754 ml of MEM B = 11 ml

After dilusion,

Final count = ~~1072, 1048, 901~~ 1127, 1141, 1041

Avg. count = 1103

Cell Conc = 1103 × 400 = 4, 41, 200 Cells/ml

Exp. #7

11/13/97

Preparation of MEMB with ^{131}IUdR

Stock ^{131}IUdR 1.0 μCi/μl on 10/14/97 at 9-30 p.m.

0.0754 μCi/μl 11/13/97 at 7-30 p.m.

$A_t = A_0 \times e^{-\lambda t}$

$= 1.0 \times e^{-\frac{0.693 \times 29.91}{8.02}}$

$= 1.0 \times e^{-2.584}$

$= 1.0 \times 0.0754$

$= \underline{0.0754}$ μCi/μl

$t = 29 \text{ days} + 22\text{h}$

$= 29 + \frac{22}{24}$

$= 29.91$ days

$t_{1/2} = 8.02$ days

Prepare 5 ml of 1.2 μCi/ml ⇒ 6 μCi needed

Stock 0.0754 μCi from 1 μl

" " $\frac{1}{0.0754}$

6 μCi : $\frac{6}{0.0754} = 79.5$ μl.

Take 80 μl of stock ^{131}IUdR, stand on RT at 3h } in hood

Add 4.920 ml of MEMB }

Expt - #7

11/13/97 - 6.50 p.m.
11/17/97 - 2.30 p.m.

72 + 12 + 7½

Tube #	Medium Count (cpm) at 2-30 p.m of 11/17	Avg cpm	Avg dpm
1	0, 0	0	0
2	1, 0	0	0
3	474, 521, 563	519.3	3657.27
4	1035, 1206, 1196	1145.6	8068.07
5	1656, 1833, 1525	1671.3	11769.95
6	0, -1	0	0
7	3, 0	0	0
8	489, 504, 487	493.3	3474.17
9	1146, 1023, 1083	1084	7633.80
10	1837, 1783, 1559	1726.3	12157.27

$$A_0 = \frac{A_t}{e^{-\frac{0.693 \times 91.5}{193.2}}} = \frac{A_t}{0.720}$$

Tube #	μCi/ml at 2-30 p.m of 11/17/97	μCi/ml at 6-50pm on 11/13/97
1	0	0
2	0	0
3	0.164	0.227
4	0.363	0.504
5	0.530	0.735
6	0	0
7	0	0
8	0.156	0.216
9	0.343	0.476
10	0.547	0.759

~~Marshall~~

Exp. #7

Tube #	Coulter count (for 100 ul cell suspension)	Avg cell count	Cells/ml (Avg. count × 400)
1	857, 855, 870	860.6	3,44,266.6
2	832, 859, 860	850.3	3,40,133.3
3	827, 814, 755	798.6	3,19,466.6
4	854, 852, 882	862.6	3,45,066.6
5	721, 733, 760	738	2,95,200
6	859, 845, 827	843.6	3,37,466.6
7	884, 872, 796	850.6	3,40,266.6
8	910, 880, 893	894.3	3,57,733.3
9	836, 865, 809	836.6	3,34,666.6
10	735, 755, 754	748	2,99,200

Tube #	Radioactivity (cpm) for 300 ul cell suspension ~~on~~ 3-10 pm on 11/17/97	Avg cpm	dpm (Avg. cpm × 1/0.142)
1	-1, 1, 1	0	0
2	0, 0	0	0
3	1845, 1853, 2000	1899.3	13375.5
4	3360, 3204, 3337	3300.3	23,241.7
5	3459, 3481, 3553	3497.6	24,631.4
6	0, 0	0	0
7	0, 0	0	0
8	2674, 2745, 2826	2748.3	19,354.4
9	4177, 4305, 4439	4307	30,330.9
10	4798, 4770, 4605	4724.3	33,269.9

Exp#7

$e^{-\frac{0.693 \times 7.75}{19.3 - 2}} = 0.757$

dpm / 666000 ↑

Tube #	μCi/~~ml~~	pCi/cell at 3-10 pm on 11/17	pCi/cell at 9-45 a.m. on 11/14 (÷ 0.757)
1	0	0	
2	0	0	
3	0.0200	0.0626	0.0826
4	0.0348	0.1008	0.1331 ✓
5	0.0369	0.1250	0.1650
6	0	0	0
7	0	0	0
8	0.0290	0.0810	0.1069
9	0.0455	0.1359	0.1794 ✓
10	0.0499	0.1667	0.2201

~~Plate #~~ (dilution) ~~# of Colonies~~ ~~Avg~~ ~~SI~~

Tube	μCi/ml at 9-45 a.m of 11/14
1	
2	
3	0.0264
4	0.0459
5	0.0484
6	
7	
8	0.0380
9	0.0597
10	0.0654

Expt #7 11/24/97

Dilution	# of colonies	Avg. Colonies (for x.2)		S/F
1.2	115, 98, 109	107.33	100.33	-
2.2	87, 45, 48	93.33		
3.2	41, 31, 38	36.66		0.3653 36×10^{-2}
4.3	146, 155 178	15.96		0.1590
5.3	112, 105, 104	10.70		0.1066
6.2	117, 143, 136	132	126.66	
7.2	117, 133, 114	121.33		
8.2	38, 57, 53	49.33		0.3894
9.3	170, 171, 176	17.23		0.1360
10.3	102, 108,	10.50		0.0828

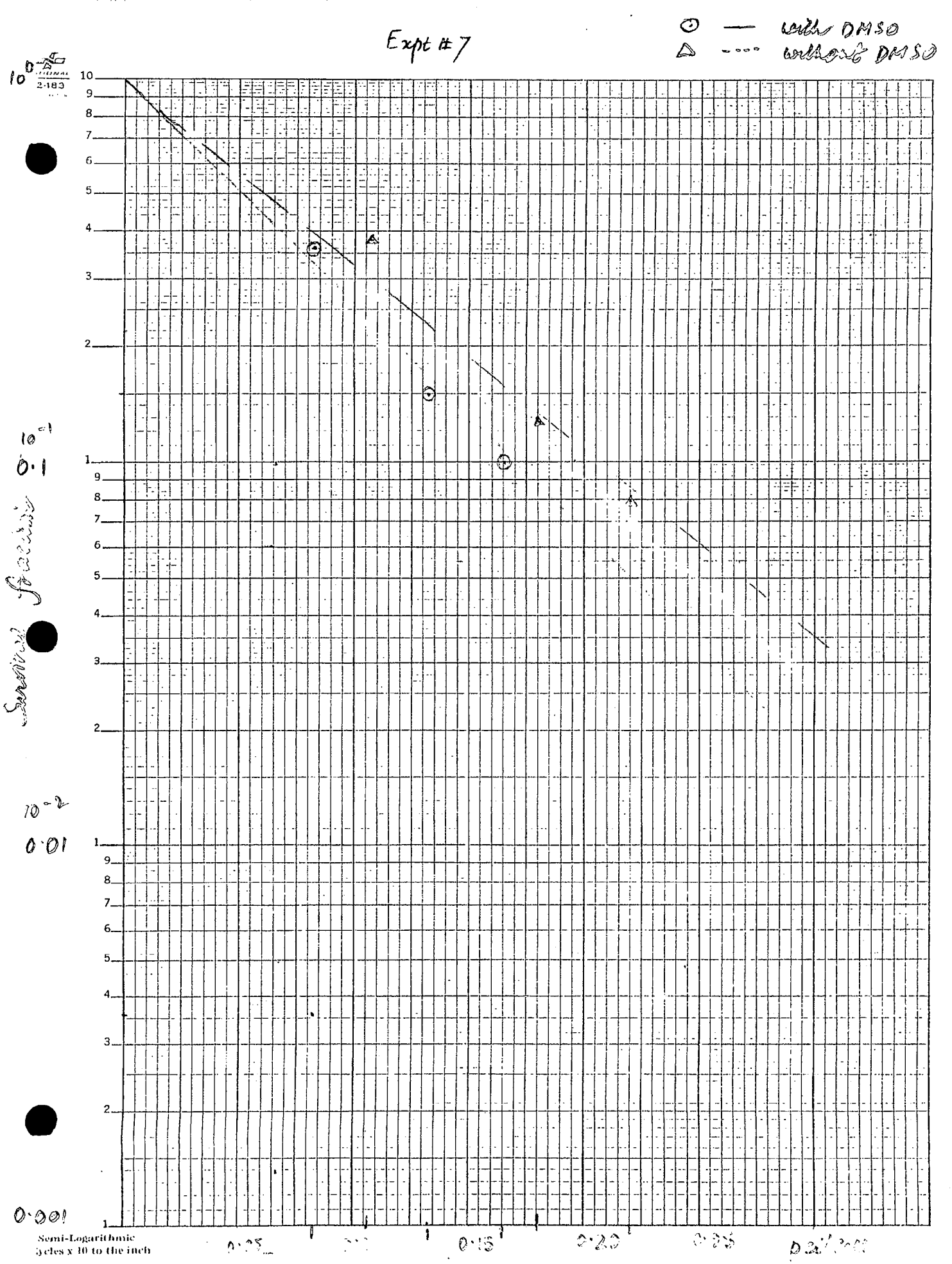
10 = 0.83

1 = 0.005 = 1 unit

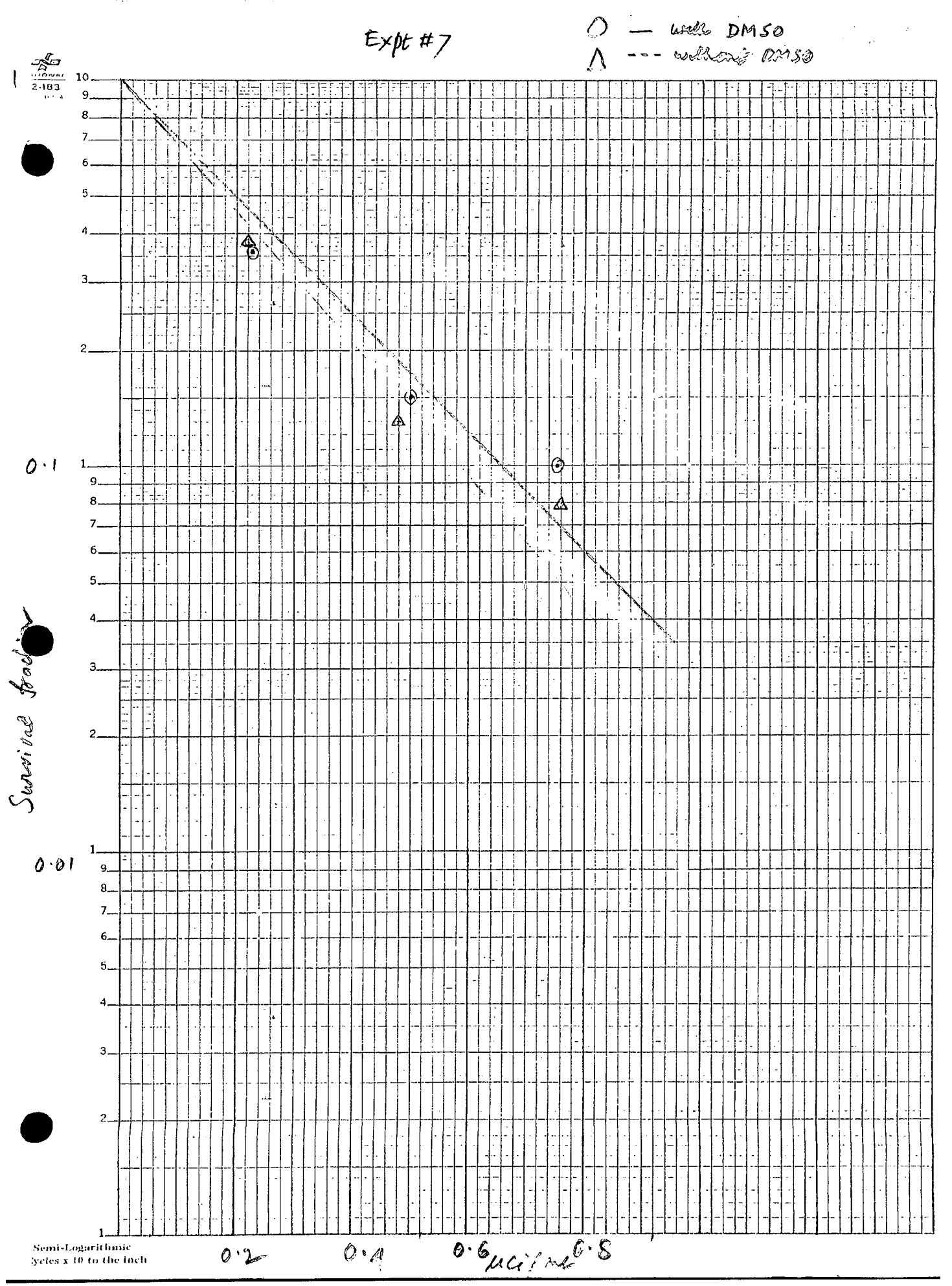
0.005 = 1

1 = 0.025

10.0 / 01 = 1 final = 10.0 = 0.01


Expt #7
⊙ — with DMSO
△ ---- without DMSO
Survival Fraction
Semi-Logarithmic
3 cles x 10 to the inch

B008434


Expt #7
○ — with DMSO
△ --- without DMSO
2-483
10
9
8
7
6
5
4
3
2
1
0·1
0·01
Surviving fraction
0·2
0·4
0·6 μCi/ml
0·8
Semi-Logarithmic
Cycles x 10 to the inch