V79 COLONY FORMING ASSAY

Experiment Name : ^{131}IUdR + 5-12.5 % DMSO; **Exp. # :** 2; **Investigator:** A. Bishayee
Date: 03/23/98

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 ~4,00,000 cells/ml in MEMB (final volume 11 ml) [Actual count : 4,53,200 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:** 03/23/98; 4-00 p.m.
5. Prepare MEMB containing radioactivity in hood
   24 µl ^{131}IUdR (prepared on 2/26/98) + 5 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:** 03/23/98; 7-30 p.m.

Tube #	^{131}IUdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{131}IUdR (ml) [1.2 uCi/ml]	MEMA+ 12.5 % DMSO (ml)	MEMA (ml)	DMSO Conc. (%)
1	0	1.0	1.0	0	0	2.0	0
2	0	1.0	1.0	0	0.8	1.2	5
3	0	1.0	1.0	0	1.2	0.8	7.5
4	0	1.0	1.0	0	1.6	0.4	10
5	0	1.0	1.0	0	2.0	0	12.5
6	0.4	1.0	0.33	0.67	0	2.0	0
7	0.4	1.0	0.33	0.67	0.8	1.2	5
8	0.4	1.O	0.33	0.67	1.2	0.8	7.5
9	0.4	1.0	0.33	0.67	1.6	0.4	10
10	0.4	1.0	0.33	0.67	2.0	0	12.5

7. Return test tubes to roller for 12 h, increase the elevation angle of the roller.
   Date/Time: 03/23/98; 7-45 p.m.

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 for 10 min (***precooled centrifuge***). **Date/Time:** 03/24/98; 9-00 a.m.
10. **During centrifugation, move roller to 10°C and obtain ice**
11. Prepare 14 ml of 12.5 % DMSO) in 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 containing 0 and/or 12.5 % DMSO as per Table. Keep on ice!
20. Transfer tubes to roller at 10°C for 72 h. **Date/Time:** 03/24/98; 11-00 a.m.
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: 03/24/98; 11-00 a.m.
21. After 72 h, remove tubes and **place on ice**, add 8 ml ***ice cold*** wash MEMA.

    Date/Time: 03/27/98; 10-15 a.m.
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 :** 03/27/98; 5-30 p.m.
35. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with crystal violet
36. Count colonies (50 or more cells). There must be between 25 and 250 colonies for the flask to be a valid data point.

IUdR + 5-12.5% DMSO

Expt #2

03/23/98

Initial count = 6314, 6129, 6050,
Avg. Cell count = 6164
Cell conc. = 6164 × 400
= 2465733 cells/ml.

For dilution,
Vol. of cell suspension taken = $\frac{4400000}{2465733}$
= 1.78 ml.

Take 1.78 ml Cell suspension + 9.22 ml MEMB = 11 ml

After dilution,
Final count = 1139, ~~1079~~, 1154, 1106
Avg. Count = 1133
Cell conc. = 1133 × 400
= 4,53,200 cells/ml.

Prepare 5 ml ~~of~~ 1·2 μCi/ml ^{131}IUdR in MEMB.
i.e. 6 μCi required

Stock

on 02/26/98 at 4-00 pm = 2·15 μCi/μl

0·24 μCi/μl

on 03/23/98 at 7-00 pm = ~~0·79~~ μCi/μl

$25 \times \cancel{24} + 3$ h

= ~~278h~~ = 603

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

$= 2{\cdot}15 \times e^{-\frac{0{\cdot}693 \times \overset{603}{\cancel{278}}}{193{\cdot}2}}$

$= 2{\cdot}15 \times e^{-\cancel{0{\cdot}997}\ 2{\cdot}16}$

$= 2{\cdot}15 \times \cancel{0{\cdot}3689}\ 0{\cdot}114 = 0{\cdot}247$

= ~~0·799 μCi/μl~~

Stock required = $\frac{6}{\cancel{0{\cdot}799}\ 0{\cdot}247}$ = ~~7·56 ml~~ 24·2 μl.

~~① Take 5 ml MEMB~~

② ~~Add~~ Take ~~7·56~~ $\overset{24}{\ }$ μl ^{131}IUdR from Stock, Keep at RT

③ Add 5 ml MEMB.

TABLE-1

Expt. # : 2 **Date/Time :** 03/24/98; 11-00 a.m

Tube #	Medium count for 10 ul (cpm)	Avg. cpm	dpm [cpm/0.142]	µCi/ml (A_t) on counting [dpm/22200]	µCi/ml (A_o) on addition [$A_t/e^{-\lambda t}$]
1	0, 0	0	0	0	0
2	0, 1	0	0	0	0
3	0, 0	0	0	0	0
4	2, 0	0	0	0	0
5	1, 1	0	0	0	0
6	869, 869, 891	876·3	6171·3	0·2779	0·2938
7	852, 865, 857	858·0	6042·2	0·2721	0·2877
8	846, 934, 902	894	6295·7	0·2835	0·2998
9	794, 939, 872	868·3	6115·0	0·2754	0·2911
10	929, 867, 874	890	6267·6	0·2823	0·2984

03/23/98; 7-30 p.m.

12hr + 3.5hr

= 15.5 h

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 15.5}{193.2}}$

$= e^{-0.055}$

$= 0.9459$

TABLE-2

Expt. # : 2 **Date/Time :** 03/27/98 ; 5-30 p.m.

Tube #	Radioactivity for 300 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.142]	µCi/ml (A_t) on counting [dpm/666000]	µCi/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1	0, 0	0	0	0	0
2	0, 0	0	0	0	0
3	0, 0	0	0	0	0
4	0, 0	0	0	0	0
5	0, 0	0	0	0	0
6	3329, 3284, 3322	3311·6	23321·5	0·0350	0·0467
7	3731, 3947, 3672	3783·3	26643·1	0·0400	0·0533
8	3182, 3327, 3186	3231·6	22758·2	0·0341	0·0456
9	3317, 3259, 3232	3269·3	23023·4	0·0345	0·0461
10	3489, 3450, 3229	3389·3	23868·5	0·0358	0·0478

03/24/98 ; 9-00 a.m.

72h + 8.5 h

= 80.5

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 80.5}{193.2}}$

$= e^{-0.288}$

$= 0.7491$

TABLE-3

Expt. # : 2 **Date/Time :** 03/27/98 ; 11-30 a.m.

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 400]	pCi/cell [uCi/cell x10^6 Cells/ml]
1	~~544~~, 464, 461, 449	458·0	183200	0
2	488, 512, 479	493	197200	0
3	617, 609, 619	615	246000	0
4	664, 668, 651	661	264400	0
5	647, 654, ~~577~~ 632, 582	644·3	257733·3	0
6	587, 572, 569	576	230400	0·2026
7	687, 689, 617	664·3	265733·3	0·2005
8	626, 595, 624	615	246000	0·1853
9	640, 622, 606	622	249066	0·1850
10	645, 652, 628	641·6	256666	0·1862

TABLE-4

Expt. #: 2 Date: 04/03/98

Colony Counts and Survival Fraction

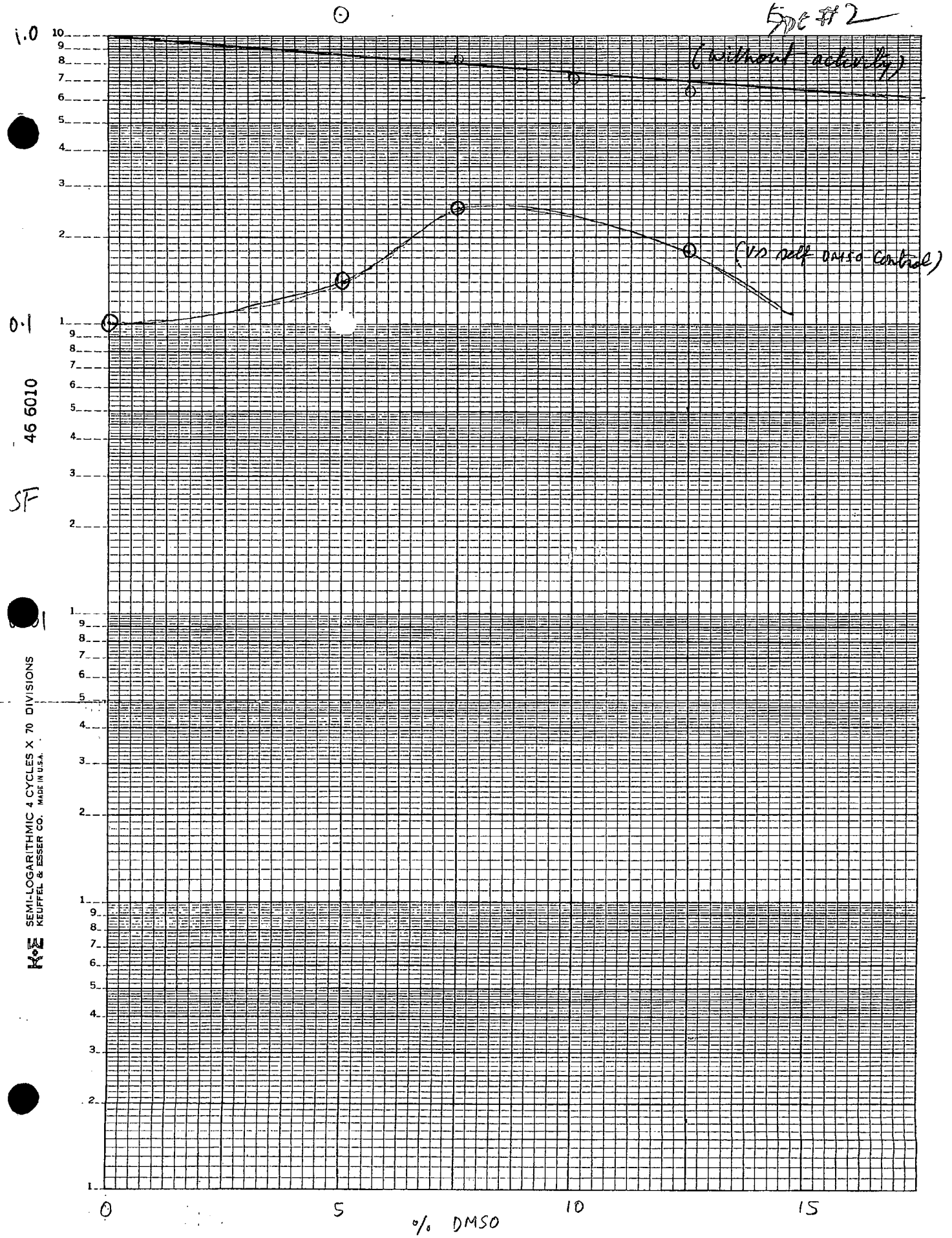
Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony for X.2	SF
1.2	95	105	119	106.33	
2.2	121	116	125	120.66	1.13
3.2	90	88	82	86.66	0.8150
4.2	80	72	76	76	0.7147
5.2	72	68	65	68.33	0.6426
6.3	110	106	112	10.9	0.1025
7.3	183	178	172	17.76	0.1670
8.3	220	216	230	22	0.2087
9.3	195	199	211	20.16	0.1896
10.3	127	121	129	12.56	0.1181

SF (6.3/1.2)

SF (vs self control)

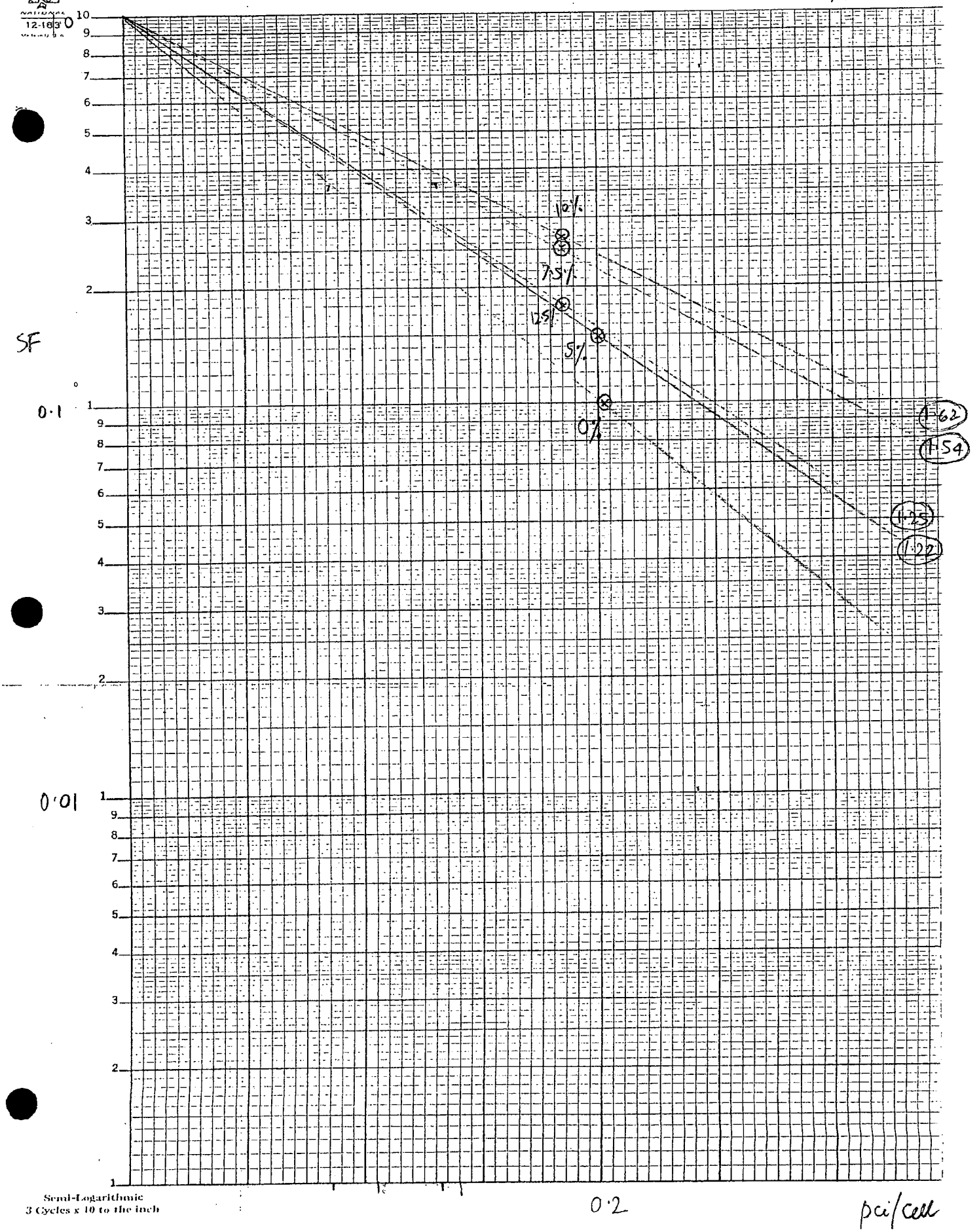
DMF

% DMSO		
0	0.1025 (6.3/1.2)	1.00
5	0.1471	~~0.143~~ 1.22
7.5	0.2538	~~2.47~~ 1.54
10	0.2652	~~2.58~~ 1.62
12.5	0.1838	~~1.79~~ 1.25


Exp #2
(without activity)
(vs self DMSO control)
1.0
0.1
46 6010
SF
SEMI-LOGARITHMIC 4 CYCLES X 70 DIVISIONS
KEUFFEL & ESSER CO. MADE IN U.S.A.
0
5
10
15
% DMSO

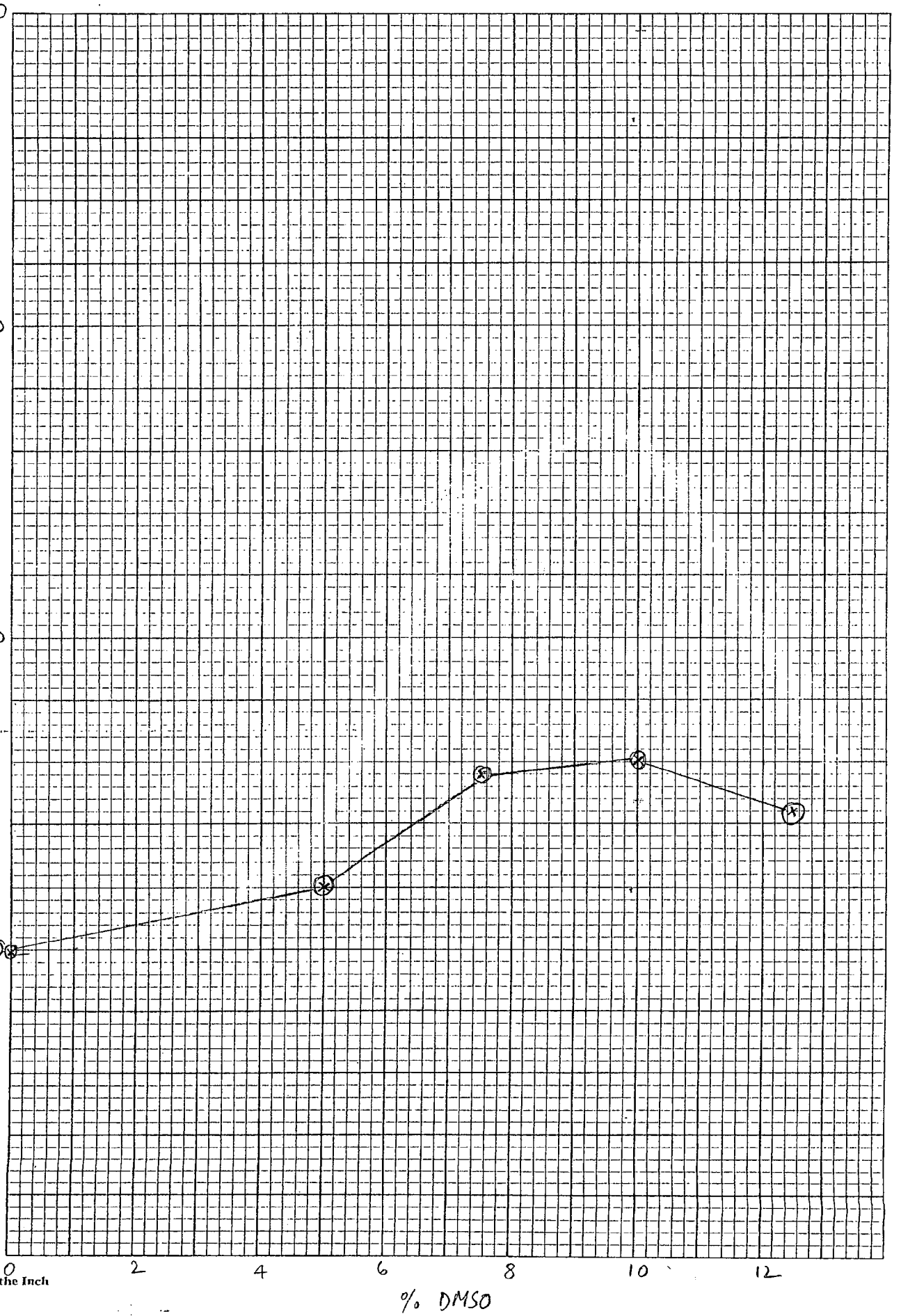
131 IUdR + 5–12.5% DMSO

Expt #2



Expt #2

NATIONAL
12-280
Made in U.S.A.



10 Squares to the Inch

B008318