V79 COLONY FORMING ASSAY

Experiment Name : ^{131}IUdR + 5-12.5 % DMSO; **Exp. # :** 1; **Investigator:** A. Bishayee
Date: 03/09/98

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 : 481466 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:** 03/09/98; 4-00 p.m.
5. Prepare MEMB containing radioactivity in hood
   7.2 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:** 03/09/98; 7-15 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	C(--)
2	0	1.0	1.0	0	0.8	1.2	5	C(+)
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	C(+-)
7	0.4	1.0	0.33	0.67	0.8	1.2	5	
8	0.4	1.0	0.33	0.67	1.2	0.8	7.5	C(++)
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: 03/09/98; 7-30 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:** 03/10/98; 8-45 a.m.
10. **During centrifugation, move roller to 10°C and obtain ice** DMSO = 1.75 ml MEMA = 12.25 ml
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:** 03/10/98; 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: 03/10/98; 11-30 a.m.
21. After 72 h, remove tubes and **place on ice**, add 8 ml ***ice cold*** wash MEMA.

    Date/Time: 03/13/98; 10-30 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 :** 03/13/98 ; 5 pm.
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.

Dilution

1·2
2·2
3·2, 3·3, 3·4
4·2, 4·3, 4·4
5·2, 5·3, 5·4
6·2, 6·3,
7·2, 7·3
8·2, 8·3, 8·4
9·2, 9·3, 9·4
~~10·2~~, 10·3, 10·4

IVdR + 5-125/ DMSO

Expt. 1

03/09/98

Initial Cell count = 1273, 1311, 1342
Avg. Cell Count = 1308.66
Cell Conc. = 1308.66 × 4000
= 5234666 Cells/ml

For dilution,

Vol. of original Cell suspension taken = $\frac{4400000}{5234666}$

= 0.84 ml

Take 0.84 ml of cells + 10.16 ml MEMB = 11 ml

After dilution,

Final Count = 1226, 1232, 1153
Avg. Count = 1203
Cell conc. = 1203 × 400
= 4,81,466 Cells/ml

Expt. 1.

03/09/98

Prepare 5 ml of 1.2 μci/ml ^{131}I/UdR in MEMB
i.e 6 μci required

Stock,

on 02/26/98 at 4-00 pm = 2.15 μci/μl

on 03/09/98 at 7-00 p.m. = 0.83 μci/μl

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11 × 24 h + 3 h
= 267 h

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

$= 2.15 \times e^{-\frac{0.693 \times 267}{193.2}}$

$= 2.15 \times e^{-0.957}$

$= 2.15 \times 0.383$

$= 0.825$ μci/μl

$\simeq 0.83$ μci/μl

Stock required $= \frac{6}{0.83} = 7.22$ μl.

① Take 5 ml of MEMB

② Add 7.22 μl of stock ^{131}I/UdR

B008323

TABLE-1

Expt. # : 1 **Date/Time :** 03/10/98; 11-30 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	1, 1	0	0	0	0
2	0, 1	0	0	0	0
3	2, 1	0	0	0	0
4	1, 1	0	0	0	0
5	0, 1	0	0	0	0
6	1107, 1121, 982*	1070	7535.2	0.3397	0.3594
7	1106, 1131, 1115	1117.3	7868.5	0.3544	0.3753
8	1119, 1107, 1128	1118	7873.2	0.3546	0.3755
9	1090, 1153, 978	1073.6	7561.0	0.3405	0.3607
10	1153, 1154, 1190	1165.6	8208.9	0.3697	0.3915

* Sample was taken later

03/09/98; 7-30 pm

12h + 4h = 16

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 16}{193.2}}$

$= e^{-0.057}$

$= 0.944$

TABLE-2

Expt. # : 1 **Date/Time :** 03/13/98 ; 5-00 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, 1	0	0	0	0
2	1, 1	0	0	0	0
3	0, 1	0	0	0	0
4	0, 1	0	0	0	0
5	1, 1	0	0	0	0
6	4823, 4784, 4937	4848	34140·8	0·0512	0·0683
7	4754, 4846, 4805	4801·6	33814·5	0·0507	0·0677
8	3992, 4016, 3961	3989·6	28096·2	0·0421	0·0562
9	4921, 5002, 4922	4948·3	34847·4	0·0523	0·0697
10	4672, 4578, 4652	4634	32633·8	0·0489	0·0653

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 80.25}{193.2}}$

$= e^{-0.287}$

$= 0.749$

03/10/98, 8-45 a.m.

72h + 8·25

= 80·25h

TABLE-3

Expt. # : 1 **Date/Time :** 03/13/98 ; 4-00 p.m

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 400]	pCi/cell [uCi/cell x 10^6 / Cells/ml]
1	1398, 1378, 1344			0
2	1486, 1469, 1463			0
3	1472, 1553, 1473			0
4	1671, 1696, 1651			0
5	1613, 1622, 1588			0
6	1823, 1832, 1845	1833.3	733333.3	0.0931
7	1695, 1651, 1708	1684.6	673866.6	0.1004
8	1568, 1593, 1551	1570.6	628266.6	0.0894
9	1692, 1663, 1709	1688	675200	0.1032
10	1562, 1525, 1540	1542.3	616933.3	0.1058

1469 1398, 1378, 1344

1486, 1469, 1463

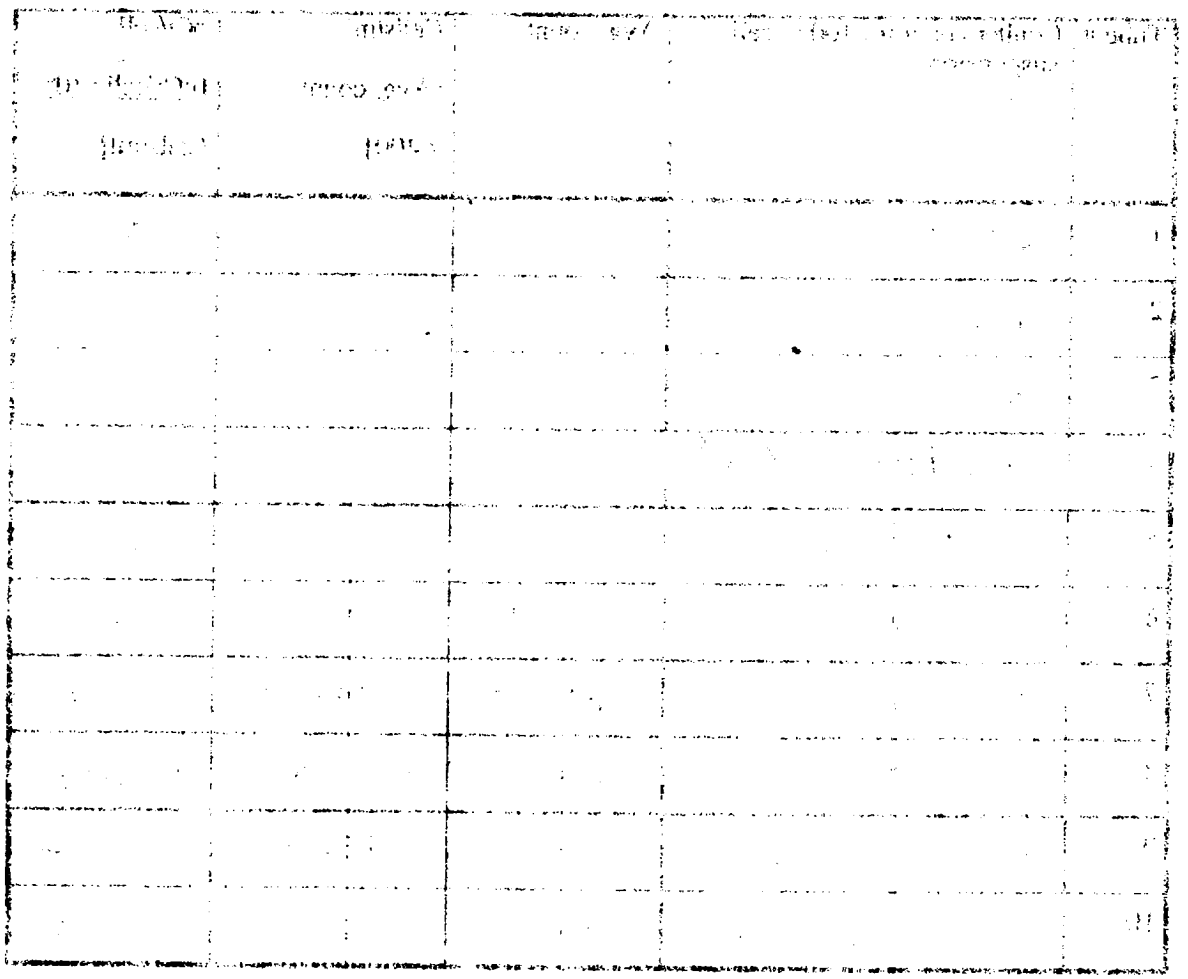


TABLE-4

Expt. #: 1 Date: 03/20/98

Colony Counts and Survival Fraction

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF	% increase
1·2	119	125	117	120·33		
2·2	135	139	130	134·66	1·11	
3·2	84	85	96	88·3	0·73	
4·2	79	78	78	78·33	0·65	
5·2	71	63	65	66·33	0·55	
6·3	59	54	47	5·3	0·044	
7·3	69	75	71	7·1	0·059	
8·3	130	127	133	13	0·108	
9·3	99	111	101	10·36	0·086	
10·3	33	38	37	3·6	0·0299	

SF (vs. 24h control)

0·044

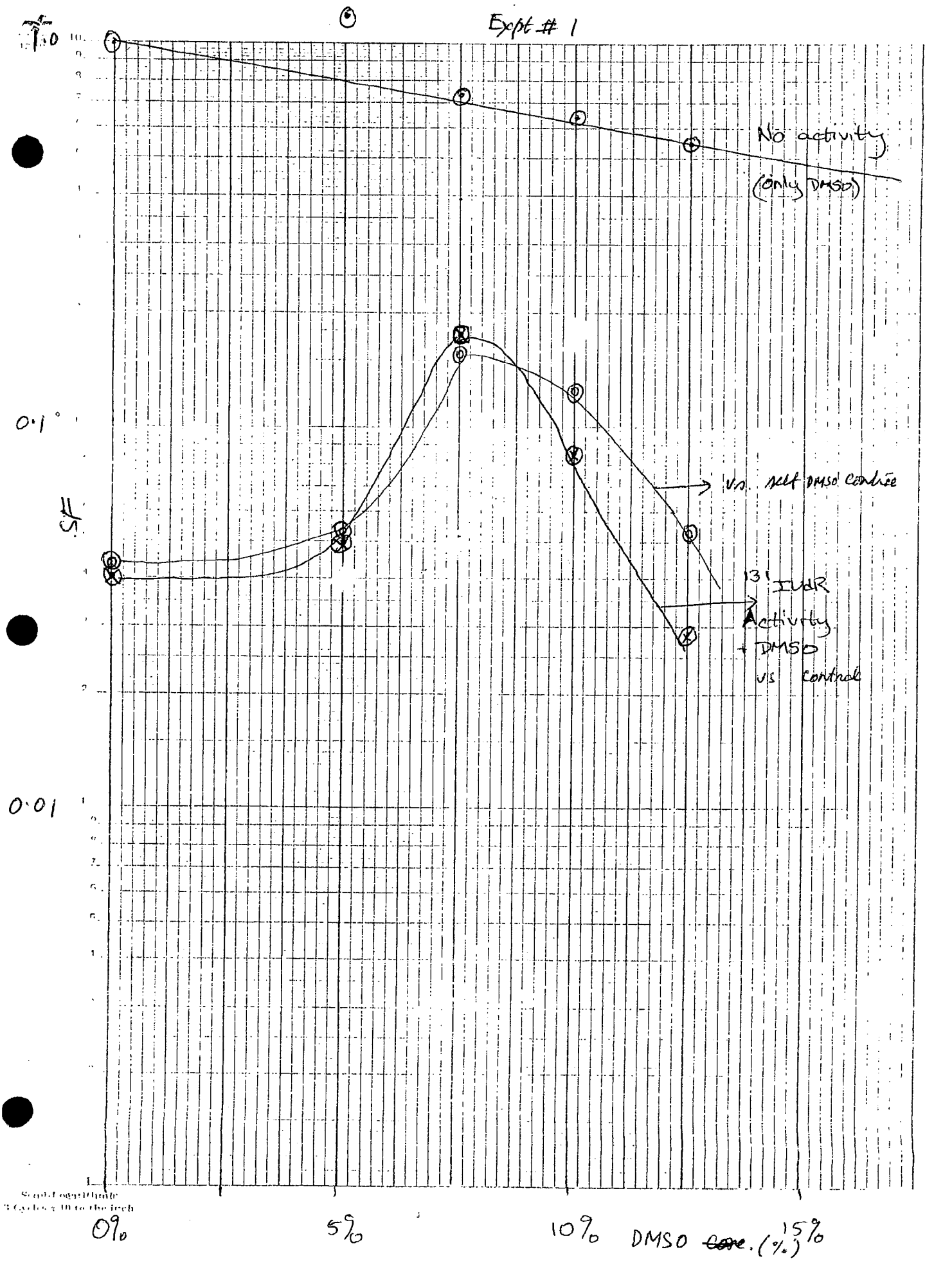
~~33%~~ 0·052

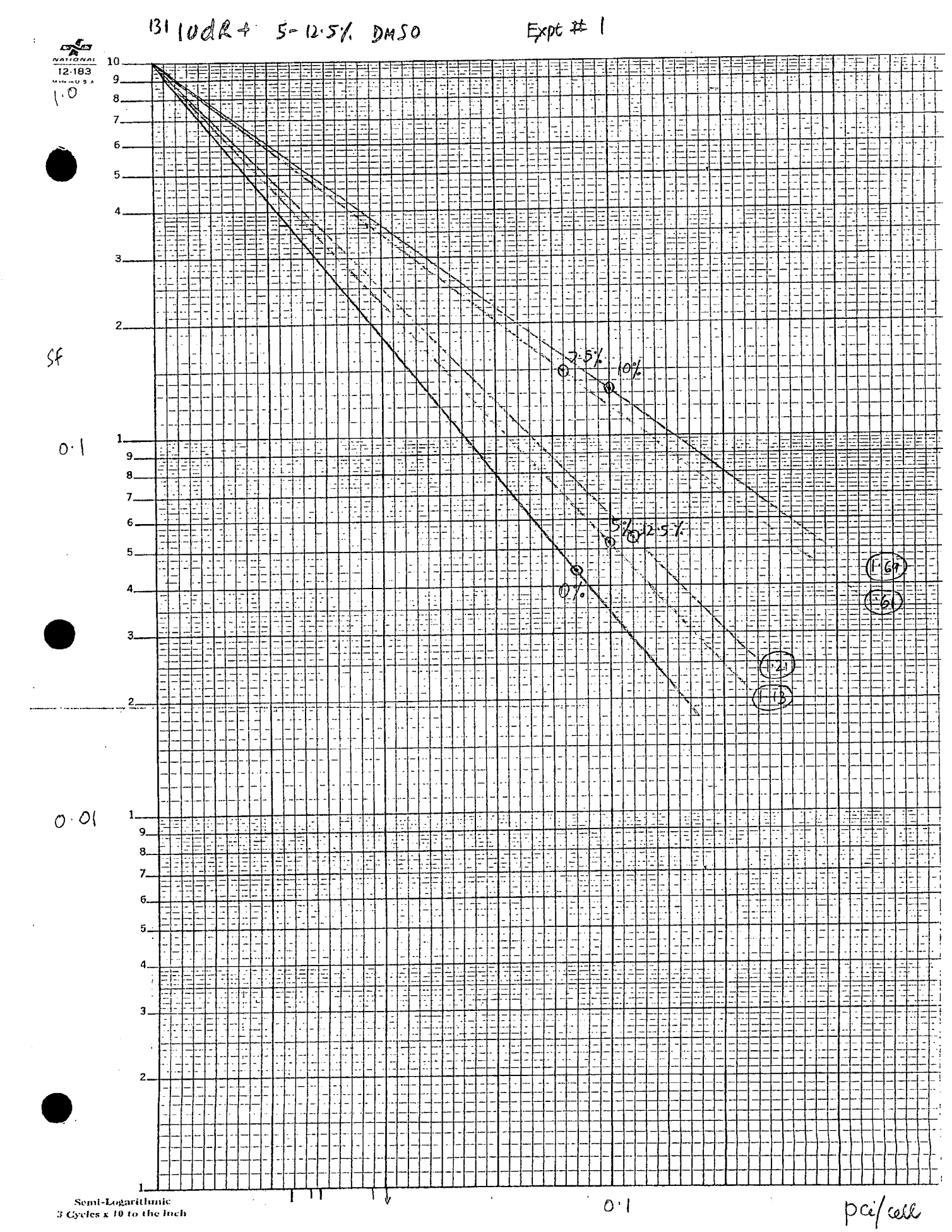
~~145%~~ 0·147

~~95%~~ 0·132

−32% 0·054

% DMSO	SF	DMF
0	0·044	
5	0·052	1·13
7·5	0·147	~~1·27~~ 1·61
10	0·132	~~1·67~~ 1·69
12·5	0·054	~~1·69~~ 1·21


Expt # 1
1.0
0.1
0.01
S/I
No activity
(only DMSO)
v.s. self DMSO control
131 IUdR
Activity
+ DMSO
vs control
0%
5%
10%
15%
DMSO (%)


131 10dR4 5-12.5% DMSO
Expt # 1
NATIONAL
12-183
1.0
SF
0.1
0.01
2.5%
10%
5%
12.5%
0%
1.69
1.61
1.21
1.13
Semi-Logarithmic
3 Cycles x 10 to the Inch
0.1
pci/cell

B008330

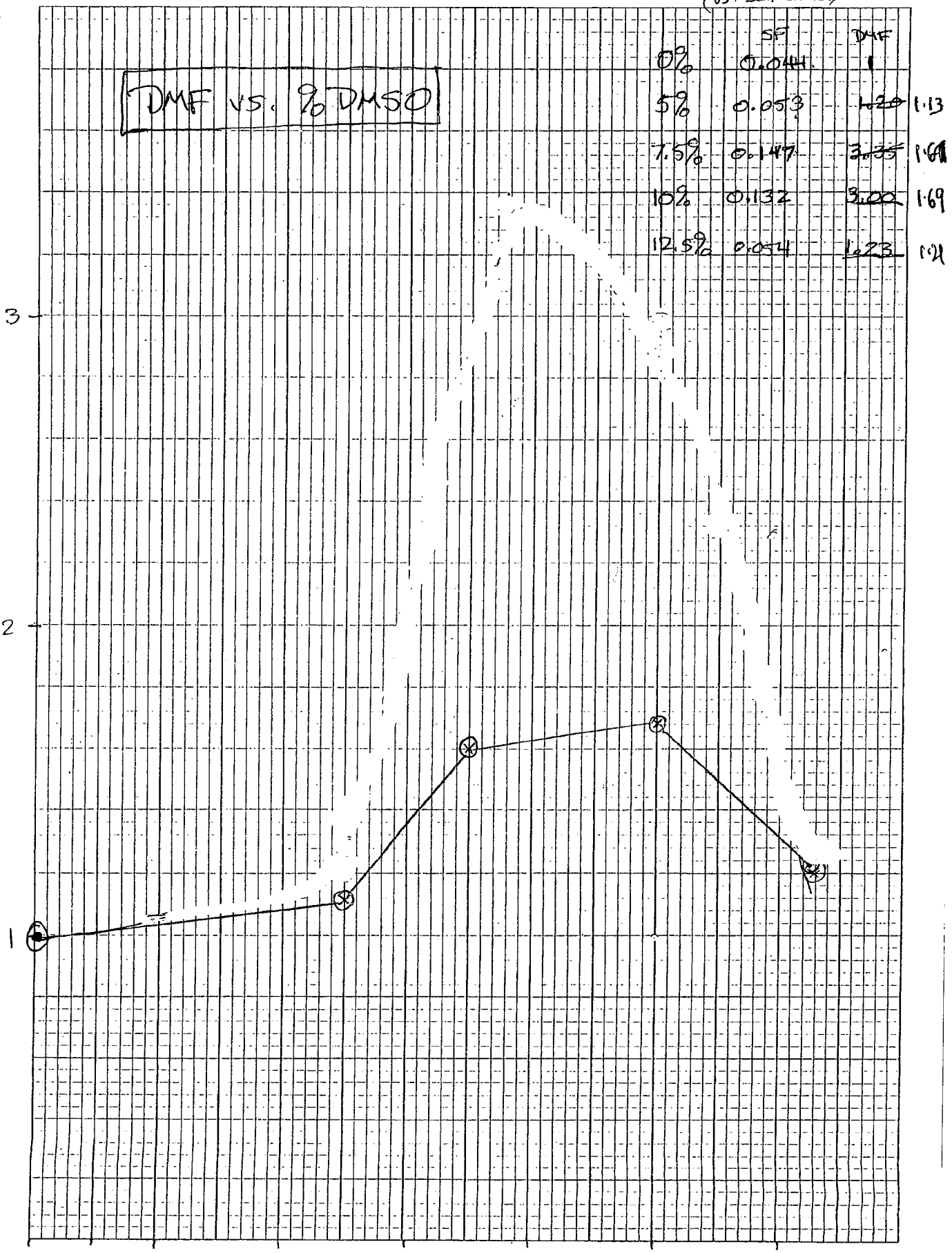
NATIONAL 12-280

Expt # 1

(vs. self-control)

DMF vs. % DMSO

	SE	DMF	
0%	0.044	1	
5%	0.053	~~1.20~~	1.13
7.5%	0.147	~~3.35~~	1.61
10%	0.132	~~3.00~~	1.69
12.5%	0.054	~~1.23~~	1.21



10 Squares to the Inch

B008331

12/24/97

^{131}IUdR + 5% DMSO

~~Ln Curve~~ Fits

Single Hit Multitarget Curve Fit

$\ln S = -n a - a_{0,37} = \frac{1}{n}$

Exp	$a_{0,37}$ (MBq/cell) DMSO	n	$a_{0,37}$ (MBq/cell) no DMSO	n	DMF
7	2.25	1.52	2.41	2.28	0.93
9	2.61	4.2	2.35	4.19	1.11
10	2.10	6.2	2.13	6.38	0.986
					1.0 ± 0.1

Ln Curve Fits

10 $a_{0,37}$ = 3.52 mBq/cell

1.0
.5
.25
.1
.05
.025
.01