V79 COLONY FORMING ASSAY

Experiment Name : ^{125}IUdR + MEA; **Exp. # :** 1; **Investigator:** A. Bishayee
Date: 04/09/98

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 : 4,54,200 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:** 04/09/98 ; 3-15 p.m.
5. Prepare MEMB containing radioactivity in hood

   2.6 µl ^{125}IUdR (prepared on 04/09/98) + 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:** 4/9/98; 7-30 p.m.

Tube #	^{125}IUdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{125}IUdR (ml) [1.0 uCi/ml]	MEMA+ MEA 100 ug/ml (1.3 mM)	MEMA	
1	0	1.0	1.0	0	2.0	0	
2	0	1.0	1.0	0	2.0	0	
3	0.01	1.0	0.98	0.02	2.0	0	
4	0.02	1.0	0.96	0.04	2.0	0	
5	0.03	1.0	0.94	0.06	2.0	0	
6	0	1.0	1.0	0	0	2.0	
7	0	1.0	1.0	0	0	2.0	
8	0.01	1.0	0.98	0.02	0	2.0	
9	0.02	1.0	0.96	0.04	0	2.0	
10	0.03	1.0	0.94	0.06	0	2.0	

7. Return test tubes to roller for 12 h, increase the elevation angle of the roller.

   Date/Time: 04/09/98 ; 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:** 04/10/98; 9-00 a.m.
10. **During centrifugation, move roller to 10°C and obtain ice**
11. Prepare 11 ml of sterile MEA (100 ug/ml) 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 or 100 ug/ml of MEA as per Table. Keep on ice!
20. Transfer tubes to roller at 10°C for 72 h. **Date/Time:** 04/10/98; 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: 04/14/98; 2-00 p.m.

21. After 72 h, remove tubes and **place on ice**, add 8 ml ***ice cold*** wash MEMA.

    Date/Time: 04/13/98; 10-00 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 :** 04/14/98 ; 3-00 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 dish to be a valid data point.

Expt # 1 04/09/98

Initial cell Count = 10,803; 10908; 11072

Avg. Cell Count = 10927

Cell Conc. = $10927.6 \times 400 = 4371066$

For dilution

Vol. of cell suspension required = $\frac{4400000}{4371066} = 1.00$

Take 1 ml of cells + 10 ml of HEMB = 11 ml

After dilution,

Final Count = 1200, 1154, 1057, 1131

Avg Cell Count = 1135.5

Cell Conc. = 1135.5×400

= 4,54,200 Cells/ml

Expt #1 04/08/98

Prepare 5 ml of 1 uci/ml = 5 uci required.

Stock 1.93 uci/ml.

$$\text{Stock required} = \frac{5}{1.93} = 2.59 = 2.6 \text{ ml}$$

(1) Take 2.6 ml ^{125}IVDP

(2) Add 5 ml of MEMB

125 IUdR + MEA (1.3mM)

4/14/98

Expt # 1

		I-125 window	open window	
1	1.00	24	244	1M
2	1.00	22	248	
3	1.00	23	289	
4	1.00	23	253	2M
5	1.00	19	240	
6	1.00	27	255	
7	1.00	157	395	3M
8	1.00	168	414	
9	1.00	176	432	
10	1.00	351	586	4M
11	1.00	299	509	
12	1.00	334	586	
13	1.00	426	653	5M
14	1.00	468	705	
15	1.00	468	709	
16	1.00	19	230	6M
17	1.00	21	241	
18	1.00	24	240	
19	1.00	20	286	7M
20	1.00	33	291	
21	1.00	19	224	
22	1.00	189	425	8M
23	1.00	150	389	
24	1.00	178	447	
25	1.00	316	544	9M
26	1.00	335	583	
27	1.00	321	561	
28	1.00	448	716	10M
29	1.00	470	724	
30	1.00	455	682	
51	1.00	1629	1978	9M
52	1.00	1770	2069	
53	1.00	1642	1940	
54	1.00	2095	2397	10M
55	1.00	2224	2478	
56	1.00	2048	2345	
57	1.00	18	262	1C
58	1.00	24	252	
59	1.00	28	262	
60	1.00	33	243	2C
61	1.00	25	271	
62	1.00	28	272	
63	1.00	954	1216	3C
64	1.00	974	1225	
65	1.00	973	1240	
66	1.00	1445	1710	4C
67	1.00	1582	1842	
68	1.00	1498	1751	
69	1.00	2005	2260	5C
70	1.00	2024	2311	
71	1.00	2152	2426	
72	1.00	27	287	6C
73	1.00	17	247	
74	1.00	21	269	
75	1.00	18	248	7C
76	1.00	28	287	
77	1.00	22	246	
78	1.00	1423	1707	8C
79	1.00	1388	1623	
80	1.00	1392	1668	
81	1.00	2255	2539	9C
82	1.00	2374	2665	
83	1.00	2377	2659	
84	1.00	2357	2672	10
85	1.00	2421	2729	

Cystamine Self-Dose Experiment

10 Ml onto tissues

Cysteamine Self Dose Experiment

300 Ml washed cells

TABLE-1

Expt. # : 4 **Date/Time :** 04/14/98 ; 2-00 p.m.

Tube #	Medium count for 10 ul (cpm) *	Avg. cpm	dpm [cpm/~~0.142~~ 0.48×1.47] cpm/0.7056 **	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	0
2	0, -4, 4	0	0	0	0
3	134, 145, 153	144	204.08	0.00919	0.0097
4	328, 276, 311	305	432.25	0.0194	0.0205
5	403, 445, 445	431	610.82	0.0275	0.0290
6	-4, -2, 1	0	0	0	0
7	-3, 10, -4	0	0	0	0
8	166, 127, 155	149.3	211.59	0.0095	0.0100
9	293, 312, 298	301	426.58	0.0192	0.0203
10	425, 447, 432	434.6	615.9	0.0277	0.0293

04/09/98 ; 7-45

4x24 + 12 + 6.25

= 114.24 h

* I-125 Window — Empty tube

* Efficiency = 0.48
  yield = 1.47
  } I-125

$e^{-\lambda t}$

$= e^{\frac{-0.693 \times 114.24}{1440}}$

= 0.9465

TABLE-2

Expt. # : 1 **Date/Time :** 04/14/98 ; 3-00 p.m

Tube #	Radioactivity for 300 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/~~0.142~~ 0.7056]	µCi/ml (A_t) on counting [dpm/666000]	µCi/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1	-5, 1, 5	0	0	0	0
2	10, 2, 5	0	0	0	0
3	931, 951, 950	944	1337.8	0.0020	0.0021
4	1422, 1559, 1475	1485.3	2105.0	0.0031	0.0033
5	1982, 2001, 2129	2037.3	2887.3	0.0043	0.0045
6	4, -6, -2	0	0	0	0
7	-5, 5, -1	0	0	0	0
8	1400, 1365, 1369	1378	1952.9	0.0029	0.0030
9	2232, 2351, 2354	2312.3	3277.1	0.0049	0.0051
10	2334, 2398, 2237	2323.	3292.23	0.0049	0.0051

04/10/98 ; 9-00 a.m

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 101}{1440}}$

$= e^{-0.0486}$

$= 0.9525$

4x24 + 5 = 101

TABLE-3

Expt. # : 1 **Date/Time :** 04/13/98; 11-30 a.m.

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 400]	pCi/cell [uCi/ml x10^6 Cells/ml]
1	~~951~~, 893, 903, 863	886·3	354533·3	0
2	416, 402, 478	432	172800	0
3	767, 771, 802	780	312000	0·00673
4	845, 802, 793	813·3	325333·3	0·01383
5	668, 575, 578	607	242800	0·01853
6	874, 876, 858	869·3	347733·3	0
7	~~874~~, —	—	—	—
8	874, 853, 807	844·6	337866·6	0·00887
9	825, 767, 777	789·6	315866·6	0·01614
10	426, 425, 413	421·3	168533·3	0·03026

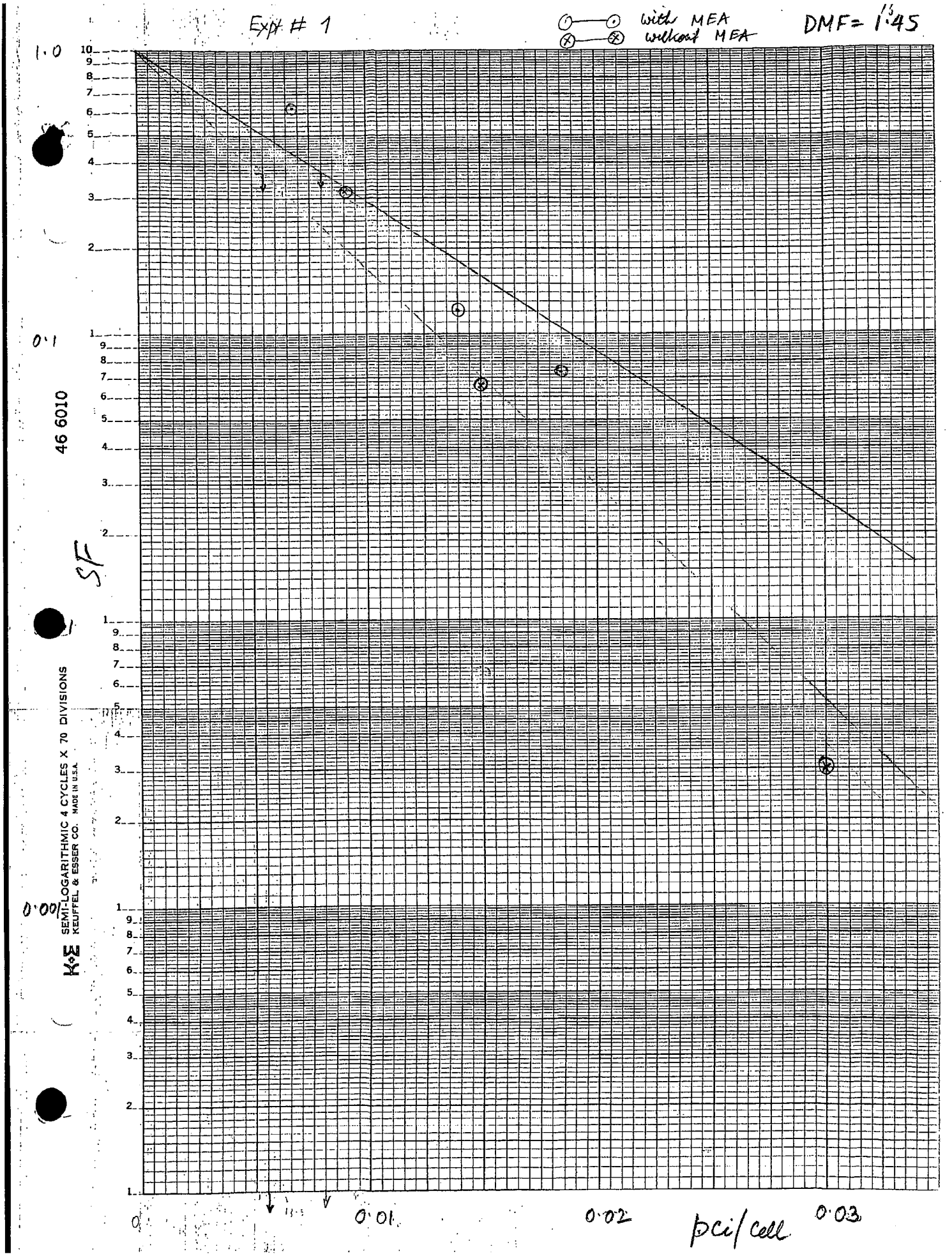
TABLE-4

Expt # : 1 **Date :** 04/20/98

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony for X·2	SF
1·2	80	68	60	69·33	
2·2	11	14	14	13*	
3·2	47	49	35	43·66	0·6298
4·3	82	90	78	8·33	0·1201
5·3	61	40	52	5·1	0·0735
6·2	72	82	95	83	
7·2**	—	—	—	—	—
8·2	20	28	32	26·66	0·3212
9·3	59	53	54	5·53	0·0666
10·4	20	27	29	0·25	0·0030

* not considered

** error during seeding not included


Exp # 1
with MEA
without MEA
DMF = 1.45
1.0
0.1
0.001
46 6010
SEMI-LOGARITHMIC 4 CYCLES X 70 DIVISIONS
KEUFFEL & ESSER CO. MADE IN U.S.A.
K+E
0
0.01
0.02
0.03
pCi/cell

B013393