V79 COLONY FORMING ASSAY

Experiment Name : ^{125}IUdR + 50-200 ug/ml MEA; **Exp. # : 2; Investigator:** A.Bishayee
Date: ~~05/14/98~~ 06/15/98.

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 : 426800 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:** 4-15 p.m.
5. Prepare MEMB containing radioactivity in hood
   3.4 µl ^{125}IUdR (prepared on 04/09/98) + 3 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:** 6/15/98 ; 8-00 p.m.

Tube #	^{125}IUdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{125}IUdR (ml) [1.0 uCi/ml]	MEMA+ MEA (200 ug/ml) (ml)	MEMA (ml)	MEA Conc. (ug/ml)
1	0	1.0	1.0	0	0	2.0	0
2	0	1.0	1.0	0	0.5	1.5	50
3	0	1.0	1.0	0	1	1	100
4	0	1.0	1.0	0	1.5	0.5	150
5	0	1.0	1.0	0	2.0	0	200
6	0.02	1.0	0.96	0.04	0	2.0	0
7	0.02	1.0	0.96	0.04	0.5	1.5	50
8	0.02	1.0	0.96	0.04	1	1	100
9	0.02	1.0	0.96	0.04	1.5	0.5	150
10	0.02	1.0	0.96	0.04	2.0	0	200

7. Return test tubes to roller for 12 h, increase the elevation angle of the roller.
   Date/Time: 06/15/98; 8-15 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:** 06/16/98; 9-10 a.m.
10. **During centrifugation, move roller to 10°C and obtain ice** MEMA = 10.89 ml
11. Prepare 11 ml of 200 ug/ml MEA in MEMA , **put on ice** MEA = 0.11 mL (20 mg/ml)
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 200 ug/ml MEA as per Table. Keep on ice!
20. Transfer tubes to roller at 10°C for 72 h. **Date/Time:** 06/16/98; 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: 06/16/98; 12-00 noon

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

    Date/Time: 06/19/98; 9-20 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 :** 06/19/98; 4-30 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.

Expt #2

06/15/98

Initial Cell count = 8693, 8716, 8638
Avg. Cell Count = 8682.3
Cell conc. = 8682.3 × 400
= 3472933 Cells/ml

For dilusion,

Vol of Cell suspension taken = $\frac{4400000}{3472933}$

= 1.26 ml

Take 1.3 ml of Cells + 9.7 ml MEMB = 11 ml.

After dilusion,

Final Cell Count = 1121, 1070, 1010
Avg. Cell Count = 1067
Cell conc. = 1067 × 400
= 426800 Cells/ml.

Expt #2

06/15/98

Preparation of ^{125}IUdR in MEMB

Prepare 3 ml of 1 µci/ml = 3 µCi required

Stock

On 4/9/98 = 1.93 µCi/µl

On 6/15/98 = 1.93 × 0.4588

= 0.88 µCi/µl.

Stock required = $\frac{3}{0.88}$ = 3.4 µl.

(i) Take 3 ml of MEMB

(ii) Take 3.4 µl Stock 125IUdR

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TABLE-1

Expt. # : 2 **Date/Time :** 06/16/98; 12-00 noon

Tube #	Medium count for 10 ul (cpm)	Avg. cpm	dpm [cpm/0.7056]	µCi/ml (A_t) on counting [dpm/22200]	µCi/ml (A_o) on addition [$A_t/e^{-\lambda t}$]	
1	0, 1, 1	0	0	0	0	
2	0, 0, 0	0	0	0	0	
3	0, 1, 0	0	0	0	0	
4	0, 0, 1	0	0	0	0	
5	1, 1, 0	0	0	0	0	
6	270, 272, 260,	267.3	378.8	0.0017	0.0017	0.017
7	246, 216, 280	247.3	350.5	0.0015	0.0015	0.015
8	293, 255, 320	289.3	410.0	0.0018	0.0018	0.018
9	252, 276, 244	257.3	364.7	0.016	0.016	0.016
10	260, 258, 236	251.3	356.1	0.016	0.016	0.016

06/15/98; 8.00 p.m.

12h + 4h

= 16h

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 16}{1440}}$

$= 0.9923$

TABLE-2

Expt. # : **Date/Time :** 06/19/98; 4-30 p.m.

Tube #	Radioactivity for 300 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.7056]	μCi/ml (A_t) on counting [dpm/666000]	μCi/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1	0, 1, 1	0	0	0	0
2	0, 1, 0	0	0	0	0
3	2, 1, 0	0	0	0	0
4	2, 2, 0	0	0	0	0
5	0, 1, 2	0	0	0	0
6	1938, 1977, 2002	1972.3	2795.2	0.00419	0.00436
7	2145, 2140, 2030	2095.6	2970.0	0.00445	0.00463
8	2183, 2114, 2002	2099.6	2975.7	0.00446	0.00464
9	1998, 1942, 1995	1978.3	2803.7	0.00420	0.00437
10	1854, 1967, 1822	1881	2665.8	0.00400	0.00415

06/16/98; 9-00 a.m

72h + 7.5

= 79.5

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 79.5}{1440}}$

$= 0.9624$

TABLE-3

Expt. # : 2. **Date/Time :** 06/19/98; 10-30 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	753, 759, 734	748.6	299466	0
2	769, 780, 752	767	306800	0
3	832, 856, 842	843.3	337333	0
4	861, 868, 867	865.3	346133	0
5	766, 708, 704	726	290400	0
6	872, 862, 869	867.6	347066	0.0125
7	869, 875, 892	878.6	351466	0.0131
8	855, 862, 872	863	345200	0.0134
9	924, 908, 904	912	364800	0.0119
10	769, 777, 748	764.6	305866	0.0135

TABLE-4

Expt. #: 2 Date: 06/26/98

Colony Counts and Survival Fraction

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony for ×.2	SF
1·2	110	119	105	113·3	–
2·2	108	99	105	104	0·9179
3·2	90	97	106	97·6	0·8620
4·2	80	66	72	72·6	0·6413
5·2	70	60	64	64·6	0·5707
6·3	59	62	57	5·93	0·0523
7·3	70	75	65	7·0	0·0673
8·3	225	220	230	22·5	0·2305
9·3	204	209	199	20·4	0·2809
10·3	71	76	66	7·1	0·1099

Conc. of MEA (µg/ml)	DMF
50	1·14
100	2·12
150	2·23
200	1·42

Expt #2