(1)

V79 COLONY FORMING ASSAY

Experiment Name : ^{131}IUdR + 5% DMSO; **Exp. # :** 6; **Investigator:** A. Bishayee

Date: 11/06/97

Set the roller at 37°C incubator, set the Coulter Counter,

1. Trypsinize cells, resuspend in 7 ml MEMB, pass five times through 3 cc syringe with 21 gauge needle, cell count
2. Dilute to 400,000 cells/ml in MEMB (final volume 11 ml) [Actual count : 4,51,200 cells/ml)
3. Transfer 1 ml of cell suspension into ten 12 ml tubes (Falcon T-tube, 17x100 mm) labeled 1-10 both on cap and wall
4. Roll for 3-4 h at 37°C, 5% CO_2 **Date/Time: 11/06/97; 2-45 p.m.** (6,)
5. Prepare MEMB containing radioactivity in hood

   44 µl ^{131}IUdR (prepared on 10/14/97) + 4.95 ml MEMB
6. After 3-4 h, remove T-tubes from roller and add MEMB ~~(1 ml) containing~~ with or without radioactivity according to Table below. **Date/Time: 11/06/97; 6-30 p.m.** (6,)

Tube #	uCi/ml IUdR	Cells (ml) 400,000 Cells/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 T-tubes to roller for 12 h, increase the elevation angle of the roller. **Date/Time: 11/06/97; 7-00 p.m.** (6,)

(4X10) VWR 13X100 glass

8. While T-tubes are rolling label 40 gamma-tubes (~~12~~ X 75 mm test tube)
9. After 12 h incubation period, remove tubes and centrifuge at 2000 rpm, 4°C.
   Date/Time: 11/07/97; 8-00 a.m. (t4)
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 T-tubes for 10 min at 2000 rpm, 4°C **(precooled centrifuge)**
15. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
16. Centrifuge T-tubes for 10 min at 2000 rpm, 4°C
17. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
18. Centrifuge T-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: 11/08/97; 11-30 a.m.** (t5)
21. Transfer 10 ul supernatant in three sets of tube from 100 ul supernatant removed earlier and count them
21. After 72 h, remove tubes and place on ice, add 8 ml ice cold wash MEMA. **Date/Time: 11/10/97; 9-00 a.m.** (t6)
22. Centrifuge T-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 T-tubes for 10 min at 2000 rpm, 4°C
26. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
27. Centrifuge T-tubes for 10 min at 2000 rpm, 4°C
28. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA
29. Determine cell concentration - transfer 100 µl to Coulter cup
30. Vortex T-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 ~~flasks~~ for 1 wk

33a. Count the gamma-tubes (petri dishes) Date / Time : 11/10/97; 2-30 pm (t6)

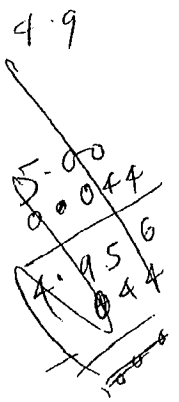
34. Wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with crystal violet ~~or trypan blue~~.
35. Count colonies (50 or more cells). There must be between 25 and 250 colonies for the flask to be a valid data point.

11/06/97

Exp#6

5-9 (4a)

Wash MEMA — Coef cum — 10%.
MEMA — FCS — 10%.



11/06/97

Initial Cell count = 9801, 9819, 9979

Avg. cell count = 9866·3

Cell conc. = 9866·3 × 400 = 3946533 /ml

For dilution,

Vol. of original cell suspension = $\frac{4400000}{3946533}$

= 1·11 ml.

1·11 ml cell suspension + 9·89 MEMB

After dilution,
Final count = 1143, 1106, 1135

Avg. count = 1128

Cell Conc. = 1128 × 400 = 4,51,200 /ml

Exp#6 11/06/97 (46)

Preparation of MEMB with ^{131}IUdR

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

0.138 μCi/μl on 11/6/97 at 7-00 p.m.

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

$\lambda t = \frac{\ln 2}{t_{1/2}} \times t$ [t = 22d 21½ h = 22d + 0.89d = 22.9 d

$= \frac{0.693 \times 22.9}{8.02}$ $t_{1/2} = 8.02$ d

$= 1.97$

$A_t = A_0 e^{-1.97}$

$= A_0 \times 0.138$

$= 1 \times 0.138$ μci/μl.

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

$\frac{6}{0.138 \text{ μci/μl}} = 43.6$ μl of Stock ^{131}IUdR

Take 43.6 μl of Stock ^{131}IUdR (~ 44 μl) } in hood

Add 4.95 ml of MEMB }

Results

Exp # 6

11/08/97
12-00 noon

Tube #	Radioactivity (10 µg for 6a Exp) cpm	Avg. cpm	Avg. dpm [cpm / efficiency] = cpm / 0.142
1	0, 1	0	
2	2, 1	0	
3	532, 646, 621	599.6	4223.0
4	1181, 1304, 1433	1306	9197.1
5	1783, 1903, 1896	1860.6	~~34304.8~~ 13103.2
6	2, 1	0	0
7	0, 0	0	0
8	573, 571, 531	558.3	3931.9
9	1185, 1258, 1313	1252.0	8816.9
10	1675, 1999, 2060	1911.3	13460.0

Tube #	µCi/ml at 12-00 noon of 11/08/97	µCi/ml at 6-30 pm of 11/07/97
1	0	0
2	0	0
3	0.190	0.202
4	0.414	0.440
5	0.590	0.628
6	0	0
7	0	0
8	0.177	0.188
9	0.397	0.422
10	0.606	0.645

$e^{-\lambda t}$
$= e^{-\frac{\ln 2 \times 17.5}{193.2}}$
$= e^{-\frac{0.693 \times 17.5}{193.2}}$
$= e^{-0.062}$
$= 0.939$

Exp # 6

11/10/97 (5)

Tube #	Coulter count (for coone cell suspension)	Avg. cell count	cells/ml (Avg. count X 400)
1	539, 543, 579	553.6	221466.6
2	628, 619, 587	611.3	244533.3
3	678, 703, 705	695.3	278133.3
4	582, 549, 543	558	223200.0
5	626, 702, 604	644	257600.0
6	732, 713, 743	729.3	291733.3
7	785, 781, 634	733.3	293333.3
8	441, 450, 469	453.3	181333.3
9	544, 550, 562	552	220800.0
10	556, 557, 550	554.3	221733.3

Tube #	Radioactivity for 300 ul cell suspension on 2-3 opm at 11/10/97 Cpm	Avg. Cpm	dpm ($\frac{cpm}{0.142}$)
1	0, -5, 0	0	0
2	0, 0	0	0
3	2645, 2605, 2589	2613	18401.4
4	4051, 3837, 4047	3978.3	28016.4
5	3244, 3069, 3254	3189	22457.7
6	0, 0	0	0
7	2, 0	0	0
8	2178, 2061, 2151	2130	15000
9	4286, 3911, 4398	4198.3	29565.7
10	3907, 3850, 3881	3879.3	27319.2

Dilusion required for Seeding of cells

Tube #	dilusions
1	1·2
2	2·2
3	3·2, 3·3
4	4·2, 4·3, 4·4
5	5·2, 5·3, 5·4
6	6·2
7	7·2
8	8·2, 8·3
9	9·2, 9·3, 9·4
10	10·3, 10·4

Total plates = 19 × 3 = 57

Exp. # 6

Tube #	μCi/ml [dpm × 1/666000]	pCi/cell at 2-30 pm on 11/10/97	pCi/cell at 8 am on 11/09/97
1	0	0	0
2	0	0	0
3	0.0276	0.0992	0.1314
4	0.0420	0.1881	0.2492
5	0.0337	0.1308	0.1733
6	0	0	0
7	0	0	0
8	0.0225	0.1240	0.1643
9	0.0443	0.2006	0.2658
10	0.0410	0.1849	0.2450

Plate # (dilution)	# of Colonies	Avg. colonies	SF

Colony formation was severely affected due to problem associated with Cox supply into the incubator.

11/12/97

Mobile phase : MPLC-H2

Analtech RPS-F
Uniplate
Cat # 52521

— NaI

● — ^{131}IUdR (purified)