B001775

[redacted] 50%. : 10%. DMSO-100mM Tris [redacted]

V79 COLONY FORMING ASSAY

Experiment Name : ^{125}IUdR cluster with 10% DMSO w/o100 uM lindane(50% label) **Exp. # :** 2;

Experiment performed by: A. Bishayee **Date:** 09/18/00

1. Set the rocker-roller at 37°C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 175 cm^2 flask, subcultured 1:2, 24h before) with PBS, trypsinize cells, each resuspend in 9 ml MEMB, pool, 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 ~2,000,000 cells/ml in MEMB [Actual count: cells/ml)
3. Transfer 1 ml of cell suspension into 14 ml tubes (Falcon plastic test tube, 17x100 mm)
4. Keep the tubes in the roller for 3-4 h at 37°C, 5% CO_2 **Date/Time:** 09/18/00, 4-00 pm
5. Prepare MEMB containing radioactivity in hood

   73 µl ^{125}IUdR (prepared on 09/08/00) + 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: 09/18/00, 8-00 pm

Tube #	^{125}IUdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{125}IUdR [6uCi/ml] (ml)	100 uM Lindane-10%DMSO in MEMA (ml)	10%DMSO in MEMA (ml)
1	0	1.0	1.0	0	0.4	0
2	0	1.0	1.0	0	0.4	0
3	0.25	1.0	0.915	0.085	0.4	0
4	0.5	1.0	0.835	0.165	0.4	0
5	1	1.0	0.665	0.335	0.4	0
6	2	1.0	0.335	0.665	0.4	0
7	3	1.0	0	1	0.4	0
8	0	1.0	1.0	0	0	0.4
9	0	1.0	1.0	0	0	0.4
10	0.25	1.0	0.915	0.085	0	0.4
11	0.5	1.0	0.835	0.165	0	0.4
12	1	1.0	0.665	0.335	0	0.4
13	2	1.0	0.335	0.665	0	0.4
14	3	1.0	0	1	0	0.4

7. Return test tubes to roller for 12 h . **Date/Time:** 09/18/00, 8-30 pm
8. Next day, while test tubes are in shaker 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:** 09/19/00, 9-00 a.m
10. Remove buckets from centrifuge and carefully remove 100 µl of supernatant and place in prelabeled gamma-tube.
11. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
12. Centrifuge tubes for 10 min at 2000 rpm, 4°C
13. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
14. Centrifuge tubes for 10 min at 2000 rpm, 4°C
15. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
16. Centrifuge tubes for 10 min at 2000 rpm, 4°C
17. Similarly wash the cells that were not labelled, pooled cells from two tubes, resuspend in 8 ml was MEMA with 10% DMSO w/o 100 uM lindane
18. Decant supernatant, click tubes, vortex, transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips
18. Again add 200 ul MEMA with 10%DMSO w/o 100 uM lindane as per the table, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
19. Centrifuge tubes for 5 min at 1000 rpm, 4°C
20. Transfer tubes at 10°C for 72 h. **Date/Time:** 09/19/00, 12-00
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: 09/19/00, 4-00 pm

22. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the content to ten 12 ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA by using pasteur pipet

Date/Time: 09/22/00, 10-00

23. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in a.a. 12 ml tubes
24. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***
25. Labeling and preparation of dilution tubes and colony dishes
    - load 60 mm petri dishes with 4 ml MEMA
    - load test tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2, X.3, X.4, X.5 etc.
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 10 ml wash MEMA
29. Centrifuge tubes for 10 min at 2000 rpm, 4°C
30. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
31. Determine cell concentration by transfering 100 µl to Coulter cup
32. Vortex tube, transfer 0.5 ml into dilusion tube X.5, vortex tube X.5, 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.
33. 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.
34. Transfer 300 µl of cell suspension (in triplicate) to gamma tubes for each tube
35. Incubate petridishes for 1 week
36. Count gamma tubes for radioactivity **Date/Time :** 09/22/00, 4:00pm
37. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
38. Count colonies. There must be between 25 and 250 colonies for the flask to be a valid data point.

09/18/00

Stock on 09/08/00 → 0·465 μci/μl

on 09/18/00 → 0·465 × 0·891

= 0·414 μci/μl

Prepare 5 ml of 6 μci/ml

= 30 μci required

Vol. required = $\frac{30}{0.414}$ = 0·730 μl

① Take 73 μl of stock
② Keep at RT for 24h
③ Add 5 ml of MEMB

P#	S#	TIME	CPMA/K	CPMB/K	FLAGS	MIN
5	91	1.00	32	152		104
5	92	1.00	24	124		105
5	93	1.00	31	182		106
5	94	1.00	25	142		107
5	95	1.00	26	160		108
5	96	1.00	27	174		110
5	97	1.00	12866	13044		111
5	98	1.00	13198	13355		112
5	99	1.00	12529	12682		113
5	100	1.00	25758	25881		114
5	101	1.00	27629	27776		116
5	102	1.00	27051	27186		117
5	103	1.00	49797	49943		118
5	104	1.00	51920	52101		119
5	105	1.00	50809	50993		120
5	106	1.00	97525	97744		122
5	107	1.00	103534	103779		123
5	108	1.00	102089	102314		124
5	109	1.00	147082	147418		125
5	110	1.00	150534	150907		127
5	111	1.00	148873	149236		128
5	112	1.00	20	157		129
5	113	1.00	25	167		130
5	114	1.00	21	157		131
5	115	1.00	24	142		133
5	116	1.00	16	145		134
5	117	1.00	21	164		135
5	118	1.00	12524	12658		136
5	119	1.00	13304	13454		137
5	120	1.00	13445	13606		139
5	121	1.00	25591	25770		140
5	122	1.00	26499	26661		141
5	123	1.00	26223	26360		142
5	124	1.00	50954	51132		144
5	125	1.00	53177	53356		145
5	126	1.00	51971	52148		146
5	127	1.00	96081	96309		147
5	128	1.00	100699	100975		148
5	129	1.00	106215	106437		149
5	130	1.00	147362	147708		151
5	131	1.00	151958	152323		152
5	132	1.00	155506	155871		153
5	133	1.00	25	164		154
5	134	1.00	23	180		156
5	135	1.00	16	156		157
5	136	1.00	127029	127899		158

61796
61927
61901
62294
62058
61644
62042

09/[illegible]/20
0.007

B001781

62017
61997
61560
61601
62374
62062
61817
61962
61964
61746
61392
62011

PROGRAM #: 10 09/20/00 16:14
BKG: 208
%CHANGE: 7.7
PEAK: 659
%RESOLUTION: 12.7
CHI-SQUARE: 19.1 NORMAL RANGE: 8.9 TO 32.8

12h + 7.5h = 19.5h 09/18/00 ; 8-30 pm

$= e^{-\lambda t}$

$= e^{-\frac{0.693 \times 19.5}{1440}} = 0.9906$

TABLE-1

Expt. # : 2 **Date/Time :** 09/19/00; 4-00 pm.

Tube #	Medium count for 10 ul (cpm)	Avg. cpm	dpm [cpm/1.09] e=0.74 y=1.47	µCi/ml (A_t) on counting [dpm/22200]	µCi/ml (A_o) on addition [$A_t/e^{-\lambda t}$]
1		} 28			
2					
3		12836	11776	0.530	0.535
4		26784	24573	1.10	1.11
5		50786	46592	2.09	2.12
6		101021	92680	4.17	4.22
7		148809	136515	6.14	6.21
8		} 21			
9					
10		13070	11990	0.5401	0.545
11		26083	23929	1.0779	1.08
12		52013	47718	2.149	2.17
13		100977	92639	4.17	4.21
14		151587	139071	6.26	6.32

62444
62132
62608
62487
61986
62037
62505
62624
61881
61923
61905
62570
62207
61913
61687
61983
61984
62282
62240
62171

PROGRAM #: 10 09/23/00 15:35
PEAK: 642
%RESOLUTION: 11.6
CHI-SQUARE: 24.2 NORMAL RANGE: 8.9 TO 32.8

P#	S#	TIME	CPMA/K	CPMB/K	FLAGS	MIN
5	1	1.00	20	154		1
5	2	1.00	32	163		3
5	3	1.00	27	157		4
5	4	1.00	27	165		5
5	5	1.00	29	152		7
5	6	1.00	34	167		8
5	7	1.00	47280	47456		9
5	8	1.00	46180	46338		10
5	9	1.00	45371	45525		11
5	10	1.00	80248	80440		12
5	11	1.00	78192	78373		14
5	12	1.00	78768	78944		15
5	13	1.00	156641	156998		16
5	14	1.00	153368	153708		17
5	15	1.00	151316	151661		19
5	16	1.00	375176	376415		20
5	17	1.00	368452	369684		21
5	18	1.00	365678	366905		22
5	19	1.00	549553	552084		24
5	20	1.00	533133	535477		25
5	21	1.00	536553	538930		26
5	22	1.00	34	186		27
5	23	1.00	32	174		29
5	24	1.00	24	168		30
5	25	1.00				
5	26	1.00	36	194		31
5	27	1.00	29	183		32
5	28	1.00	37	186		33
5	29	1.00	41316	41484		35
5	30	1.00	40449	40618		36
5	31	1.00	39948	40122		37
5	32	1.00	82449	82681		38
5	33	1.00	82492	82714		39
5	34	1.00	83135	83336		41
5	35	1.00	196437	196908		42
5	36	1.00	193284	193724		43
5	37	1.00	195488	196020		44
5	38	1.00	375206	376453		46
5	39	1.00	374247	375483		47
5	40	1.00	324914	325878		48
5	41	1.00	552226	554765		49
5	42	1.00	562397	565033		51
5	43	1.00	550243	552765		52
5	44	1.00	28	166		53
5	45	1.00	31	170		54
5	46	1.00	26	156		56
			127422	128330		57

POWER FAIL

300µl each

09/22/00

4:00 pm

09/19/00 ; 9:00 am

3x24 + 7 = 79h

TABLE-2

Expt. # : 2 **Date/Time :** 09/22/00;

Tube #	Radioactivity for 300 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/1.09]	µCi/ml (A_t) on counting [dpm/666000]	µCi/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

TABLE-3

Expt. # : **Date/Time :**

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [uCi/ml x10^6 Cells/ml]	kBq/Cluster [pCi/cellx148
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

TABLE-4

Expt # : 2 **Date :** 09/29/00

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1.2	139	122	119	131	
2.2	147	132	127		
3.2	87	77	68	77.3	0.5903
4.2	49	69	58	58.66	0.4477
5.2	45	38	31	38	0.2900
6.2	17	15	14	15.33	0.1170
7.3	57	52	48	5.23	0.0399
8.2	127	136	119	121.8	
9.2	107	117	125		
10.2	78	67	59	68	0.5583
11.2	49	41	34	41.33	0.3393
12.2	14	16	13	14.33	0.1176
13.3	57	48	39	4.8	0.039
14.4	80	76	85	0.80	0.0065

V79 COLONY FORMING ASSAY

Experiment Name : ^{125}IUdR cluster with 10% DMSO w/o100 uM lindane(50% label) **Exp. # :** 1;

Experiment performed by: A. Bishayee **Date:** 09/14/00

1. Set the rocker-roller at 37°C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 175 cm^2 flask, subcultured 1:2, 24h before) with PBS, trypsinize cells, each resuspend in 9 ml MEMB, pool, 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 ~2,000,000 cells/ml in MEMB [Actual count: 2,044,000 cells/ml)
3. Transfer 1 ml of cell suspension into 14 ml tubes (Falcon plastic test tube, 17x100 mm)
4. Keep the tubes in the roller for 3-4 h at 37°C, 5% CO_2 **Date/Time:** 09/14/00; 3-00 PM
5. Prepare MEMB containing radioactivity in hood

   70µl ^{125}IUdR (prepared on 09/08/00) + 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: 09/14/00; 7-10 PM.

Tube #	^{125}IUdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{125}IUdR [6uCi/ml] (ml)	100 uM Lindane-10%DMSO in MEMA (ml)	10%DMSO in MEMA (ml)
1	0	1.0	1.0	0	0.4	0
2	0	1.0	1.0	0	0.4	0
3	0.25	1.0	0.915	0.085	0.4	0
4	0.5	1.0	0.835	0.165	0.4	0
5	1	1.0	0.665	0.335	0.4	0
6	2	1.0	0.335	0.665	0.4	0
7	3	1.0	0	1	0.4	0
8	0	1.0	1.0	0	0	0.4
9	0	1.0	1.0	0	0	0.4
10	0.25	1.0	0.915	0.085	0	0.4
11	0.5	1.0	0.835	0.165	0	0.4
12	1	1.0	0.665	0.335	0	0.4
13	2	1.0	0.335	0.665	0	0.4
14	3	1.0	0	1	0	0.4

7. Return test tubes to roller for 12 h . **Date/Time:** 09/14/00; 7-30 PM.
8. Next day, while test tubes are in shaker 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:** 09/15/00; 9-00 a.m.
10. Remove buckets from centrifuge and carefully remove 100 µl of supernatant and place in prelabeled gamma-tube.
11. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
12. Centrifuge tubes for 10 min at 2000 rpm, 4°C
13. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
14. Centrifuge tubes for 10 min at 2000 rpm, 4°C
15. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
16. Centrifuge tubes for 10 min at 2000 rpm, 4°C
17. Similarly wash the cells that were not labelled, pooled cells from two tubes, resuspend in 8 ml was MEMA with 10% DMSO w/o 100 uM lindane
18. Decant supernatant, click tubes, vortex, transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips
18. Again add 200 ul MEMA with 10%DMSO w/o 100 uM lindane as per the table, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
19. Centrifuge tubes for 5 min at 1000 rpm, 4°C
20. Transfer tubes at 10°C for 72 h. **Date/Time:** 09/15/00; 11-30 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: 09/19/00; 4-00 pm

22. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the content to ten 12 ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA by using pasteur pipet

Date/Time: 09/18/00; 10:00 a.m.

23. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes
24. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***
25. Labeling and preparation of dilution tubes and colony dishes
    - load 60 mm petri dishes with 4 ml MEMA
    - load test tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2, X.3, X.4, X.5 etc.
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 10 ml wash MEMA
29. Centrifuge tubes for 10 min at 2000 rpm, 4°C
30. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
31. Determine cell concentration by transfering 100 µl to Coulter cup
32. Vortex tube, transfer 0.5 ml into dilusion tube X.5, vortex tube X.5, 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.
33. 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.
34. Transfer 300 µl of cell suspension (in triplicate) to gamma tubes for each tube
35. Incubate petridishes for 1 week
36. Count gamma tubes for radioactivity **Date/Time :** 09/19/00; 4:00 pm
37. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
38. Count colonies. There must be between 25 and 250 colonies for the flask to be a valid data point.

09/14/00

Stock on 09/08/00 → 0.465 μci/μl

on 09/14/00 → 0.465 × 0.933

= 0.433 μci/μl

Prepare 5 ml of 6 μci/ml

= 30 μci required

vol required = $\frac{30}{0.433}$ = ~70 μl

① Take 70 μl of stock
② Keep at RT for 3-4 hr
③ Add 5 ml of MEMB

e^{-kt}

$= e^{-\frac{0.693 \times 115.5}{1440}}$

$= e^{-0.0555}$

$= 0.946$

4 x 24 = 96 h
12 h
7.5 h
115.5

09/19/00 4-00 pm.
09/14/00; 7-30 pm

PROGRAM #: 5 09/20/00 13:11
REGION A: LL= 15 UL= 80 BKG= 0 %SIGMA= .00
REGION B: LL= 15 UL= 1000 BKG= 0 %SIGMA= .00
TIME= 1.00 SCREENING LIMITS= 0 0 K=1.000

P#	S#	TIME	CPMA/K	CPMB/K	FLAGS	MIN
5	1	1.00	25	160		2
SC TUBE JAM			1M			
2 TUBES MISSING						
5	4	1.00	31	155		7
5	5	1.00	2M 26	143		8
5	6	1.00	29	186		9
5	7	1.00	11614	11749		10
5	8	1.00	3M 12128	12249		11
5	9	1.00	12068	12229		12
5	10	1.00	20589	20730		14
5	11	1.00	4M 22520	22656		15
5	12	1.00	23251	23375		16
5	13	1.00	42806	42971		17
5	14	1.00	5M 45401	45555		19
5	15	1.00	44114	44297		20
5	16	1.00	84985	85189		21
5	17	1.00	6M 88770	89001		22
5	18	1.00	88659	88833		23
5	19	1.00	128719	129053		25
5	20	1.00	7M 133163	133482		26
5	21	1.00	133571	133852		27
5	22	1.00	31	164		28
5	23	1.00	8M 21	174		29
5	24	1.00	38	183		31
5	25	1.00	24	152		32
5	26	1.00	9M 29	182		33
5	27	1.00	29	171		34
5	28	1.00	11382	11556		35
5	29	1.00	10M 12059	12180		37
5	30	1.00	12242	12387		38
5	31	1.00	22449	22589		39
5	32	1.00	11M 22414	22581		40
5	33	1.00	23169	23299		41
5	34	1.00	12M 43727	43888		43
5	35	1.00	17	144	Sample was not added	44
5	36	1.00	33	163		45
5	37	1.00	13M 85141	85344		46
5	38	1.00	135251	135557		47
5	39	1.00	135853	136166		49
5	40	1.00	14M 126723	127036		50
5	41	1.00	134067	134405		51
5	42	1.00	136692	136998		52

TABLE-1

Expt. # : 1 **Date/Time :** 09/19/00; 4-00 pm

Tube #	Medium count for 10 ul (cpm)	Avg. cpm Test- ~~Test~~ Cont	dpm [cpm/~~0.7438~~ 1.09] e=~~0.506~~0.74 y=1.47	µCi/ml (A_t) on counting [dpm/22200]	µCi/ml (A_o) on addition [$A_t/e^{-\lambda t}$]
1		} 28			
2		}			
3		11908	10924	0.4921	0.5201
4		22092	20267	0.9129	0.9650
5		44079	40439	1.82	1.925
6		87443	80223	3.61	3.82
7		131817	120933	5.44	5.75
8		} 29			
9		}			
10		11894	10912	0.4915	0.519
11		22677	20804	0.937	0.990
12		43698	40089	1.80	1.908
13		85112	78084	3.51	3.71
14		133688	123785	5.57	5.89

$t = 24 \times 4 + 7.5$

$= 103.5$

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 103.5}{1440}}$

$= 0.9514$

0.03

9/19/00

4-00 pm

2 TUBES MISSING

P#	S#	TIME	CPMA/K	CPMB/K	FLAGS	MIN
5	49	1.00	21	146		56
5	50	1.00	30	161		57
5	51	1.00	37	199		59
5	52	1.00	35	157		60
5	53	1.00	34	184		61
5	54	1.00	27	153		62
5	55	1.00	47877	48036		63
5	56	1.00	46765	46918		65
5	57	1.00	45875	46041		66
5	58	1.00	81543	81746		67
5	59	1.00	80176	80377		68
5	60	1.00	79317	79540		69
5	61	1.00	158192	158517		71
5	62	1.00	154184	154539		72
5	63	1.00	153774	154118		73
5	64	1.00	379706	380981		74
5	65	1.00	370345	371654		76
5	66	1.00	369735	370877		77
5	67	1.00	553600	556203		78
5	68	1.00	538140	540567		79
5	69	1.00	541938	544429		81
5	70	1.00	42	191		82
5	71	1.00	29	168		83
5	72	1.00	17	162		84
5	73	1.00	28	165		86
5	74	1.00	31	157		87
5	75	1.00	23	168		88
5	76	1.00	41605	41764		89
5	77	1.00	40712	40888		90
5	78	1.00	40109	40248		91
5	79	1.00	83485	83721		93
5	80	1.00	82926	83148		94
5	81	1.00	84829	85041		95
5	82	1.00	198974	199410		96
5	83	1.00	195356	195788		97
5	84	1.00	198085	198579		99
5	85	1.00	378446	379715		100
5	86	1.00	377784	379068		101
5	87	1.00	327371	328395		103
5	88	1.00	557565	560119		104
5	89	1.00	569236	571897		105
5	90	1.00	554985	557495		106

TABLE-2

09/15/00; 9-00 a.m.

Expt. # : 1

Date/Time : 09/19/00; 4-30 pm

Tube #	Radioactivity for 300 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/1.09]	µCi/ml (A_t) on counting [dpm/666000]	µCi/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1		} 31			
2					
3		46808	42943	0·064	0·0673
4		80314	73682	0·1106	0·1163
5		155352	142525	0·214	0·225
6		373231	342413	0·514	0·5403
7		544528	499567	0·7501	0·7879
8		} 28			
9					
10		40780	37413	0·056	0·0591
11		83718	76806	0·1153	0·1212
12		197443	181140	0·2719	0·2859
13		361172	331350	0·4975	0·5226
14		560567	514281	0·772	0·8111

TABLE-3

Expt. # : 1 **Date/Time :** 09/18/00

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [uCi/ml x10^6 Cells/ml]	kBq/Cluster [pCi/cellx148
1	449, 432, 416				
2	472, 482, 461				
3	412, 435, 460	435	1742666	0·038 ~~0·298~~	
4	371, 356, 383	370	1480000	0·0785	
5	428, 456, 439	441	1764000	0·127	
6	433, 452, 449	444	1776000	0·304	
7	476, 461, 452	463	1852000	0·425	
8	516, 529, 541				
9	423, 450, 462				
10	389, 390, 415	398	1592000	0·037	
11	471, 461, 455	462	1849333	0·0655	
12	511, 526, 539	525	2101333	0·136	
13	490, 472, 463	475	1900000	0·275	
14	590, 571, 581	580	2322660	0·3496	

TABLE-4

Expt # : 1 **Date :** 09/25/00

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1.2	111	119	107	99.33	
2.2	98	89	72		
3.2	47	56	66	56.33	0.5671
4.2	43	32	24	33	0.3322
5.2	22	24	20	22	0.2215
6.3	88	78	97	8.76	0.0882
7.3	22	18	15	1.83	0.0184
8.2	109	113	110	102.83	
9.2	99	97	89		
10.2	55	45	36	45.33	0.4408
11.2	29	26	24	26.33	0.2560
12.2	14	16	12	14	0.1361
13.3	15	17	13	1.5	0.0145
14.4	70	63	56	0.63	0.0061

50% : 100 µM Lindane

V79 COLONY FORMING ASSAY

Experiment Name : ^{125}IUdR cluster with lindane (50% label) **Exp. # :** 3;

Experiment performed by: A. Bishayee **Date:** 08/10/00

1. Set the rocker-roller at 37^{0}C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 175 cm^2 flask, subcultured 1:2, 24h before) with PBS, trypsinize cells, each resuspend in 9 ml MEMB, pool, 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 ~2,000,000 cells/ml in MEMB [Actual count: cells/ml)
3. Transfer 1 ml of cell suspension into 14 ml tubes (Falcon plastic test tube, 17x100 mm)
4. Keep the tubes in the roller for 3-4 h at 37°C, 5% CO_2 **Date/Time:** 08/10/00; 3-00 pm
5. Prepare MEMB containing radioactivity in hood

   75 µl ^{125}IUdR (prepared on 07/28/00) + 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:** 08/10/00; 6-50 pm

Tube #	^{125}IUdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{125}IUdR [6uCi/ml] (ml)	100 uM Lindane in MEMA (ml)	MEMA (ml)
1	0	1.0	1.0	0	0.4	0
2	0	1.0	1.0	0	0.4	0
3	0.25	1.0	0.915	0.085	0.4	0
4	0.5	1.0	0.835	0.165	0.4	0
5	1	1.0	0.665	0.335	0.4	0
6	2	1.0	0.335	0.665	0.4	0
7	3	1.0	0	1	0.4	0
8	0	1.0	1.0	0	0	0.4
9	0	1.0	1.0	0	0	0.4
10	0.25	1.0	0.915	0.085	0	0.4
11	0.5	1.0	0.835	0.165	0	0.4
12	1	1.0	0.665	0.335	0	0.4
13	2	1.0	0.335	0.665	0	0.4
14	3	1.0	0	1	0	0.4

7. Return test tubes to roller for 12 h. **Date/Time:** 08/10/00; 7-00 p.m.
8. Next day, while test tubes are in shaker 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:** 08/11/00; 9-00 a.m.
10. Remove buckets from centrifuge and carefully remove 100 µl of supernatant and place in prelabeled gamma-tube.
11. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
12. Centrifuge tubes for 10 min at 2000 rpm, 4°C
13. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
14. Centrifuge tubes for 10 min at 2000 rpm, 4°C
15. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
16. Centrifuge tubes for 10 min at 2000 rpm, 4°C
17. Similarly wash the cells that were not labelled, pooled cells from two tubes, resuspend in 8 ml wash MEMA
18. Decant supernatant, click tubes, vortex, transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips
18. Again add 200 ul ice cold MEMA with or without 100 uM lindane as per the table, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
19. Centrifuge tubes for 5 min at 1000 rpm, 4°C
20. Transfer tubes at 10°C for 72 h. **Date/Time:** 08/11/00; 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: 08/14/00; 1-00 pm

22. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the content to ten 12 ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA by using pasteur pipet

Date/Time: 08/14/00; 2-00 pm.

23. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes
24. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***
25. Labeling and preparation of dilution tubes and colony dishes
    - load 66, 60 mm petri dishes with 4 ml MEMA
    - load 40 T-tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2, X.3, X.4, X.5 etc.
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 10 ml wash MEMA
29. Centrifuge tubes for 10 min at 2000 rpm, 4°C
30. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
31. Determine cell concentration by transfering 100 µl to Coulter cup
32. Vortex tube, transfer 0.5 ml into dilusion tube X.5, vortex tube X.5, 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.
33. 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.
34. Transfer 300 µl of cell suspension (in triplicate) to gamma tubes for each tube
35. Incubate petridishes for 1 week
36. Count gamma tubes for radioactivity **Date/Time :** 08/15/00 10:00 a.m.
37. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
38. Count colonies. There must be between 25 and 250 colonies for the flask to be a valid data point.

Preparation of ^{125}I IUdR in MEHB

Prepare 5ml of 6μci/ml

= 30 μci required

Stock on 07/28/00 0.467 μci/μl

on 08/10/00 0.467 × 0.861

= 0.40 μci/μl

$$\text{vol required} = \frac{30}{0.40} = 75\ \mu l$$

① Take 75 μl of stock
② Keep at RT for 3-4h
③ Add 5 ml of MEHB

08/11/00

10 µl maximum
08/11/00;
1-00 pm

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

1	1.00	1M	0	42
2	1.00		0	0
3	1.00		0	0
4	1.00	2M	0	0
5	1.00		0	0
6	1.00		0	0
7	1.00	3M	3352	6862
8	1.00		3517	6945
9	1.00		3656	7230
10	1.00	4M	7273	14638
11	1.00		7121	13941
12	1.00		7114	14233
13	1.00	5M	10685	21084
14	1.00		10869	21747
15	1.00		11820	23290
16	1.00	6M	19884	39452
17	1.00		20053	39468
18	1.00		18205	35614
19	1.00	7M	30984	60962
20	1.00		32067	62858
21	1.00		32913	65918
22	1.00	8M	0	37
23	1.00		0	66
24	1.00		0	38
25	1.00	9M	0	25
26	1.00		0	38
27	1.00		0	33
28	1.00	10M	3698	7574
29	1.00		3783	7745
30	1.00		3698	7520
31	1.00	11M	7285	14703
32	1.00		7271	14706
33	1.00		7280	14759
34	1.00	12M	8023	16376
35	1.00		7373	14817
36	1.00		8941	17447
37	1.00	13M	18794	37368
38	1.00		19620	39119
39	1.00		20280	40131

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

~~240~~	1.00	14M	28556	56505
~~241~~	1.00		29126	57408
~~242~~	1.00		24221	48628

$e^{-\frac{0.693 \times 17}{1440}} = 0.9918$

4

08/co/00; 7-00 pm

t = 12h + 5h = 17h

TABLE-1

Expt. # : 3 **Date/Time :** 08/11/00; 1-00 p.m.

Tube #	Medium count for 10 ul (cpm)	Avg. cpm	dpm [cpm/0.7438] e=0.506 y=1.47	µCi/ml (A_t) on counting [dpm/22200]	µCi/ml (A_o) on addition [$A_t/e^{-\lambda t}$]
1					
2					
3		3508	4716	0.212	0.214
4		7169	9638	0.434	0.437
5		11124	14956	0.673	0.679
6		19380	26056	1.17	1.18
7		31988	43006	1.9	1.95
8					
9					
10		3726	5009	0.225	0.227
11		7278	9785	0.4408	0.445
12		8112	10906	0.4912	0.4962
13		19564	26303	1.18	1.197
14		27301	36704	1.65	1.66

08/15/00 ;
10:00 a.m.

300 µl cells

1	1.00	0	280	
2	1.00	0	105	
3	1.00	0	237	1.21
4	1.00	0	194	
5	1.00	0	260	
6	1.00	0	254	
7	1.00	28054	55882	
8	1.00	27516	57219	2.0
9	1.00	26831	56979	
10	1.00	59149	123563	
11	1.00	59857	119778	2.0
12	1.00	60247	124502	
13	1.00	94391	186788	
14	1.00	89974	177132	1.97
15	1.00	92756	183236	
16	1.00	187385	371162	
17	1.00	175700	344690	1.99
18	1.00	167015	328839	
19	1.00	323110	638727	1.95
20	1.00	321100	629382	
21	1.00	276173	532357	
22	1.00	0	339	
23	1.00	0	237	
24	1.00	0	211	
25	1.00	0	176	
26	1.00	0	223	
27	1.00	0	198	
28	1.00	28644	60100	2.02
29	1.00	28439	58304	
30	1.00	29343	57562	
31	1.00	61051	121890	1.99
32	1.00	62259	124837	
33	1.00	62155	124479	
34	1.00	92953	179923	1.93
35	1.00	91140	176437	
36	1.00	89693	174372	
37	1.00	215829	421475	1.94
38	1.00	211462	411872	
39	1.00	207356	401721	
40	1.00	283733	545619	1.92
41	1.00	317429	612141	
42	1.00	273402	523713	

08/11/00 ; 9.00 a.m.

96 h + 1 h = 97 h

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 97}{1440}}$

$= 0.9544$

TABLE-2

Expt. # : 3 **Date/Time :** 08/15/00; 10.00 a.m

Tube #	Radioactivity for 300 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.7438]	µCi/ml (A_t) on counting [dpm/666000]	µCi/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1					
2					
3		27467	36927	0·055	0·058
4		59751	80332	0·1206	0·1263
5		92373	124191	0·1864	0·1954
6		176700	237563	0·3567	0·3737
7		306794	412468	0·6193	0·6489
8					
9					
10		28808	38731	0·0581	0·061
		61821	83115	0·1247	0·1307
		91262	122696	0·1842	0·193
		211549	284416	0·4270	0·4474
		291321	391935	0·5884	0·6165

TABLE-3

Expt. # : 3 **Date/Time :** 08/14/00

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [uCi/ml x10^6 Cells/ml]	kBq/cluster [pCi/cell x148
1	507, 519, 531				
2	532, 557, 525				
3	536, 542, 562	546	2186666	0·0265	3·92
4	569, 576, 582	575	2302666	0·0548	8·11
5	622, 607, 619	616	2464000	0·0793	11·74
6	551, 572, 561	561	2245333	0·1665	24·64
7	582, 571, 591	581	2325333	0·279	41·30
8	571, 581, 592				
9	566, 519, 539				
10	569, 532, 547	549	2197333	0·0277	4·11
11	539, 555, 567	553	2214666	0·059	8·73
12	572, 599, 581	584	2336000	0·0826	12·23
13	511, 514, 492	505	2022666	0·2211	32·73
14	541, 531, 561	544	2177333	0·283	41·91

TABLE-4

Expt # : **Date :**

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1·2	131	111	119	} 123·33	
2·2	143	129	107	}	
3·2	39	48	58	48·3	0·3918
4·2	22	30	37	29·6	0·2405
5·2	19	20	22	20·3	0·1648
6·3	47	55	65	5·56	0·0451
7·4	90	99	80	0·89	0·0073
8·2	151	149	161	} 145·0	
9·2	143	137	129	}	
10·2	38	48	29	38·8	0·2643
11·2	16	18	14	16	0·1103
12·3	65	72	80	7·23	0·0498
13·4	24	30	37	0·30	0·0021
14·4	9	7	6	0·07	0·0005

V79 COLONY FORMING ASSAY

Experiment Name : ^{125}IUdR cluster with lindane (50% label) **Exp. # :** 2;

Experiment performed by: A. Bishayee **Date:** 07/20/00

1. Set the rocker-roller at 37^{0}C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 175 cm^2 flask, subcultured 1:2, 24h before) with PBS, trypsinize cells, each resuspend in 9 ml MEMB, pool, 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 ~2,000,000 cells/ml in MEMB [Actual count: 2036000 cells/ml)
3. Transfer 1 ml of cell suspension into 14 ml tubes (Falcon plastic test tube, 17x100 mm)
4. Keep the tubes in the roller for 3-4 h at 37°C, 5% CO_2 **Date/Time:** 07/20/00; 3-00 pm
5. Prepare MEMB containing radioactivity in hood

   µl ^{125}IUdR (prepared on 07/13/00) + 2 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:** 07/20/00; 7-00 pm

Tube #	^{125}IUdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{125}IUdR [16 uCi/ml] (ml)	100 uM Lindane in MEMA (ml)	MEMA (ml)
1	0	1.0	1.0	0	0.4	0
2	0	1.0	1.0	0	0.4	0
3	0.1	1.0	0.987	0.013	0.4	0
4	0.2	1.0	0.975	0.025	0.4	0
5	1	1.0	0.875	0.125	0.4	0
6	2	1.0	0.75	0.25	0.4	0
7	4	1.0	0.5	0.5	0.4	0
8	0	1.0	1.0	0	0	0.4
9	0	1.0	1.0	0	0	0.4
10	0.1	1.0	0.987	0.013	0	0.4
11	0.2	1.0	0.975	0.025	0	0.4
12	1	1.0	0.875	0.125	0	0.4
13	2	1.0	0.75	0.25	0	0.4
14	4	1.0	0.5	0.5	0	0.4

92 µl → 2 ml

7. Return test tubes to roller for 12 h . **Date/Time:** 07/20/00, 7-30 pm
8. Next day, while test tubes are in shaker 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:** 07/21/00, 9-30 a.m.
10. Remove buckets from centrifuge and carefully remove 100 µl of supernatant and place in prelabeled gamma-tube.
11. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
12. Centrifuge tubes for 10 min at 2000 rpm, 4°C
13. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
14. Centrifuge tubes for 10 min at 2000 rpm, 4°C
15. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
16. Centrifuge tubes for 10 min at 2000 rpm, 4°C
17. Similarly wash the cells that were not labelled, pooled cells from two tubes, resuspend in 8 ml wash MEMA
18. Decant supernatant, click tubes, vortex, transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips
18. Again add 200 ul ice cold MEMA with or without 100 uM lindane as per the table, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
19. Centrifuge tubes for 5 min at 1000 rpm, 4°C
20. Transfer tubes at 10°C for 72 h. **Date/Time:** 07/21/00, 12-00
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: 07/21/00, 1-00 pm

22. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the content to ten 12 ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA by using pasteur pipet

Date/Time: 07/24/00, 2-00 pm

23. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes
24. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***
25. Labeling and preparation of dilution tubes and colony dishes
    - load 66, 60 mm petri dishes with 4 ml MEMA
    - load 40 T-tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2, X.3, X.4, X.5 etc.
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 10 ml wash MEMA
29. Centrifuge tubes for 10 min at 2000 rpm, 4°C
30. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
31. Determine cell concentration by transfering 100 µl to Coulter cup
32. Vortex tube, transfer 0.5 ml into dilusion tube X.5, vortex tube X.5, 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.
33. 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.
34. Transfer 300 µl of cell suspension (in triplicate) to gamma tubes for each tube
35. Incubate petridishes for 1 week
36. Count gamma tubes for radioactivity **Date/Time :** 07/24/00 4:00 pm
37. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
38. Count colonies. There must be between 25 and 250 colonies for the flask to be a valid data point.

07/20/00

Prepare 2 ml of 16 μci/ml ^{125}IVGR
= 32 μci required

Stock activity = 0.38 × 0.922 μci/ml
= 0.35 μci/ml

$$\text{Stock required} = \frac{32}{0.35} = 92 \text{ ml}$$

① Take 92 μl of stock, keep at RT for 4-5 h
② Add 2 ml of MEMB

07/21/00
10 pt medium
1:00pm ~~12:00 noon~~

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

1	1.00	0	30
2	1.00	0	0
3	1.00	0	0
4	1.00	0	0
5	1.00	0	0
6	1.00	0	0
7	1.00	474	1239
8	1.00	558	1436
9	1.00	593	1476
10	1.00	1033	2368
11	1.00	1135	2633
12	1.00	1246	2801
13	1.00	6373	13050
14	1.00	6575	13422
15	1.00	7219	14731
16	1.00	11921	24660
17	1.00	15605	31897
18	1.00	13002	26644
19	1.00	27545	55883
20	1.00	26656	54292
21	1.00	27335	55656
22	1.00	0	87
23	1.00	0	84
24	1.00	0	124
25	1.00	0	179
26	1.00	0	129
27	1.00	0	77
28	1.00	510	1384
29	1.00	570	1554
30	1.00	491	1425
31	1.00	1220	2973
32	1.00	1144	2724
33	1.00	1075	2520
34	1.00	8053	16596
35	1.00	6528	13370
36	1.00	7233	14765
37	1.00	14662	29921
38	1.00	15220	30670
39	1.00	14441	29420
40	1.00	20456	41873
41	1.00	29269	59009
42	1.00	27590	56086

TABLE-1

Expt. # : 2 **Date/Time :** 07/21/00, 1-00 pm

Tube #	Medium count for 10 ul (cpm)	Avg. cpm	dpm [cpm/0.7438] e=0.506 y=1.47	µCi/ml (A_t) on counting [dpm/22200]	µCi/ml (A_o) on addition [$A_t/e^{-\lambda t}$]
1					
2					
3		541	728	0·033	
4		1138	1529	0·068	
5		6722	9037	0·407	
6		13509	18162	0·818	
7		27178	36540	1·64	
8					
9					
10		523	704	0·032	
11		1146	1541	0·069	
12		7271	9775	0·441	
13		14774	19863	0·8947	
14		25771	34648	1·56	

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 78.5}{1440}}$

$= 0.9629$

$72h + 6.5h$

$= 78.5h$

300µl cells

07/24/00

4:00 pm

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

1	1.00	1C	0	28
2	1.00		0	19
3	1.00		0	0
4	1.00	2C	0	79
5	1.00		0	94
6	1.00		0	142
7	1.00	3C	11433	22474
8	1.00		11298	21655
9	1.00		11040	21687
10	1.00	4C	22751	43735
11	1.00		21572	42058
12	1.00		21564	41464
13	1.00	5C	65573	126043
14	1.00		66788	128660
15	1.00		67268	128345
16	1.00	6C	156547	296627
17	1.00		158722	301574
18	1.00		156644	295488
19	1.00	7C	321235	603447
20	1.00		319299	596766
21	1.00		320269	598771
22	1.00	8C	0	388
23	1.00		0	328
24	1.00		0	273
25	1.00	9C	0	381
26	1.00		0	234
27	1.00		0	267
28	1.00	10C	10614	20345
29	1.00		11134	21295
30	1.00		11194	21535
31	1.00	11C	26124	49855
32	1.00		27034	50927
33	1.00		25254	47573
34	1.00	12C	89011	165338
35	1.00		89019	167313
36	1.00		87355	161443
37	1.00	13C	178168	329580
38	1.00		175150	321662
39	1.00		173262	318814
40	1.00	14C	342958	627587
41	1.00		353186	639412
42	1.00		365542	663926

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

TABLE-2

Expt. # : 2 **Date/Time :** 07/24/00; 4-00 pm

Tube #	Radioactivity for 300 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.7438]	µCi/ml (A_t) on counting [dpm/666000	µCi/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1					
2					
3		11257	15134	0·022	0·0235
4		21902	29527	0·0443	0·0460
5		66543	89463	0·1343	0·1395
6		157304	211487	0·3175	0·3297
7		320267	430583	0·6465	0·6714
8					
9					
10		10980	14762	0·0221	0·0230
11		26137	35140	0·0527	0·0547

07/21/00; 9-30 a.m

12	88461	118932	0·1785	0·1854
13	175526	235986	0·3543	0·3679
14	353395	475793	0·7144	0·7419

TABLE-3

Expt. # : 2 **Date/Time :** 07/24/00

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [uCi/ml x10^6 Cells/ml]	kBq/cluster [pCi/cell x148]
1	407, 403, 401				
2	420, 410, 447				
3	520, 535, 541	532	2128000	0·011	1·634
4	559, 572, 566	565	2262666	0·020	3·008
5	511, 519, 531	520	2081333	0·067	9·919
6	551, 561, 535	549	2196000	0·1501	22·220
7	521, 537, 518	525	2101333	0·3195	47·287
8	532, 541, 553				
9	481, 472, 461				
10	495, 499, 519	504	2017333	0·011	1·687
11.	512, 507, 532	517	2068000	0·026	3·914
12	455, 439, 429	441	1764000	0·105	15·55
13	595, 609, 589	597	2390666	0·1538	22·775
14	575, 581, 562	572	2290666	0·3238	47·934

TABLE-4

Expt # : 2 **Date :** 07/31/06

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony (for x.2)	SF
1.2	107	119	105	103.33	
2.2	99	101	89		
3.2	73	66	60	66.33	0.6419
4.2	41	49	59	49.66	0.4806
5.2	16	25	20	20.33	0.1967
6.3	39	47	58	4.8	0.0464
7.4	55	64	73	0.64	0.0062
8.2	120	117	110	107.66	
9.2	107	100	92		
10.2	54	63	73	63.33	0.5882
11.2	29	42	35	35.33	0.3281
12.3	24	18	14	18.66	0.0173
13.4	75	84	69	0.76	0.0071
14.4	8	6	4	0.06	0.00056

w/ inhibitor

w/o inhibitor (control)

V79 COLONY FORMING ASSAY

Experiment Name : ^{125}IudR cluster with lindane (50% label) **Exp. # :** 1;

Experiment performed by: A. Bishayee **Date:** 07/13/00

1. Set the rocker-roller at 37^{0}C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 175 cm^2 flask, subcultured 1:2, 24h before) with PBS, trypsinize cells, each resuspend in 9 ml MEMB, pool, 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 ~2,000,000 cells/ml in MEMB [Actual count : **2,200,000** cells/ml)
3. Transfer 1 ml of cell suspension into 14 ml tubes (Falcon plastic test tube, 17x100 mm)
4. Keep the tubes in the roller for 3-4 h at 37°C, 5% CO_2 **Date/Time:** 07/13/00; 4-00 pm
5. Prepare MEMB containing radioactivity in hood

   84 µl ^{125}IUdR (prepared on 07/13/00) + 2 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:** 07/13/00; 7-00 pm

Tube #	^{125}IUdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{125}IUdR 16.0 [~~8.0~~ uCi/ml] (ml)	100 uM Lindane in MEMA (ml)	MEMA (ml)
1	0	1.0	1.0	0	0.4	0
2	0	1.0	1.0	0	0.4	0
3	0.1	1.0	0.987	0.013	0.4	0
4	0.2	1.0	0.975	0.025	0.4	0
5	1	1.0	0.875	0.125	0.4	0
6	2	1.0	0.75	0.25	0.4	0
7	4	1.0	0.5	0.5	0.4	0
8	0	1.0	1.0	0	0	0.4
9	0	1.0	1.0	0	0	0.4
10	0.1	1.0	0.987	0.013	0	0.4
11	0.2	1.0	0.975	0.025	0	0.4
12	1	1.0	0.875	0.125	0	0.4
13	2	1.0	0.75	0.25	0	0.4
14	4	1.0	0.5	0.5	0	0.4

7. Return test tubes to roller for 12 h. **Date/Time:** 07/13/00; 7-30 pm
8. Next day, while test tubes are in shaker 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:** 07/14/00; 9-30 a.m.
10. Remove buckets from centrifuge and carefully remove 100 µl of supernatant and place in prelabeled gamma-tube.
11. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
12. Centrifuge tubes for 10 min at 2000 rpm, 4°C
13. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
14. Centrifuge tubes for 10 min at 2000 rpm, 4°C
15. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
16. Centrifuge tubes for 10 min at 2000 rpm, 4°C
17. Similarly wash the cells that were not labelled, pooled cells from two tubes, resuspend in 8 ml wash MEMA
18. Decant supernatant, click tubes, vortex, transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips
18. Again add 200 ul ice cold MEMA with or without 100 uM lindane as per the table, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
19. Centrifuge tubes for 5 min at 1000 rpm, 4°C
20. Transfer tubes at 10°C for 72 h. **Date/Time:** 07/14/00; 11-30 pm
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: 07/14/00; 4-00 pm

22. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the content to ten 12 ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA by using pasteur pipet

Date/Time: 07/17/00; 10-00 a.m.

23. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes
24. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***
25. Labeling and preparation of dilution tubes and colony dishes
    - load 66, 60 mm petri dishes with 4 ml MEMA
    - load 40 T-tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2, X.3, X.4, X.5 etc.
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 10 ml wash MEMA
29. Centrifuge tubes for 10 min at 2000 rpm, 4°C
30. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
31. Determine cell concentration by transfering 100 µl to Coulter cup
32. Vortex tube, transfer 0.5 ml into dilusion tube X.5, vortex tube X.5, 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.
33. 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.
34. Transfer 300 µl of cell suspension (in triplicate) to gamma tubes for each tube
35. Incubate petridishes for 1 week
36. Count gamma tubes for radioactivity **Date/Time :** 07/17/00, 2:00 pm
37. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
38. Count colonies. There must be between 25 and 250 colonies for the flask to be a valid data point.

07/13/00

Preparation of ^{125}I/UdR in HEMB

Prepare 16 μci/ml of ^{125}I/UdR (≈ Total 2 ml)

= 32 μci required

0.38 μci/μl

Stock ~~155 μci/ml~~ on 07/13/00; 3:30 pm

Volume required = ~~[illegible]~~ 32 / 0.38 = 84 μl

① Take 84 μl of Stock, Keep at RT for 3-4h

② Add 2 ml of HEMB (16 μci/ml)

07/14/00

4-00 pm.

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

1	1.00	1M	0	187
2	1.00		0	45
3	1.00		0	58
4	1.00	2M	0	118
5	1.00		0	76
6	1.00		0	60
7	1.00	3M	1467	3126
8	1.00		1569	3255
9	1.00		1352	2961
10	1.00	4M	2838	5910
11	1.00		3197	6596
12	1.00		2844	5957
13	1.00	5M	15811	30993
14	1.00		15899	30951
15	1.00		12891	25125
16	1.00	6M	37208	72997
17	1.00		37388	73449
18	1.00		34078	66788
19	1.00	7M	70103	137531
20	1.00		70029	137896
21	1.00		66968	131342
22	1.00	8M	0	163
23	1.00		0	159
24	1.00		0	200
25	1.00	9M	0	108
26	1.00		0	349
27	1.00		0	136
28	1.00	10M	1460	3310
29	1.00		1505	3336
30	1.00		1412	3176
31	1.00	11M	3132	6705
32	1.00		2962	6098
33	1.00		2999	6007
34	1.00	12M	16498	32813
35	1.00		16478	32423
36	1.00		14873	29569
37	1.00	13M	33518	65738
38	1.00		37962	74354
39	1.00		35627	68940
40	1.00	14M	68902	134461
41	1.00		72363	141338
42	1.00		73490	142861

07/13/00;

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

TABLE-1

Expt. # : 1 **Date/Time :** 07/14/00, 4-00 pm

Tube #	Medium count for 10 ul (cpm)	Avg. cpm	dpm [cpm/0.7438] e=0.506 y=1.47	µCi/ml (A_t) on counting [dpm/22200]	µCi/ml (A_o) on addition [$A_t/e^{-\lambda t}$]	
1						
2						
3		1462	1966	0·088	~~0·092~~	0·087
4		2959	3979	0·1792	~~0·1863~~	0·1774
5		14867	19987	0·9003	~~0·935~~	0·891
6		36224	48702	2·19	~~2·28~~	2·16
7		69033	92811	4·18	~~4·34~~	4·13
8						
9						
10		1459	1961	0·088	~~0·0918~~	0·087
11		3031	4075	0·1835	~~0·1908~~	0·184
12		~~35702~~ 15949	21443	0·96	~~1·02~~	0·9504
13		35702	47999	2·16	~~2·24~~	2·13
14		71585	96242	4·33	~~4·50~~	4·28

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 20.5}{1440}}$

$= 0.990$

07/16/00, 7-30 pm

$t = 12h + 8.5h$

$= 20.5h$

07/17/00

2-00 pm

300 µl cells

2	1.00	0	200
3	1.00	1c 0	250
4	1.00	0	178
5	1.00	0	255
6	1.00	2c 0	187
7	1.00	0	150
8	1.00	11077	21815
9	1.00	3c 11887	23419
10	1.00	12127	24114
11	1.00	25144	49217
12	1.00	4c 24454	48055
13	1.00	25064	49357
14	1.00	105262	204659
15	1.00	5c 106244	206480
16	1.00	105065	203949
17	1.00	241651	466790
18	1.00	6c 231587	446096
19	1.00	238454	458209
20	1.00	396328	756090
21	1.00	7c 397126	752160
22	1.00	403185	760704
23	1.00	6830	13236
24	1.00	8c 6158	12253
25	1.00	6316	12376
26	1.00	745	1868
27	1.00	9c 788	1990
28	1.00	888	2065
29	1.00	16186	31294
30	1.00	10c 16173	31400
31	1.00	15919	30687
32	1.00	27975	53567
33	1.00	11c 26169	49986
34	1.00	27382	52705
35	1.00	116620	222201
36	1.00	12c 115108	218844
37	1.00	113314	214527
38	1.00	208262	391749
39	1.00	13c 212894	400376
40	1.00	202175	378429
41	1.00	398384	738413
42	1.00	14c 406322	752714
43	1.00	414473	757622
1	.51	0	41

High count! tubes must have been contaminated!

TABLE-2

Expt. # : 1 **Date/Time :** 07/17/00; 2-00 pm.

Tube #	Radioactivity for 300 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.7438]	µCi/ml (A_t) on counting [dpm/666000]	µCi/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1					
2					
3	~~612, 633, 649~~	11697	15726	0·0236	0·0244
4		24887	33459	0·0502	0·0521
5		105523	141871	0·213	0·2210
6		237230	318944	0·478	0·4968
7		398879	536272	0·8052	0·8354
8 9		0 0			
~~9~~10 11		16092 27175	21635 36535	0·032 0·0548	0·0337 0·0569
~~10~~12		115014	154630	0·2324	0·2408
13		207777	279345	0·4194	0·4351
14		406393	546374	0·820	0·8511

07/14/00; 9-30 a.m

t = 72h + 4.5h
= 76.5

$e^{-\lambda t} = e^{-\frac{0.693 \times 76.5}{1440}} = 0.9638$

TABLE-3

Expt. # : 1 **Date/Time :** 07/17/00

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [uCi/ml x10^6 Cells/ml]	kBq/cluster [pCi/cell x148
1	612, 648, 631				
2	652, 639, 627				
3	612, 633, 649	631	2525333	0·009	1·43
4	656, 677, 642	658	263333	0·019	2·93
5	607, 592, 580	593	2372000	0·093	13·79
6	657, 671, 662	663	2653333	0·1872	27·71
7	641, 639, 621	633	2534666	0·329	48·78
8	526, 536, 546				
9	630, 659, 662				
10	599, 619, 640	619	2477333	0·013	2·01
11	613, 637, 651	633	2534666	0·022	3·32
12	673, 685, 666	674	2698666	0·0892	13·21
13	581, 574, 583	579	2317333	0·1877	27·78
14	679, 659, 651	663	2692000	0·320	47·49

TABLE-4

Expt # : 1 **Date :** 07/24/00

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1·2	135	161	149	144·5	
2·2	122	149	151		
3·2	97	108	88	97·6	0·6758
4·2	65	75	87	75·6	0·5236
5·3	160	142	176	159·3	0·1102
~~6·3~~	~~35~~	~~44~~	~~54~~	~~4·43~~	~~0·0306~~
7·4	66	73	80	0·73	0·0051
8·2	156	166	169	158·5	
9·2	161	149	150		
10·2	63	82	71	72	0·4542
11·2	52	59	66	59	0·3722
12·3	91	80	72	8·1	0·0511
13·3	111	121	98	11	0·00694
14·4	9	14	20	0·14	0·0009

[redacted] 50%

V79 COLONY FORMING ASSAY

Experiment Name : ^{125}IUdR toxicity (50% label) **Exp. # : 3; Experiment**
performed by: A. Bishayee **Date:** 06/22/00

1. Set the rocker-roller at 37^{0}C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 175 cm^2 flask, subcultured 1:2, 24h before) with PBS, trypsinize cells, each resuspend in 9 ml MEMB, pool, 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 ~2,000,000 cells/ml in MEMB [Actual count : 2,240,000cells/ml)
3. Transfer 1 ml of cell suspension into 14 ml tubes (Falcon plastic test tube, 17x100 mm)
4. Keep the tubes in the roller for 3-4 h at 37°C, 5% CO_2 **Date/Time:** 06/22/00 4-00 pm
5. Prepare MEMB containing radioactivity in hood

   72 µl ^{125}IUdR (prepared on 06/12/00) + 2 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:** 06/22/00; 6-00 pm

Tube #	^{125}IUdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{125}IUdR [8.0 uCi/ml]
1	0	1.0	1.0	0
2	0	1.0	1.0	0
3	0.01	1.0	0.997	0.003
4	0.05	1.0	0.987	0.013
5	0.1	1.0	0.975	0.025
6	0.2	1.0	0.950	0.05
7	0.5	1.0	0.875	0.125
8	1	1.0	0.75	0.25
9	2	1.0	0.5	0.5
10	4	1.0	0	1.0

7. Return test tubes to roller for 12 h . **Date/Time:** 06/22/00; 6-30 pm
8. Next day, while test tubes are in shaker 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:** 06/23/00; 9-00 a.m.
10. Remove buckets from centrifuge and carefully remove 100 µl of supernatant and place in prelabeled gamma-tube.
11. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
12. Centrifuge tubes for 10 min at 2000 rpm, 4°C
13. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
14. Centrifuge tubes for 10 min at 2000 rpm, 4°C
15. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
16. Centrifuge tubes for 10 min at 2000 rpm, 4°C
17. Similarly wash the cells that were not labelled, pooled cells from two tubes, resuspend in 8 ml wash MEMA
18. Decant supernatant, click tubes, vortex, transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips
18. Again add 200 ul ice cold MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
19. Centrifuge tubes for 5 min at 1000 rpm, 4°C
20. Transfer tubes at 10°C for 72 h. **Date/Time:** 06/23/00; 12-00
21. Transfer 10 ul supernatant in three sets of tubes containing small pieces of tissue paper from 100 ul supernatant removed earlier (Step 12) and count them for radioactivity

    Date/Time: 06/23/00; 1-00 pm
22. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the content to ten 12 ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA by using pasteur pipet

    Date/Time: 06/26/00; 2-00 pm.
23. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes
24. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***
25. Labeling and preparation of dilution tubes and colony dishes
    - load 66, 60 mm petri dishes with 4 ml MEMA
    - load 40 T-tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2, X.3, X.4, X.5 etc.
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 10 ml wash MEMA

29. Centrifuge tubes for 10 min at 2000 rpm, 4°C
30. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
31. Determine cell concentration by transfering 100 µl to Coulter cup
32. Vortex tube, transfer 0.5 ml into dilusion tube X.5, vortex tube X.5, 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.
33. 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.
34. Transfer 300 µl of cell suspension (in triplicate) to gamma tubes for each tube
35. Incubate petridishes for 1 week
36. Count gamma tubes for radioactivity **Date/Time :** 06/26/00; 5-00 pm.
37. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
38. Count colonies. There must be between 25 and 250 colonies for the flask to be a valid data point.

06/22/00

Stock on 06/19/00 = 0.23 μci/ml

Stock on 06/21/00 = 0.23 × 0.977

= 0.22 μci/ml

e^{-xt}

$= e^{-\frac{0.693 \times 2 \times 24}{1440}}$

= 0.977

Prepare 2 ml of 8 μci/ml = 16 μci required

Vol. of stock required = $\frac{16}{0.22}$ = 71.2 ml

① Take 72 ml of stock
② Keep at RT for 4-5h
③ Add 2 ml of MEM/3

10/pl medium

06/23/00
1-00 PM

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

1	1.00		0	0
2	1.00	1M	0	0
3	1.00		0	0
4	1.00		0	0
5	1.00	2M	0	29
6	1.00		0	37
7	1.00		44	342
8	1.00	3M	30	351
9	1.00		68	436
10	1.00		635	1526
11	1.00	4M	704	1668
12	1.00		639	1600
13	1.00		1300	2868
14	1.00	5M	1329	2821
15	1.00		1229	2665
16	1.00		2153	4433
17	1.00	6M	2247	4691
18	1.00		2034	4333
19	1.00		5880	11546
20	1.00	7M	6019	11843
21	1.00		6143	12180
22	1.00		12667	24928
23	1.00	8M	12154	23728
24	1.00		11136	21652
25	1.00		24443	47386
26	1.00	9M	25992	50346
27	1.00		27560	54145
28	1.00		49149	94762
29	1.00	10M	52390	100507
30	1.00		51008	97768

TABLE-1

Expt. # : 3 **Date/Time :** 06/~~22~~/00, 1-00 pm

Tube #	Medium count for 10 ul (cpm)	Avg. cpm	dpm [cpm/0.7438] e=0.506 y=1.47	µCi/ml (A_t) on counting [dpm/22200]	µCi/ml (A_o) on addition [$A_t/e^{-\lambda t}$]
1					
2					
3		47.3	63	0.0028	0.0029
4		659	886	0.039	0.040
5		1286	1728	0.077	0.078
6		2144	2883	0.129	~~0.3110~~ 0.1310
7		6014	8085	0.3642	0.3674
8		11985	16114	0.725	0.7323
9		25998	34953	1.57	1.58
10		50849	68363	3.07	3.11

06/29/00, 6-30 pm

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 18.5}{1440}}$

$= 0.9911$

t : 12h + 6.5h

= 18.5h

300 µl cells

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

1	1.00	1c	0	179
2	1.00		0	78
3	1.00		0	79
4	1.00	2c	0	61
5	1.00		0	52
6	1.00		0	20
7	1.00	3c	1699	3681
8	1.00		1775	3779
9	1.00		1874	3951
10	1.00	4c	5905	11837
11	1.00		11463	22755
12	1.00		5934	12043
13	1.00	5c	5890	11682
14	1.00		11837	23669
15	1.00		12371	24596
16	1.00	6c	19942	39463
17	1.00		21439	42168
18	1.00		21303	41825
19	1.00	7c	53337	105262
20	1.00		52894	104090
21	1.00		50722	100018
22	1.00	8c	101126	199225
23	1.00		103605	204534
24	1.00		105948	208564
25	1.00	9c	167928	331408
26	1.00		177736	347191
27	1.00		183647	360508
28	.84	10c	520318	1006424
29	1.00		480650	929947
30	1.00		150732	292323

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

TABLE-2

Expt. # : 3 **Date/Time :** 06/26/00; 5-00 pm

Tube #	Radioactivity for 300 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.7438]	µCi/ml (A_t) on counting [dpm/666000]	µCi/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1					
2					
3		1782	2396	0·0035	0·0037
4		5909	7945	0·0119	0·0124
5		11890	15985	0·024	0·0249
6		20894	28091	0·0421	0·0438
7		52317	70338	0·1056	0·1097
8		103559	139230	0·2090	0·2172
9		176437	237210	0·3562	0·3701
10		383900	516133	0·7749	0·8053

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 80}{1440}}$

$= 0.9623$

06/23/00 : 9-00 a.m.

$t = 72 + 8 h$

$= 80 h$

TABLE-3

Expt. # : 3 **Date/Time :** 06/26/00, 4:00 pm

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [uCi/ml x10^6 Cells/ml]	kBq/cluster [pCi/cell x148]
1	611, 629, 633	624			
2	590, 631, 619	613			
3	582, 571, 585	579	2317333	0.0016	0.236
4	662, 679, 649	663	2653333	0.0046	0.692
5	562, 582, 592	578	2314666	0.0107	1.59
6	637, 639, 655	643	2574666	0.017	2.52
7	581, 609, 622	604	2416000	0.045	6.72
8	629, 639, 641	636	2545333	0.0853	12.63
9	693, 688, 672	684	2737333	0.1352	20.01
10	762, 777, 749	762	3050666	0.2639	39.07

TABLE-4

Expt # : 3 **Date :** 07/03/00

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1·2	135	147	129	126·83	
2·2	121	111	118		
3·2	107	114	124	115	0·9067
4·2	110	119	99	109.3	0·8620
5·2	75	67	60	67·3	0·5308
6·2	38	45	56	46·3	0·3653
7·2	32	27	21	26·6	0·2102
8·3	83	101	117	10·03	0·0791
9·4	80	120	160	1·2	0·009
10·4	11	7	4	0·07	0·0005

V79 COLONY FORMING ASSAY

Experiment Name : ^{125}IUdR toxicity (50% label) **Exp. # :** 2;
Experiment performed by: A. Bishayee **Date:** 09/27/99

1. Set the rocker-roller at 37°C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 150 cm^2 flusk, subcultured 1:2, 24h before) with PBS, trypsinize cells, each resuspend in 9 ml MEMB, pool, 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 ~2,000,000 cells/ml in MEMB [Actual count : cells/ml)
3. Transfer 1 ml of cell suspension into 14 ml tubes (Falcon plastic test tube, 17x100 mm)
4. Keep the tubes in the roller for 3-4 h at 37°C, 5% CO_2 **Date/Time:** 09/27/99 4-00 p.m.
5. Prepare MEMB containing radioactivity in hood
   70 µl ^{125}IUdR (prepared on 08/24/99) + 2 ~~ml~~ MEMB
6. After 3-4 h, remove test tubes from ~~shaker~~ roller and add MEMB with or without radioactivity according to Table below. **Date/Time:** 09/27/99; 7-10 p.m.

Tube #	^{125}IUdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{125}IUdR [8.0 uCi/ml]
1	0	1.0	1.0	0
2	0	1.0	1.0	0
3	0.01	1.0	0.997	0.003
4	0.05	1.0	0.987	0.013
5	0.1	1.0	0.975	0.025
6	0.2	1.0	0.950	0.05
7	0.5	1.0	0.875	0.125
8	1	1.0	0.75	0.25
9	2	1.0	0.5	0.5
10	4	1.0	0	1.0

7. Return test tubes to shaker for 12 h . **Date/Time:** 09/27/99; 7-30 p.m.
8. Next day, while test tubes are in ~~shaker~~ roller 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:** 09/28/99; 9-00 a.m.
10. Remove buckets from centrifuge and carefully remove 100 µl of supernatant and place in prelabeled gamma-tube.
11. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
12. Centrifuge tubes for 10 min at 2000 rpm, 4°C
13. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
14. Centrifuge tubes for 10 min at 2000 rpm, 4°C
15. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
16. Centrifuge tubes for 10 min at 2000 rpm, 4°C
17. Similarly wash the cells that were not labelled, pooled cells from two tubes, resuspend in 8 ml wash MEMA
18. Decant supernatant, click tubes, vortex, transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips
18. Again add 200 ul ice cold MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
19. Centrifuge tubes for 5 min at 1000 rpm, 4°C
20. Transfer tubes at 10°C for 72 h. **Date/Time:** 09/28/99; 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 (Step 12) and count them for radioactivity
Date/Time: 09/28/99; 12-00
22. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the content to ten 12 ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA by using pasteur pipet
Date/Time: 10/01/99; 10-30 a.m.
23. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes
24. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***
25. Labeling and preparation of dilution tubes and colony dishes
    - load 66, 60 mm petri dishes with 4 ml MEMA
    - load 40 T-tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2, X.3, X.4, X.5 etc.
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 10 ml wash MEMA
29. Centrifuge tubes for 10 min at 2000 rpm, 4°C
30. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times

through 3 cc syringe with 21 gauge needle

31. Determine cell concentration by transfering 100 µl to Coulter cup
32. Vortex tube, transfer 0.5 ml into dilusion tube X.5, vortex tube X.5, 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.
33. 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.
34. Transfer 300 µl of cell suspension (in triplicate) to gamma tubes for each tube
35. Incubate petridishes for 1 week
36. Count gamma tubes for radioactivity **Date/Time :** 10/01/99, 12-00 noon
37. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
38. Count colonies. There must be between 25 and 250 colonies for the flask to be a valid data point.

09/27/99

Stock on 09/23/99 0.25 μci/μl

Stock on 09/27/99 0.25 × 0.9548

e^{-rt} = 0.23 μci/μl

$= e - \frac{0.693 \times 4 \times 24}{1440}$

$= e - 0.0462$

$= 0.9548$

Prepare 2 ml of 8 μci/ml = 16 μci required

Vol taken = $\frac{16}{0.23}$ = 69.5 μl

Take 70 μl ^{125}I/UdR + 2 ml MEM

09/28/99

12-00 gram

1	1.00	Back 58	0	
2	1.00	99	0	
3	1.00	1M 81	0	1M
4	1.00	81	0	
5	1.00	2M 75	0	
6	1.00	81	0	
7	1.00	3M 204	0	
8	1.00	203	0	
9	1.00	4M 582	465	4M
10	1.00	666	534	
11	1.00	5M 1044	1446	5M
12	1.00	1140	1620	
13	1.00	6M 1992	3368	6M
14	1.00	2081	3601	
15	1.00	7M 5258	10371	7M
16	1.00	5484	10559	
17	1.00	8M 10393	20985	8M
18	1.00	11615	23718	
19	1.00	9M 22358	45939	9M
20	1.00	22979	47752	
21	1.00	10M 45805	95077	10M
22	1.00	50226	104972	

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE

TABLE-1

Expt. # : 7 ✓ **Date/Time :** 09/28/99; 12-00 noon

Tube #	Medium count for 10 ul (cpm)	Avg. cpm	dpm [cpm/0.7438] e=0.506 y=1.47	µ Ci/ml (A_t) on counting [dpm/22200]	µ Ci/ml (A_o) on addition [$A_t/e^{-\lambda t}$]
1		2			
2		-1			
3		125	167	0·0075	0·0076
4		545	732	0·033	0·033
5		1013	1361	0·061	0·061
6		1957	2631	0·1185	0·1194
7		5292	7114	0·3204	0·3229
8		10925	14688	0·6616	0·6662
9		22589	30370	1·36	1·38
10		47936	64448	2·90	2·92

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 15.5}{1440}}$

$= 0.9925$

09/28/99 ; 7-30 pm

= 12 + 3.5 hr

= 15.5 hr

10/01/99; 12-00

1	1.00	75	0
2	1.00	70	0
3	1.00	94	0
4	1.00	64	0
5	1.00	86	0
6	1.00	87	0
7	1.00	98	0
8	1.00	91	0
9	1.00	87	0
10	1.00	1956	3024
11	1.00	2019	3217
12	1.00	1923	2984
13	1.00	6910	12843
14	1.00	6721	12603
15	1.00	7008	13119
16	1.00	13161	25316
17	1.00	13304	25394
18	1.00	13485	26106
19	1.00	24587	48037
20	1.00	24658	47434
21	1.00	24712	47953
22	1.00	50750	99587
23	1.00	49266	96635
24	1.00	48985	95539
25	1.00	100828	196996
26	1.00	102526	200296
27	1.00	102885	200530
28	1.00	180707	353352
29	1.00	189491	369617
30	1.00	186903	363556
31	1.00	374297	727542
32	1.00	381225	737237
33	1.00	387641	744944
34	1.00	134	0
35	1.00	138	0
36	1.00	144	0
37	1.00	127	0
38	1.00	126	0
39	1.00	108	0
40	1.00	112	0
41	1.00	116	0
42	1.00	107	0
43	1.00	113	0
44	1.00	116	0

TABLE-2

Expt. # : 2 ✓ **Date/Time :** 10/01/99 ; 12-00 noon

Tube #	Radioactivity for 300 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.7438]	µ Ci/ml (A_t) on counting [dpm/666000]	µ Ci/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1		-1			
2		12			
3		1886	2535.6	0.0038	0.0039
4		6799	9141	0.0137	0.014
5		13236	17796	0.026	0.027
6		24572	33036	0.049	0.051
7		49587	66667	0.1001	0.1038
8		102999	137133	0.2059	0.2134
9		185620	249556	0.3747	0.3885
10		380974	512199	0.7690	0.7973

$e^{-\frac{0.693 \times 75}{1440}}$

09/28/99 ; 9-00 a.m

2 0.9645

72h + 3h = 75h

TABLE-3

Expt. # : 2 Date/Time :

KBq/cell [nCi/cluster x 0.037]	Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [uCi/ml x 10^6 Cells/ml]	nCi/cluster [pCi/cell x 4000]
	1	750, 769, 742				
	2	695, 685, 672				
0.197	3	711, 739, 742	730	2922666	0.0013	5.33
0.772	4	675, 666, 672	671	2684000	0.005	20.86
1.33	5	766, 742, 752	753	3013333	0.0089	35.84
2.92	6	639, 652, 647	646	2584000	0.0197	78.94
5.32	7	709, 721, 735	721	2886666	0.0359	143.83
10.81	8	741, 729, 721	730	2921333	0.073	292.19
18.76	9	762, 777, 759	766	3064000	0.1267	507.18
39.09	10	756, 742, 766	754	3018666	0.2641	1056

TABLE-4

Expt # : 2 **Date :** 10/08/99

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1·1	160	141	157	150·33	
~~1·2~~	137	149	158		
3·2	131	138	126	131·66	0·8758
4·2	106	125	115	115·33	0·7672
5·2	89	95	102	95·33	0·6341
6·2	48	53	42	47·66	0·3171
7·2	30	36	42	36	0·2395
8·3	38	47	29	38	0·025
9·4	51	56	61	0·56	0·0037
10·4	2	3	4	0·03	0·00019

V79 COLONY FORMING ASSAY

Experiment Name : ^{125}IUdR toxicity (50% label) **Exp. # :** 1;
Experiment performed by: A. Bishayee **Date:** 09/23/99

1. Set the rocker-roller at 37°C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 150 cm^2 flusk, subcultured 1:2, 24h before) with PBS, trypsinize cells, each resuspend in 9 ml MEMB, pool, 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 ~2,000,000 cells/ml in MEMB [Actual count : cells/ml)
3. Transfer 1 ml of cell suspension into 14 ml tubes (Falcon plastic test tube, 17x100 mm)
4. Keep the tubes in the roller for 3-4 h at 37°C, 5% CO_2 **Date/Time:** 09/23/99 4-00 PM.
5. Prepare MEMB containing radioactivity in hood
   65 µl ^{125}IUdR (prepared on 08/29/99) + 2 ml MEMB
6. After 3-4 h, remove test tubes from shaker and add MEMB with or without radioactivity according to Table below. **Date/Time:** 09/23/99 ; 7-15 PM.

Tube #	^{125}IUdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{125}IUdR [8.0 uCi/ml]
1	0	1.0	1.0	0
2	0	1.0	1.0	0
3	0.01	1.0	0.997	0.003
4	0.05	1.0	0.987	0.013
5	0.1	1.0	0.975	0.025
6	0.2	1.0	0.950	0.05
7	0.5	1.0	0.875	0.125
8	1	1.0	0.75	0.25
9	2	1.0	0.5	0.5
10	4	1.0	0	1.0

7. Return test tubes to shaker for 12 h . **Date/Time:** 09/23/99; 7-30 p.m.
8. Next day, while test tubes are in shaker 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:** 09/24/99; 9-00 a.m.
10. Remove buckets from centrifuge and carefully remove 100 µl of supernatant and place in prelabeled gamma-tube.
11. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
12. Centrifuge tubes for 10 min at 2000 rpm, 4°C
13. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
14. Centrifuge tubes for 10 min at 2000 rpm, 4°C
15. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
16. Centrifuge tubes for 10 min at 2000 rpm, 4°C
17. Similarly wash the cells that were not labelled, pooled cells from two tubes, resuspend in 8 ml wash MEMA
18. Decant supernatant, click tubes, vortex, transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips
18. Again add 200 ul ice cold MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
19. Centrifuge tubes for 5 min at 1000 rpm, 4°C
20. Transfer tubes at 10°C for 72 h. **Date/Time:** 09/24/99; 11-30 a.m.
21. Transfer 10 ul supernatant in three sets of tubes containing small pieces of tissue paper from 100 ul supernatant removed earlier (Step 12) and count them for radioactivity

    Date/Time: 09/24/99; 12-00
22. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the content to ten 12 ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA by using pasteur pipet

    Date/Time: 09/27/99; 9-30 a.m.
23. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes
24. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***
25. Labeling and preparation of dilution tubes and colony dishes
    - load 66, 60 mm petri dishes with 4 ml MEMA
    - load 40 T-tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2, X.3, X.4, X.5 etc.
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 10 ml wash MEMA
29. Centrifuge tubes for 10 min at 2000 rpm, 4°C
30. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times

through 3 cc syringe with 21 gauge needle

31. Determine cell concentration by transfering 100 µl to Coulter cup
32. Vortex tube, transfer 0.5 ml into dilusion tube X.5, vortex tube X.5, 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.
33. 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.
34. Transfer 300 µl of cell suspension (in triplicate) to gamma tubes for each tube
35. Incubate petridishes for 1 week
36. Count gamma tubes for radioactivity **Date/Time :** 09/27/99, 1200
37. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
38. Count colonies. There must be between 25 and 250 colonies for the flask to be a valid data point.

09/23/99

Stock on 08/24/99 = 0.35 μCi/ml

Stock on 09/23/99 = 0.35 × 0.71

= 0.25 μCi/ml

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 30 \times 24}{1440}}$

$= e^{-0.34}$

$= 0.71$

Prepare 2 ml of 8 μCi/ml ^{125}IUdR

= 16 μCi required

Vol taken = $\frac{16}{0.25}$ = 64 ml

Take 65 μl ^{125}IUdR (Stock) + 2 ml HEMS

09/24/99
12-00

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

11	1.00	1M	95	0 (1M)
12	1.00		84	0
13	1.00		89	0
14	1.00	2M	90	0 (2M)
15	1.00		111	0
16	1.00		95	0
17	1.00	3M	224	0 (3M)
18	1.00		216	0
19	1.00		207	0
20	1.00	4M	597	578 — 4M
21	1.00	7M	5895	11563 — 7M
22	1.00	8M	11268	22668 (8M)
23	1.00		11612	23980
24	1.00		11928	23744
25	1.00	9M	25509	52008 (9M)
26	1.00		26302	53695
27	1.00		25441	52391
28	1.00	COM	38824	79426 (10M)
29	1.00		40597	83844
30	1.00		39981	82687
31	1.00	4M	630	641 — 4M
32	1.00		667	633 — 4M
33	1.00	5M	1264	1845 (5M)
34	1.00		1250	1890
35	1.00		1283	1887
36	1.00	6M	2192	3917 (6M)
37	1.00		2342	4050
38	1.00		2265	3950
39	1.00	7M	5845	11440 (7M)
40	1.00		6198	11956

Background 82

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

TABLE-1

Expt. # : 1 **Date/Time :** 09/24/99; 12-00

Tube #	Medium count for 10 ul (cpm)	Avg. cpm	dpm [cpm/0.7056]	µ Ci/ml (A_t) on counting [dpm/22200]	µ Ci/ml (A_o) on addition [$A_t/e^{-\lambda t}$]
1		7			
2		16			
3		133	189	0·008	
4		549	778	0·04	
5		1265	1793	0·081	
6		2184	3095	0·14	
7		5897	8357	0·38	
8		11520	16327	0·74	
9		25668	36378	1·64	
10		39818	56290	2·54	

09/27/99, 12:00

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

Background 30

1	1.00	1C	38	0
2	1.00		27	0
3	1.00	2C	41	0
4	1.00		33	0
5	1.00	3C	1380	2012
6	1.00		1472	2180
7	1.00	4C	6037	11011
8	1.00		5686	10750
9	1.00	5C	10873	20942
10	1.00		11187	21733
11	1.00	6C	16306	32138
12	1.00		16048	31734
13	1.00	7C	41170	82986
14	1.00		41815	83967
15	1.00	8C	79910	159894
16	1.00		80255	161927
17	1.00	9C	124017	250109
18	1.00		126571	257465
19	1.00	10C	173517	351410
20	1.00		167761	339576

1.83
1.86
1.99
1.97
2.0
2.0
2.0
2.0

TABLE-2

Expt. # : 1 **Date/Time :** 09/27/99; 12-00

Tube #	Radioactivity for 300 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.7056]	μ Ci/ml (A_t) on counting [dpm/666000]	μ Ci/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1		7·5			
2		17			
3		1396	1978	0·003	0·0031
4		5831	8264	0·012	0·012
5		11000	15589	0·023	0·024
6		16147	22884	0·034	0·035
7		41462	58762	0·088	0·091
8		80052	113453	0·171	0·177
9		125264	177528	0·266	0·275
10		170609	241792	0·363	0·376

09/24/99; 9-00 a.m

3×24+3h

= 75h

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 75}{1440}}$

= 0·9645

TABLE-3

Kbq/cluster [nCi/c x 0.037)

Expt. # : 1

Date/Time : 09/27/99 ; 1200

nCi/cluster [pCi/cell x 4000)

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [uCi/ml x 10^6 Cells/ml]	
1	609, 595, 575				
2	578, 566, 571				
0.1776 3	613, 622, 635	623	2493333	0.0012	4.8
0.6464 4	575, 560, 582	572	2289333	0.0043	17.47
1.704 5	535, 519, 511	521	2086666	0.0115	46.06
2.336 6	569, 555, 539	554	2217333	0.0157	63.13
5.68 7	591, 599, 602	597	2389333	0.038	152.3
10.5 8	621, 635, 607	621	2484000	0.071	285
18.67 9	545, 551, 539	545	2180000	0.126	504
27.05 10	511, 503, 529	514	2057333	0.182	731

TABLE-4

Expt # : **Date :** 10/04/99

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1·2	165	141	153	149·16	
2·2	135	152	149		
3·2	129	134	140	134·3	0·9003
4·2	109	119	128	118·6	0·7956
5·2	108	97	87	97·3	0·6525
6·2	67	74	61	67·3	0·4514
7·2	20	30	41	30·3	0·2034
8·3	60	68	76	6·8	0·0456
9·4	40	30	21	0·303	0·002
10·4	6	9	11	0·08	0·00058

[redacted]

[redacted]

[redacted]

B001864

V79 COLONY FORMING ASSAY

Experiment Name : ^{125}IUdR toxicity with 10% DMSO w/o lindane (10% label) **Exp. # :** 9

Experiment performed by: A. Bishayee **Date:** 10/30/00

1. Set the rocker-roller at 37^{0}C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 150 cm^2 flask, subcultured 1:2, 24h before) with PBS, trypsinize cells, each resuspend in 9 ml MEMB, pool, 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 ~400,000/0.5 ml and 3,600,000 cells/ml in MEMB [Actual count : cells/ml)
3. Transfer 1 ml of cell suspension into 14 ml tubes (Falcon plastic test tube, 17x100 mm) labeled 1-10 both on cap and wall
4. Keep the tubes in the roller for 3-4 h at 37°C, 5% CO_2 **Date/Time:** 10/30/00, 4-00 pm

Tube #	^{125}IUdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{125}IUdR [30 uCi/ml]	100 uM lindane-10%DMSO MEMA (ml)	10% DMSO in MEMA (ml)
1	0	0.5	0.5	0	0.4	
2	0	0.5	0.5	0	0.4	
3	0.2	0.5	0.493	0.007	0.4	
4	1	0.5	0.465	0.035	0.4	
5	5	0.5	0.335	0.165	0.4	
6	10	0.5	0.165	0.335	0.4	
7	15	0.5	0.0	0.5	0.4	
8	0	0.5	0.5	0		0.4
9	0	0.5	0.5	0		0.4
10	0.2	0.5	0.493	0.007		0.4
11	1	0.5	0.465	0.035		0.4
12	5	0.5	0.335	0.165		0.4
13	10	0.5	0.165	0.335		0.4
14	15	0.5	0.0	0.5		0.4

5. Prepare MEMB containing radioactivity in hood

   201 µl ^{125}IUdR (prepared on 10/27/00) + 2.2 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:** 10/30/00, 7-15 pm

7. Return test tubes to roller for 12 h . **Date/Time:** 10/30/00, 7-30 pm

8. Next day, while test tubes are in roller 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:** 10/31/00, 9-00 a.m.

10. Remove buckets from centrifuge and carefully remove 100 µl of supernatant and place in prelabeled gamma-tube.

11. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA

12. Centrifuge tubes for 10 min at 2000 rpm, 4°C

13. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA

14. Centrifuge tubes for 10 min at 2000 rpm, 4°C

15. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA with unlabeled cells

16. Centrifuge tubes for 10 min at 2000 rpm, 4°C

17. Decant supernatant, click tubes, vortex, transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips

18. Again add 200 ul ice cold MEMA with 10%DMSO with or without 100 uM lindane as per the table, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)

19. Centrifuge tubes for 5 min at 1000 rpm, 4°C

20. Transfer tubes at 10°C for 72 h. **Date/Time:** 10/31/00, 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 (Step 12) and count them for radioactivity **Date/Time:** 10/31/00 ; 12-00 noon

22. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the content to ten 12 ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA by using pasteur pipet **Date/Time:** 11/03/00 ; 10-00 a.m.

23. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes

24. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***

25. Labeling and preparation of dilution tubes and colony dishes

- load 66, 60 mm petri dishes with 4 ml MEMA
- load 40 T-tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2, X.3, X.4, X.5 etc.

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 10 ml wash MEMA
29. Centrifuge tubes for 10 min at 2000 rpm, 4°C
30. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
31. Determine cell concentration by transfering 100 µl to Coulter cup
32. Vortex tube, transfer 0.5 ml into dilusion tube X.5, vortex tube X.5, 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.
33. 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.
34. Transfer 300 µl of cell suspension (in triplicate) to gamma tubes for each tube
35. Incubate petridishes for 1 week
36. Count gamma tubes for radioactivity **Date/Time :** 11/07/00 ; 4:00 pm
37. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
38. Count colonies. There must be between 25 and 250 colonies for the flask to be a valid data point.

10/30/00

Prepare 2.2 ml of 30 uci/ml

= 66 uci required

Stock on 10/27/00 3pm 0.34 uci/ul

10/30/00 7pm 0.34×0.964

$= 0.327$ uci/ul

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 76}{1440}}$

$= 0.9640$

Stock required $= \frac{66}{0.327}$

$= 201$ ul

① Take 201 ul stock

② Keep at RT for 3-4h

③ Add 2.2 ml of MEM/B

10/31/00
12-00 door

10 µl medium

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

1	1.00	1M	0	358
2	1.00		0	300
3	1.00		0	126
4	1.00	2M	0	152
5	1.00		0	76
6	1.00		0	94
7	1.00	3M	2373	5761
8	1.00		2115	5430
9	1.00		1976	5186
10	1.00	4M	10726	25281
11	1.00		11167	26055
12	1.00		11604	26642
13	1.00	5M	56785	132723
14	1.00		53750	125418
15	1.00		56935	130522
16	1.00	6M	112195	253882
17	1.00		133449	300770
18	1.00		120397	268167
19	1.00	7M	176493	404147
20	1.00		187201	421509
21	1.00		176049	406679
22	1.00	8M	0	172
23	1.00		0	113
24	1.00		0	87
25	1.00	9M	0	107
26	1.00		0	114
27	1.00		0	106
28	1.00	10M	2351	5706
29	1.00		2282	5346
30	1.00		2385	5589
31	1.00	11M	11551	26308
32	1.00		12777	29431
33	1.00		12253	27961
34	1.00	12M	54012	120368
35	1.00		57789	129870
36	1.00		59109	129226
37	1.00	13M	128176	283160
38	1.00		122076	265123
39	1.00		132345	286410
40	1.00	14M	200908	440615
41	1.00		200996	439096
42	1.00		192043	414909

$e^{-\lambda t} = e^{-\frac{0.693 \times 16.5}{1640}}$

$= 0.992$

16.5h

10/30/00; 7-30

TABLE-1

Expt. # : 2

Date/Time : 10/31/00; 12-00

Tube #	Medium count for 10 ul (cpm)	Avg. cpm	dpm [cpm/1.09] e=~~0.74~~ 0.506 y=1.47	µCi/ml (A_t) on counting [dpm/22200]	µCi/ml (A_o) on addition [$A_t/e^{-\lambda t}$]
1					
2					
3		2154	2896	0.130	0.1315
4		11165	15011	0.676	0.682
5		55823	75051	3.38	3.41
6		122013	164040	7.38	7.44
7		179914	241885	10.89	10.98
8					
9					
10		2339	3145	0.141	0.143
11		12193	16393	0.738	0.744
12		56970	76593	3.45	3.47
13		127532	171460	7.72	7.78
14		197982	266176	11.98	12.08

10/31/00 : 9-06 a.m.

11/03/00

4-00 pm

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

1	1.00	0	131
402	1.00	0	70
403	.25	0	0
404	1.00	0	91
405	1.00	0	115
406	1.00	0	35
407	1.00	10256	22163
408	1.00	10718	23571
409	1.00	11156	23908
410	1.00	29973	63807
411	1.00	30411	64162
412	1.00	29722	62452
413	1.00	236486	497758
414	1.00	237180	499090
415	1.00	251109	525850
416	1.00	392271	818394
417	1.00	429916	883844
418	1.00	434845	891262
419	1.00	410675	835756
420	1.00	439963	880424
421	1.00	447052	895571
422	1.00	0	327
423	1.00	0	259
424	1.00	0	235
425	1.00	0	202
426	1.00	0	240
407	1.00	0	143
408	1.00	12144	25224
409	1.00	12815	26967
410	1.00	13143	26908
411	1.00	35912	73496
412	1.00	34867	72084
413	1.00	36427	75766

3x24+7

= 72+7

= 79

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

1	1.00	284404	587171
2	1.00	296718	606194
3	1.00	304803	622444
4	.95	489862	1006481
5	.93	497372	1009141
6	.91	492014	1003224
7	1.00	420268	832508
8	1.00	427980	857500

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

8	1.00	410475	814908

TABLE-2

Expt. # : **Date/Time :**

Tube #	Radioactivity for 300 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/1.09]	μCi/ml (A_t) on counting [dpm/666000]	μCi/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

TABLE-3

Expt. # : **Date/Time :**

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [uCi/ml x10^6 Cells/ml]	kBq/Cluster [pCi/cellx148
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

TABLE-4

Expt # : 2 **Date :** 11/10/00

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1.2	152	165	132	155.83	
2.2	165	172	149		
3.2	137	127	119	127.66	0.8192
4.2	78	86	92	85.66	0.5476
5.2	11	14	16	13.66	0.0877
6.3	55	66	42	5.43	0.0348
7.3	25	43	50	3.93	0.0252
8.2	161	171	162	144.5	
9.2	112	132	129		
10.2	82	87	92	87	0.6020
11.2	52	60	48	53.33	0.3690
12.3	26	30	22	2.6	0.0179
13.4	101	111	93	1.01	0.0070
14.4	38	48	29	0.38	0.0026

V79 COLONY FORMING ASSAY

Experiment Name : ^{125}IUdR toxicity with 10% DMSO w/o lindane (10% label) **Exp. #** : 1;
Experiment performed by: A. Bishayee **Date:** 09/21/00

1. Set the rocker-roller at 37^{0}C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 150 cm^2 flask, subcultured 1:2, 24h before) with PBS, trypsinize cells, each resuspend in 9 ml MEMB, pool, 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 ~400,000/0.5 ml and 3,600,000 cells/ml in MEMB [Actual count : cells/ml)
3. Transfer 1 ml of cell suspension into 14 ml tubes (Falcon plastic test tube, 17x100 mm) labeled 1-10 both on cap and wall
4. Keep the tubes in the roller for 3-4 h at 37°C, 5% CO_2 **Date/Time:** 09/21/00; 4:00 PM.

Tube #	^{125}IUdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{125}IUdR [30 uCi/ml]	100 uM lindane-10%DMSO MEMA (ml)	10% DMSO in MEMA (ml)
1	0	0.5	0.5	0	0.4	
2	0	0.5	0.5	0	0.4	
3	0.2	0.5	0.493	0.007	0.4	
4	1	0.5	0.465	0.035	0.4	
5	5	0.5	0.335	0.165	0.4	
6	10	0.5	0.165	0.335	0.4	
7	15	0.5	0.0	0.5	0.4	
8	0	0.5	0.5	0		0.4
9	0	0.5	0.5	0		0.4
10	0.2	0.5	0.493	0.007		0.4
11	1	0.5	0.465	0.035		0.4
12	5	0.5	0.335	0.165		0.4
13	10	0.5	0.165	0.335		0.4
14	15	0.5	0.0	0.5		0.4

5. Prepare MEMB containing radioactivity in hood

   167 µl ^{125}IUdR (prepared on 09/08/00) + 2.2~~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:** 09/21/00, 7-00 PM

7. Return test tubes to roller for 12 h . **Date/Time:** 09/21/00, 7-15 PM

8. Next day, while test tubes are in roller 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:** 09/22/00, 9-00 a.m.

10. Remove buckets from centrifuge and carefully remove 100 µl of supernatant and place in prelabeled gamma-tube.

11. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA

12. Centrifuge tubes for 10 min at 2000 rpm, 4°C

13. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA

14. Centrifuge tubes for 10 min at 2000 rpm, 4°C

15. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA with unlabeled cells

16. Centrifuge tubes for 10 min at 2000 rpm, 4°C

17. Decant supernatant, click tubes, vortex, transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips

18. Again add 200 ul ice cold MEMA with 10%DMSO with or without 100 uM lindane as per the table, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)

19. Centrifuge tubes for 5 min at 1000 rpm, 4°C

20. Transfer tubes at 10°C for 72 h. **Date/Time:** 09/22/00, 11-30 a.m.

21. Transfer 10 ul supernatant in three sets of tubes containing small pieces of tissue paper from 100 ul supernatant removed earlier (Step 12) and count them for radioactivity

    Date/Time: 09/22/00, 3-00 PM.

22. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the content to ten 12 ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA by using pasteur pipet

    Date/Time: 09/25/00, 10-00 a.m.

23. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes

24. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***

25. Labeling and preparation of dilution tubes and colony dishes

- load 66, 60 mm petri dishes with 4 ml MEMA
- load 40 T-tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2, X.3, X.4, X.5 etc.

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 10 ml wash MEMA
29. Centrifuge tubes for 10 min at 2000 rpm, 4°C
30. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
31. Determine cell concentration by transfering 100 µl to Coulter cup
32. Vortex tube, transfer 0.5 ml into dilusion tube X.5, vortex tube X.5, 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.
33. 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.
34. Transfer 300 µl of cell suspension (in triplicate) to gamma tubes for each tube
35. Incubate petridishes for 1 week
36. Count gamma tubes for radioactivity **Date/Time :** 09/29/00, 10:40 a.m.
37. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
38. Count colonies. There must be between 25 and 250 colonies for the flask to be a valid data point.

09/21/00

Prepare 2.2 ml of 30 μci/ml

= 30 × 2.2 = 66 μci required

Stock on 09/08/00 0.46 μci/ml

on 09/21/00 0.46 × 0.861

= 0.396 μci/ml

Stock required = $\frac{66}{0.396}$

= 167 ml.

① Take 167 ml of ^{125}IUdR

② Keep at RT for 3-4 h

③ Add 2.2 ml of MEMB

10 µl medium

09/22/00 : 3-00pm

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

1	1.00	0	494
2	1.00	0	211
3	1.00	0	223
4	1.00	0	216
5	1.00	0	174
6	1.00	0	170
7	1.00	2436	5810
8	1.00	2204	5423
9	1.00	2283	5574
10	1.00	11332	26007
11	1.00	11899	26972
12	1.00	11750	26560
13	1.00	54530	125925
14	1.00	60205	135842
15	1.00	59936	132805
16	1.00	117946	257447
17	1.00	124815	270495
18	1.00	141306	306009
19	1.00	194247	426759
20	1.00	187155	412193
21	1.00	186578	412905
22	1.00	0	347
23	1.00	0	207
24	1.00	0	186
25	1.00	0	161
26	1.00	0	144
27	1.00	0	142
28	1.00	2457	5654
29	1.00	2396	5569
30	1.00	2436	5777
31	1.00	12280	27215
32	1.00	13344	28680
33	1.00	13692	30001
34	1.00	61934	131366
35	1.00	56304	122343
36	1.00	58166	128533
37	1.00	123697	265733
38	1.00	132478	285465
39	1.00	193542	416065
40	1.00	131137	286952
41	1.00	205392	441263
42	1.00	206634	443045
44	.01	0	0

12h + 7.75 h

$e - \lambda t$ $= 19.75 h$ (4)

$= e - \frac{0.693 \times 19.75}{1440}$

$= 0.9905$

TABLE-1

Expt. # : 1 **Date/Time :** 09/21/00 ; 7-15pm
09/22/00 ; 3-pm

Tube #	Medium count for 10 ul (cpm)	Avg. cpm	dpm [cpm/~~1.09~~ 0.7438] e=~~0.740~~506 y=1.47	µCi/ml (A_t) on counting [dpm/22200]	µCi/ml (A_o) on addition [$A_t/e^{-\lambda t}$]
1					
2					
3		2307	3102	0.139	0.1403
4					
5					
6					
7					
8					
9					
10					

09/22/00 : 9-00

09/29/00 ; 10-30

300 µl cells

7x24 + 1.5

= 168 + 1.5

= 169.5

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

1	1.00	14	494
2	1.00	1	496
3	1.00	0	447
4	1.00	0	387
5	1.00	0	343
6	1.00	0	383
7	1.00	9665	20877
8	1.00	10606	22265
9	1.00	10470	22474
10	1.00	24313	51151
11	1.00	25714	54535
12	1.00	20898	45832
13	1.00	229727	480277
14	1.00	244381	501912
15	1.00	250016	523517
16	1.00	346281	718131
17	1.00	367651	764729
18	1.00	376693	788924
19	1.00	404429	851650
20	1.00	443747	887234
21	1.00	444682	922073
22	1.00	45	481
23	1.00	100	628
24	1.00	47	636
25	1.00	55	650
26	1.00	66	595
27	1.00	21	528
28	1.00	10315	20967
29	1.00	10186	18864
30	1.00	11714	23421
31	1.00	28523	57552
32	1.00	30139	60399
33	1.00	31293	63085
34	1.00	233268	472848
35	1.00	247271	498468
36	1.00	253294	505275
37	1.00	397601	801532
38	1.00	407798	808828
39	1.00	415065	834110
40	.98	503947	1000281
41	.93	502494	1001218
42	.92	502009	1008598

TABLE-2

Expt. # : **Date/Time :**

Tube #	Radioactivity for 300 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/1.09]	µCi/ml (A_t) on counting [dpm/666000]	µCi/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

TABLE-3

Expt. # : **Date/Time :**

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [uCi/ml x10^6 Cells/ml]	kBq/Cluster [pCi/cellx148
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

TABLE-4

Expt # : 1 **Date :** 10/02/00

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1.2	137	142	157	130.8	
2.2	119	109	121		
3.2	102	112	94	102.66	0.7847
4.2	80	90	72	80.66	0.6165
5.2	11	14	16	13.66	0.1044
6.3	34	40	47	4.0	0.3082
7.3	36	27	19	2.7	0.0208
8.2	117	121	132	134.83	
9.2	149	151	139		
10.2	78	88	99	88.33	0.6551
11.2	55	62	70	62.33	0.4622
12.3	38	44	31	3.76	0.0279
13.4	81	90	72	0.81	0.006
14.4	29	34	40	0.34	0.0025

[redacted] 10%.: 100µM Lin.

V79 COLONY FORMING ASSAY

Experiment Name : ^{125}IUdR toxicity w/o lindane (10% label) **Exp. # :** 2;

Experiment performed by: A. Bishayee **Date:** 08/07/00

1. Set the rocker-roller at 37^{0}C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 150 cm^2 flask, subcultured 1:2, 24h before) with PBS, trypsinize cells, each resuspend in 9 ml MEMB, pool, 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 ~400,000/0.5 ml and 3,600,000 cells/ml in MEMB [Actual count : cells/ml)
3. Transfer 1 ml of cell suspension into 14 ml tubes (Falcon plastic test tube, 17x100 mm) labeled 1-10 both on cap and wall
4. Keep the tubes in the roller for 3-4 h at 37°C, 5% CO_2 **Date/Time:** 08/07/00; 3-00pm

Tube #	^{125}IUdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{125}IUdR [30 uCi/ml]	100 uM lindane in MEMA (ml)	MEMA (ml)
1	0	0.5	0.5	0	0.4	
2	0	0.5	0.5	0	0.4	
3	0.2	0.5	0.493	0.007	0.4	
4	1	0.5	0.465	0.035	0.4	
5	5	0.5	0.335	0.165	0.4	
6	10	0.5	0.165	0.335	0.4	
7	15	0.5	0.0	0.5	0.4	
8	0	0.5	0.5	0		0.4
9	0	0.5	0.5	0		0.4
10	0.2	0.5	0.493	0.007		0.4
11	1	0.5	0.465	0.035		0.4
12	5	0.5	0.335	0.165		0.4
13	10	0.5	0.165	0.335		0.4
14	15	0.5	0.0	0.5		0.4

5. Prepare MEMB containing radioactivity in hood
   161 µl ^{125}IUdR (prepared on 7/28/00) + 2.2ml MEMB
6. After 3-4 h, remove test tubes from roller and add MEMB with or without radioactivity according to Table below. **Date/Time:** 08/07/00; 7-15 pm
7. Return test tubes to roller for 12 h. **Date/Time:** 08/07/00; 7-30 pm
8. Next day, while test tubes are in roller 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:** 08/08/00; 9-30 a.m.
10. Remove buckets from centrifuge and carefully remove 100 µl of supernatant and place in prelabeled gamma-tube.
11. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
12. Centrifuge tubes for 10 min at 2000 rpm, 4°C
13. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
14. Centrifuge tubes for 10 min at 2000 rpm, 4°C
15. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA with unlabeled cells
16. Centrifuge tubes for 10 min at 2000 rpm, 4°C
17. Decant supernatant, click tubes, vortex, transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips
18. Again add 200 ul ice cold MEMA with or without 100 uM lindane as per the table, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
19. Centrifuge tubes for 5 min at 1000 rpm, 4°C
20. Transfer tubes at 10°C for 72 h. **Date/Time:** 08/08/00; 12-00 noon
21. Transfer 10 ul supernatant in three sets of tubes containing small pieces of tissue paper from 100 ul supernatant removed earlier (Step 12) and count them for radioactivity **Date/Time:** 08/08/00; ~~12-00 noon~~ 2-30 pm.
22. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the content to ten 12 ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA by using pasteur pipet **Date/Time:** 08/11/00; 1-30 pm.
23. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes
24. Centrifuge the tubes for 10 min at 2000 rpm, 4°C (***precooled centrifuge***)
25. Labeling and preparation of dilution tubes and colony dishes
    - load 66, 60 mm petri dishes with 4 ml MEMA
    - load 40 T-tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2,

X.3, X.4, X.5 etc.

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 10 ml wash MEMA
29. Centrifuge tubes for 10 min at 2000 rpm, 4°C
30. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
31. Determine cell concentration by transfering 100 µl to Coulter cup
32. Vortex tube, transfer 0.5 ml into dilusion tube X.5, vortex tube X.5, 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.
33. 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.
34. Transfer 300 µl of cell suspension (in triplicate) to gamma tubes for each tube
35. Incubate petridishes for 1 week
36. Count gamma tubes for radioactivity **Date/Time :** 08/11/00; 4-00 pm
37. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
38. Count colonies. There must be between 25 and 250 colonies for the flask to be a valid data point.

08/07/00

Prepare 2.2 ml of 30 μci/ml of ^{125}I-IUDR

= 30 × 2.2 = 66 μci required

Stock on 07/28/00 = 0.47 μci/μl

on 08/07/00 = 0.47 × 0.891

= 0.41 μci/μl

Stock required = $\frac{66}{0.41}$ = $\frac{160.9}{}$ ~~158 μl~~

≈ 161 μl

① Take 161 μl of stock
② Keep at RT for 3-4 hr
③ Add 2.2 ml MEM

10 ml nebraska 08/08/00

2-30 pm

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

1	1.00		0	207
2	1.00	1M	0	375
3	1.00		0	325
4	1.00		0	275
5	1.00	2M	0	224
6	1.00		0	157
7	1.00		3082	6631
8	1.00	3M	3145	6441
9	1.00		2424	5189
10	1.00		13644	27096
11	1.00	4M	13408	26660
12	1.00		13405	26655
13	1.00		70163	138431
14	1.00	5M	70392	139208
15	1.00		68699	135044
16	1.00		157903	309856
17	1.00	6M	153675	304029
18	1.00		149480	295883
19	1.00		243081	478371
20	1.00	7M	244716	481707
21	1.00		238977	464468
22	1.00		0	398
23	1.00	8M	0	341
24	1.00		0	243
25	1.00		0	248
26	1.00	9M	0	176
27	1.00		0	139
28	1.00		3562	8071
29	1.00	10M	2810	5871
30	1.00		2661	5617
31	1.00		13241	28110
32	1.00	11M	13123	28091
33	1.00		12848	27142
34	1.00		83527	175218
35	1.00	12M	77983	163342
36	1.00		76741	161437
37	1.00		177278	367601
38	1.00	13M	173727	361036
39	1.00		166307	347003
40	1.00		259501	541846
41	1.00	14M	259804	544472
42	1.00		242517	510034

$e^{-\lambda t}$

$= e^{\frac{-0.693 \times 19}{1440}}$

$= 0.9908$

08/07/00; 7-30pm

$= t = 12 + 7 = 19$

TABLE-1

Expt. # : 2

Date/Time : 08/08/00 ; 2-30 pm

Tube #	Medium count for 10 ul (cpm)	Avg. cpm	dpm [cpm/0.7438] e=0.506 y=1.47	µCi/ml (A_t) on counting [dpm/22200]	µCi/ml (A_o) on addition [$A_t/e^{-\lambda t}$]
1					0
2					0
3		2883	3876	0.1746	0.1762
4		13485	18130	0.8167	0.8242
5		69751	93777	4.22	4.26
6		153686	206622	9.30	9.39
7		242258	325703	14.67	14.80
8, 9					
10		3011	4048	0.182	0.184
~~11~~		~~13070~~	~~17572~~	0.79	0.7989
12		79417	106771	4.80	4.85
~~13~~				~~10.44~~	~~10.53~~
14		172437	231832		
		253940	341409	15.37	15.52

08/11/00

300µL cells

4-00 pm

08/08/00 - 9-30 a.m

72h + 6.5h

= 78.5h

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

1	1.00	0	140
2	1.00	10 0	54
3	1.00	0	59
4	1.00	0	52
5	1.00	20 0	79
6	1.00	0	125
7	1.00	8016	16374
8	1.00	30 8204	16570
9	1.00	8228	16905
10	1.00	18403	36298
11	1.00	40 18076	35907
12	1.00	16786	33098
13	1.00	173646	396214
14	1.00	50 178238	394328
15	1.00	185213	414880
16	1.00	240166	534962
17	1.00	60 314196	690254
18	1.00	343459	742309
19	1.00	300036	643497
20	1.00	70 327571	693193
21	1.00	342613	716565
22	1.00	0	375
23	1.00	80 0	215
24	1.00	0	169
25	1.00	0	221
26	1.00	90 0	283
27	1.00	0	164
28	1.00	10505	19290
29	1.00	100 9840	18066
30	1.00	10150	18399
31	1.00	19657	35792
32	1.00	110 19174	34679
33	1.00	17749	31966
34	1.00	121373	253670
35	1.00	120 124277	260386
36	1.00	124648	259875
37	1.00	266785	558820
38	1.00	130 259584	539378
39	1.00	264935	548454
40	1.00	320032	651784
41	1.00	140 330841	667552
42	1.00	298882	601461

e^{-kt}

$= e^{\frac{-0.693 \times 78.5}{1440}}$

$= 0.9629$

TABLE-2

Expt. # : 2 **Date/Time :** 08/4/00; 4-00 pm

Tube #	Radioactivity for 300 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.7438]	µCi/ml (A_t) on counting [dpm/666000]	µCi/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1					
2					
3		8149	10956	0·0164	0·0171
4		17755	23870	0·0358	0·0372
5		179032	240699	0·3614	0·3753
6		299273	402357	0·6041	0·6274
7		323406	434803	0·6528	0·6781
8					
9					
10		10165	13666	0·0205	0·021
11		18860	25356	0·0381	0·0395
12		123432	165948	0·2491	0·2587
13		263768	354622	0·5324	0·5529
14		316585	425631	0·639	0·6637

TABLE-3

Expt. # : 2 **Date/Time :** 08/11/00

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [uCi/ml x10^6 Cells/ml]	kBq/Cluster [pCi/cellx148
1	537, 555, 562				
2	511, 509, 531				
3	569, 577, 552	566	2264000	0.0075	1.11
4	511, 529, 537	525	2102666	0.0176	2.62
5	581, 572, 562	571	2286666	0.1641	24.29
6	601, 592, 589	594	2376000	0.2640	39.08
7	532, 549, 562	547	2190666	0.3095	45.81
8	532, 547, 561				
9	517, 509, 528				
10	581, 599, 572	584	2336000	0.0089	1.33
11	549, 567, 550	555	2221333	0.0177	2.63
12	489, 499, 513	500	2001333	0.129	19.13
13	533, 561, 547	547	2188000	0.2526	37.39
14	561, 574, 539	558	2232000	0.2973	44.01

TABLE-4

Expt # : 2 **Date :** 8/18/00

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1.2	165	155	147	149.33	
2.2	139	149	141		
3.2	115	105	97	105.66	0.7075
4.2	95	84	75	84.66	0.5669
5.2	10	13	15	13	0.0870
6.3	43	52	34	43	0.02879
7.3	13	30	21	21	0.0141
8.2	123	137	141	126.16	
9.2	111	119	126		
10.2	92	82	74	82.66	0.6552
10.2	— 83	73	62	72.66	0.5759
12.3	— 69	76	82	7.5	0.0598
13.4	— 70	63	57	0.63	0.0049
14.4	— 24	30	19	0.24	0.0019

V79 COLONY FORMING ASSAY

Experiment Name : ^{125}IUdR toxicity w/o lindane (10% label) **Exp. # :** 1;

Experiment performed by: A. Bishayee **Date:** 08/03/00

1. Set the rocker-roller at 37^{0}C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 150 cm^2 flask, subcultured 1:2, 24h before) with PBS, trypsinize cells, each resuspend in 9 ml MEMB, pool, 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 ~400,000/0.5 ml and 3,600,000 cells/ml in MEMB [Actual count : cells/ml)
3. Transfer 1 ml of cell suspension into 14 ml tubes (Falcon plastic test tube, 17x100 mm) labeled 1-10 both on cap and wall
4. Keep the tubes in the roller for 3-4 h at 37°C, 5% CO_2 **Date/Time:** 08/03/00, 3-00 pm.

Tube #	^{125}IUdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{125}IUdR [30 uCi/ml]	100 uM lindane in MEMA (ml)	MEMA (ml)
1	0	0.5	0.5	0	0.4	
2	0	0.5	0.5	0	0.4	
3	0.2	0.5	0.493	0.007	0.4	
4	1	0.5	0.465	0.035	0.4	
5	5	0.5	0.335	0.165	0.4	
6	10	0.5	0.165	0.335	0.4	
7	15	0.5	0.0 ✓	0.5	0.4	
8	0	0.5	0.50 ✓	0		0.4
9	0	0.5	0.5	0		0.4
10	0.2	0.5	0.493	0.007		0.4
11	1	0.5	0.465	0.035		0.4
12	5	0.5	0.335	0.165		0.4
13	10	0.5	0.165	0.335		0.4
14	15	0.5	0.0	0.5		0.4

5. Prepare MEMB containing radioactivity in hood
   151 µl ^{125}IUdR (prepared on 7/28/00) + 2.2ml MEMB
6. After 3-4 h, remove test tubes from roller and add MEMB with or without radioactivity according to Table below. **Date/Time:** 08/03/00; 7-00 pm
7. Return test tubes to roller for 12 h . **Date/Time:** 08/03/00; 7-30 a.m
8. Next day, while test tubes are in roller 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:** 08/04/00; 9-30 a.m
10. Remove buckets from centrifuge and carefully remove 100 µl of supernatant and place in prelabeled gamma-tube.
11. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
12. Centrifuge tubes for 10 min at 2000 rpm, 4°C
13. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
14. Centrifuge tubes for 10 min at 2000 rpm, 4°C
15. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA with unlabeled cells
16. Centrifuge tubes for 10 min at 2000 rpm, 4°C
17. Decant supernatant, click tubes, vortex, transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips
18. Again add 200 ul ice cold MEMA with or without 100 uM lindane as per the table, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
19. Centrifuge tubes for 5 min at 1000 rpm, 4°C
20. Transfer tubes at 10°C for 72 h. **Date/Time:** 08/04/00; 12-00 noon
21. Transfer 10 ul supernatant in three sets of tubes containing small pieces of tissue paper from 100 ul supernatant removed earlier (Step 12) and count them for radioactivity
    Date/Time: 08/04/00; 12-00 noon
22. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the content to ten 12 ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA by using pasteur pipet
    Date/Time: 08/07/00; 10-30 a.m.
23. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes
24. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***precooled centrifuge***
25. Labeling and preparation of dilution tubes and colony dishes
    - load 66, 60 mm petri dishes with 4 ml MEMA
    - load 40 T-tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2,

X.3, X.4, X.5 etc.

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 10 ml wash MEMA
29. Centrifuge tubes for 10 min at 2000 rpm, 4°C
30. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
31. Determine cell concentration by transfering 100 µl to Coulter cup
32. Vortex tube, transfer 0.5 ml into dilusion tube X.5, vortex tube X.5, 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.
33. 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.
34. Transfer 300 µl of cell suspension (in triplicate) to gamma tubes for each tube
35. Incubate petridishes for 1 week
36. Count gamma tubes for radioactivity **Date/Time :** 08/07/00; 3-00 pm.
37. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
38. Count colonies. There must be between 25 and 250 colonies for the flask to be a valid data point.

08/03/00

Prepare 30 μci/ml of ^{125}I IOR

Prepare 2.2 ml.

= $30 \times 2.2 =$ 66 μci required

Stock on 07/28/00 0.47 μci/ml

on 08/03/00 0.47×0.933

$= 0.44$ μci/ml

Stock required $= \frac{66}{0.44} = 151$ ml.

① Take 150 ml of ^{125}I IOR

② Keep at RT for 3-4 h

③ Add 2.2 ml of MBMB

31	1.00	0	155
32	1.00	1M 0	132
33	1.00	0	112
34	1.00	0	62
35	1.00	2M 0	195
36	1.00	0	227
37	1.00	2575	5539
38	1.00	3M 2495	5307
39	1.00	2430	4961
40	1.00	12443	24800
41	1.00	4M 11231	22658
42	1.00	9837	19628
43	1.00	54956	107725
44	1.00	5M 52835	103892
45	1.00	56175	110551
46	1.00	137491	269419
47	1.00	6M 146984	286281
48	1.00	141633	273337
49	1.00	234066	453480
50	1.00	7M 239537	470221
51	1.00	228160	448581
52	1.00	0	315
53	1.00	8M 0	395
54	1.00	0	316
55	1.00	0	265
56	1.00	9M 0	327
57	1.00	0	297
58	1.00	2250	4660
59	1.00	10M 2045	4530
60	1.00	1801	3902
61	1.00	11799	23435
62	1.00	11M 12046	23559
63	1.00	11096	21840
64	1.00	65319	127994
65	1.00	12M 63241	124353
66	1.00	60330	117698
67	1.00	124051	241252
68	1.00	13M 141227	272241
69	1.00	137215	264998
70	1.00	234853	445447
71	1.00	14M 250643	479387
72	1.00	230555	434736

10M reading
08/04/00
12-00 noon

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 16.5}{1440}}$

$= 0.9920$

08/09/00 - 7-30 pm

$= 12 + 4.5$ 4

$= 16.5$ h

TABLE-1

Expt. # : 1 **Date/Time :** 08/04/00; 12-00 noon

Tube #	Medium count for 10 ul (cpm)	Avg. cpm	dpm [cpm/0.7438] e=0.506 y=1.47	µCi/ml (A_t) on counting [dpm/22200]	µCi/ml (A_o) on addition [$A_t/e^{-\lambda t}$]
1					
2					
3		2500	3361	0.151	0.152
4		11170	15017	0.676	0.682
5		54655	73481	3.31	3.34
6		142036	190959	8.60	8.67
7		233921	314494	14.16	14.28
8					
9					
10		2032	2731	0.123	0.124
11.		11647	15658	0.7053	0.711
12		62963	84650	3.810	3.84
13		134164	180376	8.125	8.19
14		238683	320897	1445	14.57

31	1.00	0	35
32	1.00	0	89
33	1.00	0	73
34	1.00	0	78
35	1.00	0	39
36	1.00	0	69
37	1.00	13280	24426
38	1.00	14425	26726
39	1.00	14233	26037
40	1.00	28604	52896
41	1.00	28425	52166
42	1.00	29739	54435
43	1.00	121351	220831
44	1.00	120143	218837
45	1.00	123168	222533
46	1.00	262286	471394
47	1.00	268053	477499
48	1.00	278996	499906
49	1.00	432031	768222
50	1.00	438431	772019
51	1.00	452908	794986
52	1.00	0	178
53	1.00	0	164
54	1.00	0	97
55	1.00	0	93
56	1.00	0	126
57	1.00	0	77
58	1.00	17486	31284
59	1.00	18249	32754
60	1.00	17523	31370
61	1.00	30775	55044
62	1.00	29915	53608
63	1.00	28660	51343
64	1.00	126508	224664
65	1.00	130463	231800
66	1.00	123421	220004
67	1.00	229581	404979
68	1.00	224986	398429
69	1.00	220327	384712
70	1.00	431822	749612
71	1.00	434562	757660
72	1.00	443708	764317

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300 µl cells

08/07/00

3-00 pm

08/04/00

9-30 a.m

$t = 72h + 5.5h$

$= 77.5\ h$

e^{-kt}

$= e^{-\frac{0.693 \times 77.5}{1440}}$

$= 0.9634$

TABLE-2

Expt. # : 2 **Date/Time :** 04/07/00 ; 3-00pm

Tube #	Radioactivity for 300 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.7438]	µCi/ml (A_t) on counting [dpm/666000]	µCi/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1					
2					
3		13979	18794	0.0282	0.0293
4		28922	38885	0.0584	0.061
5		121554	163422	0.2453	0.2547
6		269778	362702	0.5445	0.5653
7		441123	593067	0.8904	0.9243
8, 9					
9/10		17752	23867	0.0358	0.0371
~~10~~/11		29783	40042	0.0601	0.0624
12		126797	170472	0.2559	0.2656
13		224964	302453	0.4541	0.4713
14		436697	587116	0.8815	0.9150

9
2.58
2.11
3.82
9.0
17

TABLE-3

Expt. # : 1 **Date/Time :** 08/07/00;

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [uCi/ml x10^6 Cells/ml]	kBq/Cluster [pCi/cellx148
1	659, 672, 681				
2	613, 635, 622				
3	679, 689, 661	676	2705333	0·0108	1·60
4	652, 632, 619	634	2537333	0·024	3·56
5	617, 632, 642	630	2521333	~~0·396~~ 0·1010	14·95
6	677, 690, 670	679	2716000	0·2081	30·80
7	609, 631, 621	620	2481333	0·372	55·13
8	649, 666, 672				
9	633, 642, 655				
10	661, 649, 657	655	2622666	0·014	2·09
11	671, 689, 666	675	2701333	0·023	3·42
12	639, 659, 642	646	2586666	0·1026	15·19
13	622, 619, 601	614	2456000	0·1918	28·41
14	688, 671, 659	672	2690666	0·3400	50·32

TABLE-4

Expt # : 1 **Date :** 08/14/00

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1·2	138	156	121	} 128·66	
2·2	129	119	109	}	
3·2	105	95	87	95·66	0·7435
4·2	70	81	68	73	0·5674
5·2	19	20	22	20·3	0·1581
6·3	78	68	99	8·16	0·0634
7·4	155	165	148	1·56	0·0121
8·2	151	142	138	} 135·66	
9·2	117	129	137	}	
10·2	63	90	76	76·3	0·5624
11·2	58	60	71	63	0·4643
12·3	95	107	87	9·6	0·0710
13·3	17	26	37	2·6	0·0196
14·4	48	54	60	054	0·0039

[redacted]

10%

V79 COLONY FORMING ASSAY

Experiment Name : ^{125}IUdR toxicity (10% label) **Exp. # :** 2;

Experiment performed by: A. Bishayee **Date:** 06/19/00

1. Set the rocker-roller at 37^{0}C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 150 cm^2 flask, subcultured 1:2, 24h before) with PBS, trypsinize cells, each resuspend in 9 ml MEMB, pool, 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 ~400,000/0.5 ml and 3,600,000 cells/ml in MEMB [Actual count : cells/ml)
3. Transfer 1 ml of cell suspension into 14 ml tubes (Falcon plastic test tube, 17x100 mm) labeled 1-10 both on cap and wall
4. Keep the tubes in the roller for 3-4 h at 37°C, 5% CO_2 **Date/Time:** 06/19/00; 4:00 pm
5. Prepare MEMB containing radioactivity in hood
   261 µl ^{125}IUdR (prepared on 06/12/00) + 15ml MEMB
6. After 3-4 h, remove test tubes from shaker and add MEMB with or without radioactivity according to Table below. **Date/Time:** 06/19/00; 7-10 pm

Tube #	^{125}IUdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{125}IUdR [40 uCi/ml] (ml)
1	0	0.5	0.5	0
2	0	0.5	0.5	0
3	0.1	0.5	0.497	0.003
4	0.5	0.5	0.488	0.012
5	1	0.5	0.475	0.025
6	2	0.5	0.45	0.05
7	5	0.5	0.375	0.125
8	10	0.5	0.25	0.25
9	15	0.5	0.125	0.375
10	20	0.5	0	0.5

7. Return test tubes to roller shaker for 12 h . **Date/Time:** 06/19/00; 7-30 pm
8. Next day, while test tubes are in shaker 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:** 06/20/00; 9-00 a.m.
10. Remove buckets from centrifuge and carefully remove 100 µl of supernatant and place in prelabeled gamma-tube.
11. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
12. Centrifuge tubes for 10 min at 2000 rpm, 4°C
13. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
14. Centrifuge tubes for 10 min at 2000 rpm, 4°C
15. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA with unlabeled cells
16. Centrifuge tubes for 10 min at 2000 rpm, 4°C
17. Decant supernatant, click tubes, vortex, transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips
18. Again add 200 ul ice cold MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
19. Centrifuge tubes for 5 min at 1000 rpm, 4°C
20. Transfer tubes at 10°C for 72 h. **Date/Time:** 06/20/00; 11-30 a.m.
21. Transfer 10 ul supernatant in three sets of tubes containing small pieces of tissue paper from 100 ul supernatant removed earlier (Step 12) and count them for radioactivity

    Date/Time: 06/20/00;
22. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the content to ten 12 ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA by using pasteur pipet

    Date/Time: 06/23/00; 1-00 pm
23. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes
24. Centrifuge the tubes for 10 min at 2000 rpm, 4°C (***precooled centrifuge***)
25. Labeling and preparation of dilution tubes and colony dishes
    - load 66, 60 mm petri dishes with 4 ml MEMA
    - load 40 T-tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2, X.3, X.4, X.5 etc.
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 10 ml wash MEMA

29. Centrifuge tubes for 10 min at 2000 rpm, 4°C
30. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
31. Determine cell concentration by transfering 100 µl to Coulter cup
32. Vortex tube, transfer 0.5 ml into dilusion tube X.5, vortex tube X.5, 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.
33. 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.
34. Transfer 300 µl of cell suspension (in triplicate) to gamma tubes for each tube
35. Incubate petridishes for 1 week
36. Count gamma tubes for radioactivity **Date/Time :** 06/23/00; 3:30 pm
37. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
38. Count colonies. There must be between 25 and 250 colonies for the flask to be a valid data point.

06/19/00

Stock on 06/12/00 = 0.25 μci/ml

Stock on 06/19/00 = 0.25 × 0.9223
= 0.23 μci/ml

$e^{-\frac{0.693 \times 7 \times 24}{1440}}$

= 0.9223

Prepare 1.5 ml of 40 μci/ml = 60 μci required

Vol required = $\frac{60}{0.23}$ = 261 ml

μci/ml		MEMB	MEMB + ITLS (40 μci/ml)
0	0.5	0.5	0
0		0.5	0
0.1		0.497 →	0.003
0.5		0.488 → 0.475 →	0.012
1		0.450 →	0.025
2			0.05
5		0.375 →	0.125
10		0.25 —	0.25
15		0.125 ←	0.375
20		0 —	0.5

B001912

06/23/00
3-30 pm
300 µl cells

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1	1.00	1C	6	157
2	1.00		0	0
3	1.00		0	0
4	1.00	2C	0	0
5	1.00		0	0
6	1.00		0	0
7	1.00	3C	4009	7842
8	1.00		4221	7952
9	1.00		4287	8344
10	1.00	4C	7146	13552
11	1.00		7163	13505
12	1.00		7115	13441
13	1.00	5C	18565	34383
14	1.00		19230	35864
15	1.00		19060	35740
16	1.00	6C	24083	44856
17	1.00		25980	48335
18	1.00		26745	49102
19	1.00	7C	42830	79762
20	1.00		42261	78593
21	1.00		43109	79469
22	1.00	8C	162664	301289
23	1.00		158849	290135
24	1.00		159859	294689
25	1.00	9C	200414	366625
26	1.00		222199	407442
27	1.00		223713	407455
28	1.00	10C	283161	518412
29	1.00		292563	532792
30	1.00		0 *	96

* Sample was not added by mistake

TABLE-2

Expt. # : 2 **Date/Time :** 06/23/00; 3-30 pm

Tube #	Radioactivity for 300 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.7438]	µCi/ml (A_t) on counting [dpm/666000]	µCi/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1					
2					
3		4172	5609	0.0084	0.0087
4		7141	9601	0.0144	0.0149
5		18951	25479	0.0382	0.0397
6		25602	34421	0.0516	0.0536
7		42733	57452	0.0862	0.0895
8		160457	215726	0.3239	0.336
9		215442	289650	0.4349	0.4516
10		287880	387040	0.58114	0.6035

06/20/00; 900 a.m

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 78.5}{1440}}$

$= 0.9629$

$t = 72h + 6.5h$

$= 78.5\ h$

Table-3

Expt#: 2

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [uCi/ml x10^6 Cells/ml]	kBq/Cluster [pCi/cellx148
1	568, 588, 571				
2	579, 593, 569				
3	592, 575, 561	576	2304000	0·0037	0·559
4	531, 511, 520	520	2082666	0·0071	1·06
5	576, 556, 587	573	2292000	0·0173	2·56
6	566, 589, 572	575	2302666	0·0232	3·45
7	609, 632, 621	620	2482666	0·036	5·33
8	545, 519, 562	542	2168000	0·1549	22·94
9	575, 559, 561	565	2260000	0·1998	29·57
10	615, 589, 602	602	2408000	0·2506	37·09

TABLE-4

Expt #.: 2 **Date :** 06/30/00

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1·2	110	109	117	111·5	
2·2	97	115	121		
3·2	110	100	90	100	0·8968
4·2	82	87	78	82·3	0·7384
5·2	59	77	68	68	0·6098
6·2	67	58	49	58	0·5201
7·2	41	50	33	41·3	0·3707
8·3	67	78	54	6·63	0·0595
9·3	20	28	39	2·9	0·0260
10·3	17	26	12	1·8	0·0164

V79 COLONY FORMING ASSAY

Experiment Name : ^{125}IUdR toxicity (10% label) **Exp. #:** 1;
Experiment performed by: A. Bishayee **Date:** 10/07/99

1. Set the rocker-roller at 37°C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 150 cm^2 flusk, subcultured 1:2, 24h before) with PBS, trypsinize cells, each resuspend in 9 ml MEMB, pool, 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 ~400,000/0.5 ml and 3,600,000 cells/ml in MEMB [Actual count : cells/ml)
3. Transfer 1 ml of cell suspension into 14 ml tubes (Falcon plastic test tube, 17x100 mm) labeled 1-10 both on cap and wall
4. Keep the tubes in the roller for 3-4 h at 37°C, 5% CO_2 **Date/Time:** 10/11/99; 4-00 P.
5. Prepare MEMB containing radioactivity in hood
   150 µl ^{125}IUdR (prepared on 08/24/99) + 1.5 ml MEMB
6. After 3-4 h, remove test tubes from ~~shaker~~ roller and add MEMB with or without radioactivity according to Table below. **Date/Time:** 10/11/99, 7-00 p.m.

Tube #	^{125}IUdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{125}IUdR [20 uCi/ml] (ml)
1	0	0.5	~~1.005~~	0
2	0	0.5	~~1.005~~	0
3	0.05	0.5	0.497	0.003
4	0.1	0.5	0.495	0.005
5	0.5	0.5	0.475	0.025
6	1	0.5	0.45	0.05
7	2	0.5	0.4	0.1
8	5	0.5	0.25	0.25
9	7.5	0.5	0.125	0.375
10	10	0.5	0	0.5

7. Return test tubes to ~~shaker~~ roller for 12 h . **Date/Time:** 10/11/99; 7-20 p.m.
8. Next day, while test tubes are in shaker 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:** 10/12/99; 9-00 a.m.
10. Remove buckets from centrifuge and carefully remove 100 µl of supernatant and place in prelabeled gamma-tube.
11. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
12. Centrifuge tubes for 10 min at 2000 rpm, 4°C
13. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA
14. Centrifuge tubes for 10 min at 2000 rpm, 4°C
15. Decant supernatant, click tubes, vortex, resuspend in 8 ml wash MEMA with unlabeled cells
16. Centrifuge tubes for 10 min at 2000 rpm, 4°C
17. Decant supernatant, click tubes, vortex, transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips
18. Again add 200 ul ice cold MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
19. Centrifuge tubes for 5 min at 1000 rpm, 4°C
20. Transfer tubes at 10°C for 72 h. **Date/Time:** 10/12/99; 11-30 a.m.
21. Transfer 10 ul supernatant in three sets of tubes containing small pieces of tissue paper from 100 ul supernatant removed earlier (Step 12) and count them for radioactivity

    Date/Time: 10/12/99; 12-00 noon
22. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the content to ten 12 ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA by using pasteur pipet

    Date/Time: 10/15/99; 9-30 a.m.
23. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes
24. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***
25. Labeling and preparation of dilution tubes and colony dishes
    - load 66, 60 mm petri dishes with 4 ml MEMA
    - load 40 T-tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2, X.3, X.4, X.5 etc.
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 10 ml wash MEMA
29. Centrifuge tubes for 10 min at 2000 rpm, 4°C

30. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
31. Determine cell concentration by transfering 100 µl to Coulter cup
32. Vortex tube, transfer 0.5 ml into dilusion tube X.5, vortex tube X.5, 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.
33. 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.
34. Transfer 300 µl of cell suspension (in triplicate) to gamma tubes for each tube
35. Incubate petridishes for 1 week
36. Count gamma tubes for radioactivity **Date/Time :** 10/18/99 ; 12:00 noon
37. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
38. Count colonies. There must be between 25 and 250 colonies for the flask to be a valid data point.

10/11/99

Stock on 09/27/99 = 0.23 μCi/μl

Stock on 10/11/99 = 0.23 × 0.8605

= 0.19 μCi/μl

$e^{-\frac{0.693 \times 13 \times 24}{1440}}$

$= e^{-0.15}$

$= 0.8605$

Prepare 1.5 ml of 20 μCi/ml = 30 μCi required

Vol required $= \frac{30}{0.19}$

= 158 μl.

① Take 158 μl of stock, Keep on hood 1-2 h

② Add 1.5 ml HBHB

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE

1	1.00	Blank	106	0
2	1.00		182	0
3	1.00	1M	175	0
4	1.00		128	0
5	1.00	2M	150	0
6	1.00		90	0
7	1.00	3M	954	1333
8	1.00		1075	1359
9	1.00	4M	2105	3437
10	1.00		2250	3665
11	1.00	5M	6547	11933
12	1.00		6368	12033
13	1.00	6M	13689	25875
14	1.00		13662	24445
15	1.00	7M	29707	58621
16	1.00		29336	56481
17	1.00	8M	75123	150010
18	1.00		73781	148350
19	1.00	9M	127702	256020
20	1.00		123148	247139
21	1.00	10M	183511	375755
22	1.00		174844	358774

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE

1	.05	7	0

TABLE-1

Expt. # : 1 **Date/Time :** 10/12/99; 12:00 noon

Tube #	Medium count for 10 ul (cpm)	Avg. cpm	dpm [cpm/0.7438] e=0.506 y=1.47	μ Ci/ml (A_t) on counting [dpm/22200]	μ Ci/ml (A_o) on addition [$A_t/e^{-\lambda t}$]
1		7.5			
2		-24			
3		870	1169	0.052	0.053
4		2033	2733	0.1231	0.1241
5		6313	8487	0.382	0.385
6		13531	18191	0.8194	0.826
7		29377	39495	1.77	1.79
8		~~74452~~ 74308	99903	4.50	4.53
9		125281	168433	7.58	7.64
10		179033	240700	10.84	10.92

$e^{-\lambda t}$

$= e^{\frac{-0.693 \times 16.5}{1440}}$

$= 0.992$

10/11/99 - 7-20

12h + 4.5

= 16.5

18	0.0028
17	0.0504
16	0.1339
15	0.3086
14	0.6602
13	0.8086
12	0.921
11	0.9665
10	1
9	0.002
8	0.0099
7	0.0895
6	0.2002
5	0.5007
4	0.6037

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE

1	1.00	Back 43	0
2	1.00	55 } 56	0
3	1.00	69	0
4	1.00	56	0
5	1.00	1C 75	0
6	1.00	59	0
7	1.00	75	0
8	1.00	2C 76	0
9	1.00	78	0
10	1.00	3136	5379
11	1.00	3C 3057	5385
12	1.00	2862	4935
13	1.00	3972	7148
14	1.00	4C 4065	7284
15	1.00	3863	6805
16	1.00	5939	10834
17	1.00	5C 6057	10881
18	1.00	6016	11026
19	1.00	16547	31605
20	1.00	6C 15959	30427
21	1.00	16028	30790
22	1.00	19193	36720
23	1.00	7C 18968	36595
24	1.00	18426	35884
25	1.00	35416	69357
26	1.00	8C 35029	68677
27	1.00	30790	60281
28	1.00	73998	145038
29	1.00	9C 75626	148687
30	1.00	76013	150194
31	1.00	132494	260434
32	1.00	10C 128844	253370
33	1.00	120107	236415

TABLE-2

Expt. # : 1 **Date/Time :** 10/18/99 ; 12-00 noon

Tube #	Radioactivity for 300 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.7438]	µ Ci/ml (A_t) on counting [dpm/666000]	µ Ci/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1		7.3			
2		20			
3		2962	3982	0.0059	0.0064
4		3910	5256	0.0078	0.0084
5		5948	7996	0.012	0.0128
6		16122	16122	0.0242	0.0259
7		18806	25283	0.0379	0.0407
8		33689	45293	0.068	0.0729
9		75156	101043	0.1517	0.1628
10		127092	170868	0.2565	0.2753

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 147}{1440}}$

$= 0.9317$

10/12/99; 9-00 a.m

6x24h + 3h

= 147h

TABLE-3

Expt. # : 1 **Date/Time :** 10/15/99; 11-00

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [uCi/ml x 10^6 Cells/ml]	nCi/cluster [pCi/cell x 4000]
1	527, 509, 512				
2	545, 562, 571				
3	551, 563, 549	554	2217333	0.0028	11.55
4	490, 485, 472	482	1929333	0.0043	17.41
5	567, 579, 582	576	2304000	0.00555	22.2
6	579, 561, 555	565	2260000	0.01146	45.8
7	611, 592, 589	597	2389333	0.017	68.1
8	542, 562, 559	554	2217333	0.0328	131.5
9	499, 522, 535	518	2074666	0.0784	313.8
10	572, 559, 563	564	225866	0.1218	487.5

[nCi/cluster x Kbq/cell 0.037]

0.427
0.644
0.822
1.696
2.521
4.865
11.613
18.037

TABLE-4

Expt # : 1 Date : 10/22/99

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1·2	137	123	145	130·33	
2·2	119	127	131		
3·2	132	126	121	126·33	0·9693
4·2	106	115	124	115	0·8823
52	99	107	116	107·3	0·8235
6·2	91	95	100	95·3	0·7314
7·2	72	65	59	65·3	0·5012
8·2	32	49	60	47	0·3606
9·2	24	30	19	24·33	0·1867
10·3	91	99	84	9·13	0·0700