3

V79 COLONY FORMING ASSAY

Experiment Name : ^{3}HTdR toxicity (cluster, 50% labeling); **Exp. # : ;** 3
Investigator: R. Howell **Date:** 5/3/2001

1. Set the rocker-roller at 37°C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 175 cm^2 flasks, seeded with $20x10^6$ cells 24 h before) with PBS, trypsinize cells with 2 ml trypsin 3 min at 37°C, resuspend in 8 ml MEMB, pool, pass five times through 10 cc syringe with 21 gauge needle, perform cell count by transferring 100 ul in Coulter cup containing 20 ml Isotone II (Coulter balanced electrolyte solution).
2. Dilute to ~2,000,000 cells/ml in MEMB [Actual count : 1.98×10^6 cells/ml)
   $[(4996+4893+9965)/3] \times 400 = 1.98 \times 10^6$ cells/ml
3. Transfer 1 ml of cell suspension into 20 12 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:** 5/3 2:30pm
5. Prepare MEMB containing radioactivity in hood
   800 µl ^{3}HTdR (Stock : 1.0 µCi/µl on 4/16/01) + 4.2 ml MEMB
   Manufacturer: NEN NET-027Z Lot #: 3106-421 Calibration: 4/16/01 12:00
6. After 3-4 h, remove first set of 10 test tubes from roller and add MEMB with or without radioactivity according to Table below. Also add 1 ml MEMB to each of second set of tubes.
   Date/Time:

Tube #	^{3}HTdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{3}HTdR (ml) [160uCi/ml]	
1	0	1.0	1.0	0	
2	0	1.0	1.0	0	
3	10	1.0	0.875	0.125	-
4	20	1.0	0.75	0.25	-
5	30	1.0	0.625	0.375	-
6	40	1.0	0.5	0.5	-
7	50	1.0	0.375	0.625	-
8	60	1.0	0.25	0.75	-
9	70	1.0	0.125	0.875	-
10	80	1.0	0	1.0	-

6.5ml + 10ml = 16.5ml 4.5ml Make 5ml @ 160uCi/ml → 800uCi

7. Return test tubes to roller for 12-14 h . **Date/Time:** 5/3/2001 5:15pm
8. Next day, while test tubes are in roller label 10 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:** 6:50 am 5/4/2001
10. Remove buckets from centrifuge and carefully remove 150 µl of supernatant from tubes containing radioactivity and place in pre-labeled gamma-tube.
11. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
12. Centrifuge tubes for 10 min at 2000 rpm, 4°C
13. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
14. Centrifuge tubes for 10 min at 2000 rpm, 4°C
15. Decant supernatant, click tubes, vortex, resuspend in 10 ml of MEMA
16. Centrifuge tubes for 10 min at 2000 rpm, 4°C
17. Decant supernatant, click tubes, vortex, resuspend unlabeled cells in 5 ml wash MEMA.
18. Transfer unlabeled cells to the corresponding tubes containing 2,000,000 labelled cells.
19. Wash unlabeled cell tube with 5 ml MEMA and transfer to corresponding labeled cell tube.
20. Syringe the pooled cells 5 times with 5 ml syringe with 21 G needle.
21. Centrifuge tubes for 10 min at 2000 rpm, 4°C.
22. Decant supernatant completely, click tubes, vortex.
23. Transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tip.
24. Again add 200 ul ice cold MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
25. Centrifuge tubes for 5 min at 1000 rpm, 4°C
26. Transfer tubes at 10.5°C for 72 h. **Date/Time:** 10:08am 5/4/2001
27. Transfer 10 ul supernatant in three sets of 7 ml scintillation vials and add 6 ml liquid scintillation cocktail (Aquasol) from 150 ul supernatant removed earlier and count them for radioactivity **Date/Time:** 12:00 am 5/4/2001
28. 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:** 11:00am 5/7/01
29. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes
30. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***
31. Labeling and preparation of dilution tubes and colony dishes
    - load 66, 60 mm petri dishes with 4 ml MEMA
    - load 40 sterile 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.

32. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
33. Centrifuge tubes for 10 min at 2000 rpm, 4°C
34. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
35. Centrifuge tubes for 10 min at 2000 rpm, 4°C
36. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 5 cc syringe with 21 gauge needle
37. Determine cell concentration by transferring 100 µl to Coulter cup
38. Vortex tube, transfer 0.5 ml into dilution 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.
39. 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.
40. Transfer 200 µl of cell suspension (in triplicate) to ~~20~~ 7 ml scintillation vial containing 6 ml cocktail (Aquasol)
41. Incubate petri dishes for 1 week
42. Count vials for radioactivity **Date/Time :**
43. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
44. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.

Tube	Dilution	Colony Counts
1	1.2	161, 143, 123
2	2.2	132, 141, 124
3	3.2	88, 69, 70
4	4.2	77, 65, 55
5	5.2	72, 71, 80
6	6.2	62, 73, 58
7	7.2	73, 80, 78
8	8.2	89, 76, 85
9	9.2	58, 74, 85
10	10.2	83, 90, 71

Exp. 3 5/7/2001

Coulter Counts (100µl cells, Coulter set at 500µl)

Mutagenesis T75 + 12.5ml + below

1)	8985, 8822, 8917	3.56×10^6/ml	0.56ml for 2×10^6
2)	9664, 9567, 9312	3.81×10^6/ml	0.53 ml for 2×10^6
3)	9476, 9505, 9323	3.77×10^6/ml	0.53 ml for 2×10^6
4)	7770, 7907, 7819	3.13×10^6/ml	0.64 ml
5)	7901, 7830, 7715	3.13×10^6/ml	0.64 ml
6)	7279, 7241, 7095	2.88×10^6/ml	0.69 ml
7)	7231, 7150, 7128	2.87×10^6/ml	0.70 ml
8)	7590, 6689, 7508, 7655	3.03×10^6/ml	0.66 ml
9)	6853, 6826, 6447	2.68×10^6/ml	0.75 ml
10)	7188, 6886, 6972	2.81×10^6/ml	0.71 ml

Background - 8 counts

Mode - 500µl.

Exp 3

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 TUE 08 MAY 2001 12:08
[redacted]PLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
[redacted]: 1 AQC:N QCF:N RCM:N
CHANNEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:@ 1.00000
HALF LIFE(DAYS):N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR	
1	**- 1	1	15.00	51.64	1.00	1.43	75.0		1M
2	**- 2	1	~~14.00~~	16.78	1.00	3.06	76.0		1M
3	**- 3	1	8.00	70.71	1.00	4.63	75.0		1M
4	**- 4	1	12.00	57.74	1.00	6.20	76.0		2M
5	**- 5	1	15.00	51.64	1.00	7.77	73.0		2M
6	**- 6	1	12.00	57.74	1.00	9.33	74.0		2M
7	**- 7	1	91020.00	1.71	0.15	10.03	77.0		3M
8	**- 8	1	93060.00	1.69	0.15	10.73	77.0		3M
9	**- 9	1	90346.66	1.72	0.15	11.49	76.0		3M
10	**-10	1	188060.00	1.46	0.10	12.20	76.0		4M
11	**-11	1	174733.33	1.24	0.15	12.91	76.0		4M
12	**-12	1	170833.33	1.25	0.15	13.62	76.0		4M
13	**-13	1	285273.31	0.97	0.15	14.33	77.0		5M
14	**-14	1	270320.00	0.99	0.15	15.05	77.0		5M
15	**-15	1	287660.00	0.96	0.15	15.77	77.0		5M
16	**-16	1	399359.97	0.82	0.15	16.49	77.0		6M
17	**-17	1	374033.31	0.84	0.15	17.23	75.0		6M
[redacted]	**-18	1	382166.66	0.84	0.15	17.96	77.0		6M
[redacted]	28- 1	1	502586.66	0.73	0.15	18.74	75.0		7M
20	28- 2	1	476146.66	0.75	0.15	19.48	76.0		7M
21	28- 3	1	506266.66	0.73	0.15	20.22	77.0		7M
22	28- 4	1	476353.31	0.75	0.15	20.95	77.0		8M
23	28- 5	1	625933.31	0.65	0.15	21.70	75.0		8M
24	28- 6	1	591526.62	0.67	0.15	22.44	77.0		8M
25	28- 7	1	673533.31	0.63	0.15	23.19	76.0		9M
26	28- 8	1	673346.62	0.63	0.15	23.94	76.0		9M
27	28- 9	1	596840.00	0.67	0.15	24.69	76.0		9M
28	28-10	1	712086.62	0.61	0.15	25.46	75.0		10M
29	28-11	1	766946.62	0.59	0.15	26.22	75.0		10M
30	28-12	1	760160.00	0.73	0.10	26.98	77.0		10M
31	28-13	1	13.00	55.47	1.00	28.60	100.0		1C
32	28-14	1	7.00	75.59	1.00	30.17	100.0		1C
33	28-15	1	16.00	50.00	1.00	31.74	98.0		1C
34	28-16	1	7.00	75.59	1.00	33.36	96.0		2C
35	28-17	1	9.00	66.67	1.00	34.92	97.0		2C
36	28-18	1	7.00	75.59	1.00	36.49	97.0		2C
37	**- 1	1	66793.33	2.00	0.15	37.24	97.0		3C
38	**- 2	1	62370.00	1.79	0.20	38.00	96.0		3C
39	**- 3	1	62560.00	1.79	0.20	38.75	95.0		3C
40	**- 4	1	110099.99	1.56	0.15	39.45	97.0		4C
41	**- 5	1	96940.00	1.66	0.15	40.16	98.0		4C
42	**- 6	1	96473.33	1.66	0.15	40.87	97.0		4C
[redacted]	**- 7	1	167326.66	1.26	0.15	41.58	98.0		5C
[redacted]	**- 8	1	161726.66	1.28	0.15	42.29	95.0		5C
45	**- 9	1	163960.00	1.28	0.15	43.00	96.0		5C
46	**-10	1	~~163106.66~~	1.28	0.15	43.71	95.0		

Exp 3

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
[redacted]	**-11	1	203566.66	1.14	0.15	44.42	96.0	6C
48	**-12	1	218086.66	1.11	0.15	45.14	99.0	
49	**-13	1	287373.31	0.96	0.15	45.86	96.0	7C
50	**-14	1	272426.66	0.99	0.15	46.57	96.0	
51	**-15	1	296146.66	0.95	0.15	47.31	98.0	
52	**-16	1	330700.00	0.90	0.15	48.03	97.0	8C
53	**-17	1	316020.00	0.92	0.15	48.76	97.0	
54	**-18	1	323900.00	0.91	0.15	49.48	98.0	
55	**- 1	1	437546.66	0.78	0.15	50.27	97.0	9C
56	**- 2	1	378186.66	0.84	0.15	50.99	97.0	
57	**- 3	1	439199.97	0.78	0.15	51.72	99.0	
58	**- 4	1	475906.66	0.75	0.15	52.46	98.0	10C
59	**- 5	1	468266.66	0.75	0.15	53.19	97.0	
60	**- 6	1	445526.66	0.77	0.15	53.93	98.0	
62	**- 8	1	29708.57	1.96	0.35	54.89	0.0	

#3 standard.
DPM = 98200

Parameters

Date	4/19/01		
Experiment No.	3		
Investigator	R. Howell		
Cell Line	V79		
Modifier	None		
Radionuclide	H-3		
Half-life (days)	4500.45	I-125=59.408, H-3=4500.45, Po-210=138.376, I-131=8.02	
Radiation Yield	1	I-125=1.47, H-3=1.0, Po-210=1.0, I-131=8.02	
Radiochemical	H3 thymidine NET-027Z		
Manufacturer/Lot	NEN/3106-421		
Original Calibration Date/Time	4/16/01 12:00	Original Activity Concentration (MBq/ml)	37
Present Calibration Date/Time	5/4/01 12:00	Time Elapsed Since Original Calibration (d)	18
Fraction of Cells Labeled	0.5	Present Activity Concentration (MBq/ml)	36.90
Liquid Scintillation Cocktail	Ecolume		
Volume of LSC Cocktail (ml)	6		
Volume/Type Counting Vial	7		
Model of Counter	Beckman 5800		
Counting Efficiency	0.65		
Activity Added (Date/Time)	5/3/01 17:15		
Cells Washed (Date/Time)	5/4/01 6:50	Time Elapsed Between Add and Wash (hr)	13.50
Medium Tubes Counted (Date/Time)	5/8/01 12:08	Time Elapsed Between Add and Count (hr)	112.75
Cell Tubes Counted (Date/Time)	5/8/01 12:08	Time Elapsed Between Wash and Count (hr)	101.00
Vol. Supernatant Counted (µl)	10		
Vol. Suspension Counted Cell Activity (µl)	200		
Vol. Suspension Coulter (µl)	100		
Coulter Manometer Volume (µl)	500		
Average Coulter Background Counts	8		
Coulter Calibration Parameter	400		
Hemocytometer Counting (Yes or No)?	No		

Background		
Coulter 1	Coulter 2	Coulter 3
8	8	8

MediumActivity

Experiment: 3
Date: 4/19/2001

Tube #	Medium count (CPM) 1st	2nd	3rd	CPM Average	CPM corrected for control	DPM CPM/(y e)	At µCi/ml on counting	Ao µCi/ml at addition [At/e-0.693t/T]	Ao kBq/ml at addition
1	*15*		*8*	*12*	*0*	*0*	*0*	*0*	*0*
2	*12*	*15*	*12*		*0*	*0*	*0*	*0*	*0*
3	91020	93060	90347	91476	91463	140713	6.3384	6.3430	234.6909
4	188060	174733	170833	177875	177863	273635	12.3259	12.3348	456.3888
5	285273	270320	287660	281084	281072	432418	19.4783	19.4924	721.2188
6	399360	374033	382167	385187	385174	592576	26.6926	26.7119	988.3410
7	502587	476147	506267	495000	494988	761520	34.3027	34.3275	1270.1183
8	476353	625933	591527	564604	564592	868603	39.1263	39.1546	1448.7193
9	673533	673346	596840	647906	647894	996760	44.8991	44.9316	1662.4687
10	712087	766947	760160	746398	746386	1148286	51.7246	51.7620	1915.1942

CellSuspension

Experiment: 3
Date: 04/19/01

Tube #	Suspension count (CPM) 1st	2nd	3rd	CPM Average	CPM corrected for control	DPM CPM/(y e)	A_i µCi/ml on counting	A_o µCi/ml after uptake	A_o kBq/ml after uptake
1	*13*	*7*	*16*	*10*	*0*	*0*	*0.00000*	*0*	*0.0000*
2	*7*	*9*	*7*		*0*	*0*	*0.00000*	*0*	*0.0000*
3	66793	62370	62560	63908	63898	98304	0.22141	0.22155	8.1973
4	110100	96940	96473	101171	101161	155633	0.35052	0.35075	12.9778
5	167327	161727	163960	164338	164328	252813	0.56940	0.56977	21.0814
6		203567	218087	210827	210817	324334	0.73048	0.73096	27.0454
7	287373	272427	296147	285316	285306	438932	0.98859	0.98923	36.6014
8	330700	316020	323900	323540	323530	497739	1.12103	1.12176	41.5051
9	437547	378187	439200	418311	418302	643541	1.44942	1.45036	53.6632
10	475907	468267	445527	463234	463224	712652	1.60507	1.60611	59.4262

CoulterSurvival

riment: 3
e/Time: 4/19/01

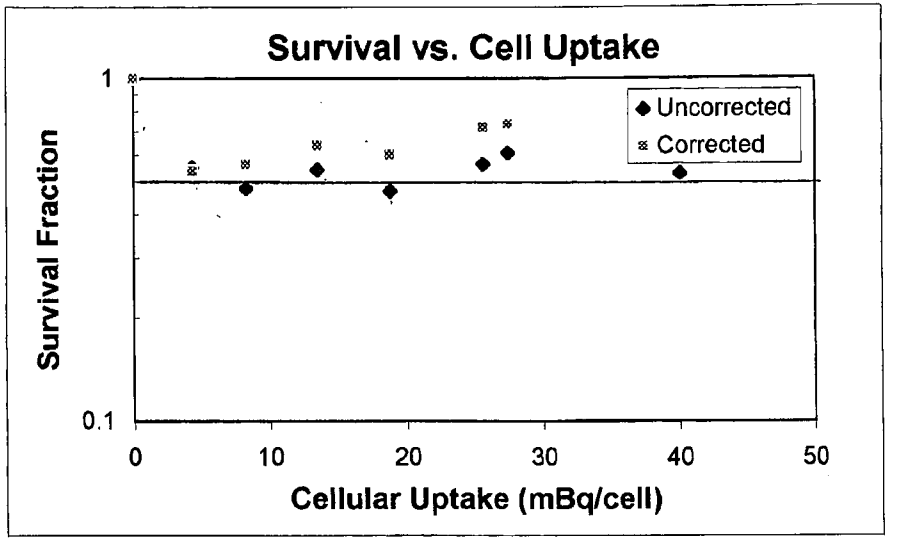
Tube #	Coulter count 1st	2nd	3rd	Average	Cells/ml	Hemocytometer Count in Grid 1st	2nd	3rd	4th
1	*8985*	*8822*	*8917*	*8908*	*3560000*				
2	*9664*	*9567*	*9312*	*9514*	*3802533*				
3	9476	9505	9323	9435	3770667				
4	7770	7907	7819	7832	3129600				
5	7901	7830	7715	7815	3122933				
6	7279	7241	7095	7205	2878800				
7	7231	7150	7128	7170	2864667				
8	7590	7508	7655	7584	3030533				
9	6853	6826	6447	6709	2680267				
10	7188	6886	6972	7015	2802933				

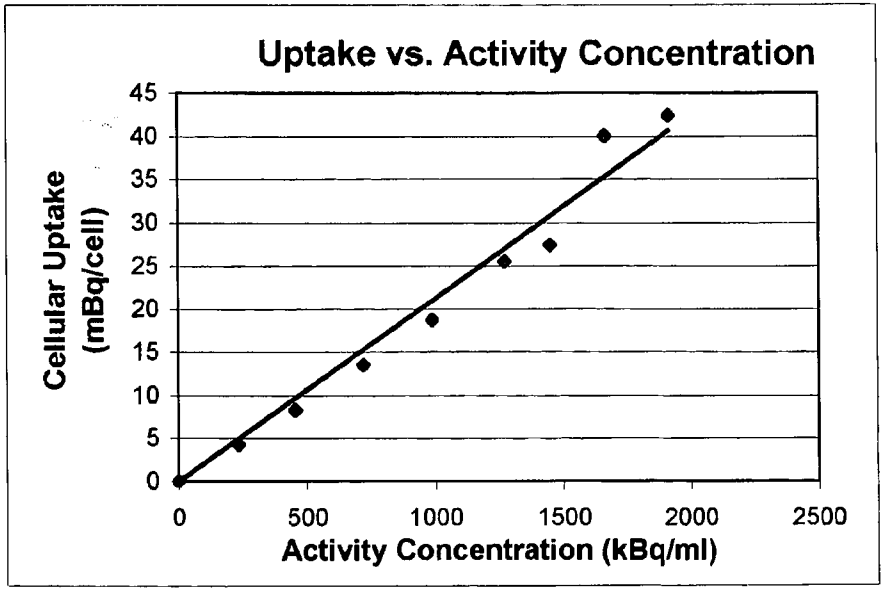
Tube #	Predicted # Cells Seeded	Actual # Cells Seeded	Colony count 1st	2nd	3rd	Average	PE (%)	SF Uncorrected	SF Corrected
1	200	*356*	*161*	*143*	*123*	*137*	*37.306*	*1.00*	*1.0000*
2	200	*380*	*132*	*141*	*124*				
3	200	377	88	69	70	76	20.067	0.5510	0.5379
4	200	313	77	65	55	66	20.982	0.4782	0.5624
5	200	312	72	71	80	74	23.802	0.5413	0.6380
6	200	288	62	73	58	64	22.347	0.4684	0.5990
7	200	286	73	80	78	77	26.879	0.5607	0.7205
8	200	303	89	76	85	83	27.498	0.6068	0.7371
9	200	268	58	74	85	72	26.987	0.5267	0.7234
10	200	280	83	90	71	81	29.017	0.5922	0.7778

Summary

Experiment:
Date/Time: 4/19/01

Tube #	Activity Conc. (kBq/ml)	Activity/Cell (mBq/cell)	Survival Uncorrected	Survival Corrected
1	*0.000*	*0.000*	*1.0000*	*1.0000*
2	*0.000*	*0.000*		
3	234.691	4.348	0.5510	0.5379
4	456.389	8.294	0.4782	0.5624
5	721.219	13.501	0.5413	0.6380
6	988.341	18.789	0.4684	0.5990
7	1270.118	25.554	0.5607	0.7205
8	1448.719	27.391	0.6068	0.7371
9	1662.469	40.043	0.5267	0.7234
10	1915.194	42.403	0.5922	0.7778





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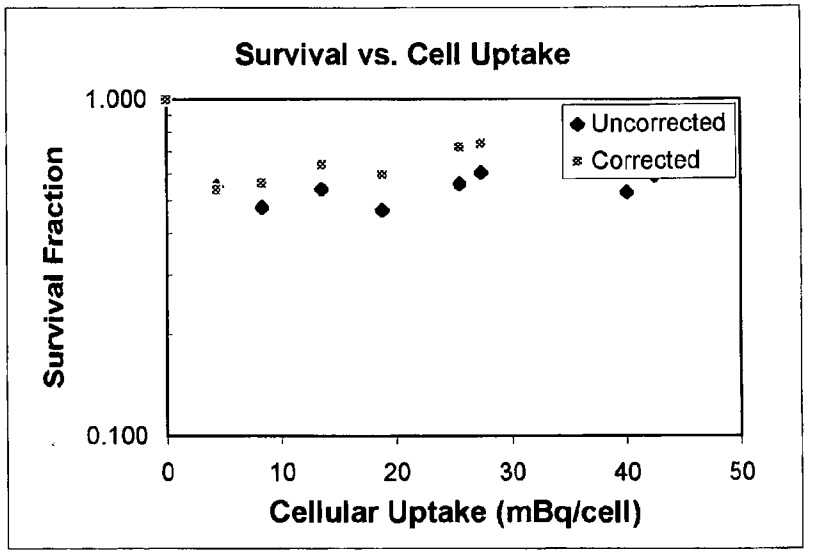
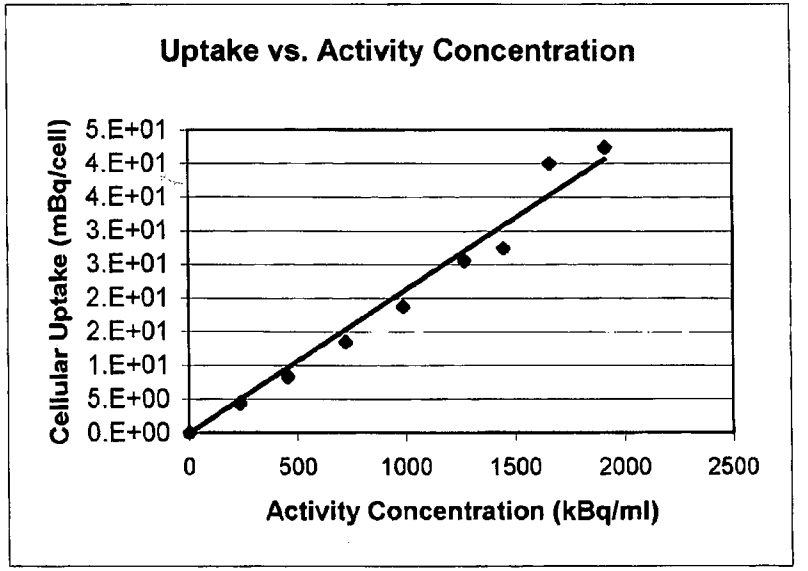
Experiment:
Date/Time: 4/19/01

Tube #	Activity Conc. (kBq/ml)	Activity/Cell (mBq/cell)	Survival Uncorrected	Survival Corrected
1	*0.000*	*0.000*	*1.0000*	*1.0000*
2	*0.000*	*0.000*		
3	234.691	4.348	0.5510	0.5379
4	456.389	8.294	0.4782	0.5624
5	721.219	13.501	0.5413	0.6380
6	988.341	18.789	0.4684	0.5990
7	1270.118	25.554	0.5607	0.7205
8	1448.719	27.391	0.6068	0.7371
9	1662.469	40.043	0.5267	0.7234
10	1915.194	42.403	0.5922	0.7778





HPRT mutant expression datasheet - Day 3

Date: 10-May-01
Experiment: V79, HTdR, 50% cluster, Roger's exp # 3

Sample #	Coulter count			Coulter bckgr.	Coulter Mode (µl)	# of cells per ml	Total # of cells x 10^6 (in 5 ml)	Cell susp. volume for 1000000 cells (ml)
1	9369	9103	9227	4	500	3691600	18.5	0.27
2	9727	9585	9579	4	500	3850533	19.3	0.26
3	8693	8509	8454	4	500	3419200	17.1	0.29
4	7737	7611	7600	4	500	3058133	15.3	0.33
5	7020	6816	6977	4	500	2773467	13.9	0.36
6	8526	8421	8221	4	500	3354133	16.8	0.30
7	7227	7173	7246	4	500	2884533	14.4	0.35
8	6389	6438	6286	4	500	2546800	12.7	0.39
9	6780	6713	6284	4	500	2635333	13.2	0.38
10	6926	7072	6991	4	500	2796933	14.0	0.36

Protocol:
1. Wash cells 1x with 10 ml PBS (no Ca++, no Mg++)
2. Add 2 ml trypsinize/ T75 & keep at RT for 3-4 min.
3. Add 10 ml medium (MEMA) & resuspend the cells.
4. Transfere cell suspension into 15 ml conical tube.
5. Spin the cells down: 4-5 min, 2K rpm, RT.
6. Aspirate supernatant.
7. Resuspend cells in 5-7 ml regular MEMA medium.
8. Siringe cells 3x with 21 gauge.
7. Count the cells: 100 µl cell susp. + 20 ml Isotone
8. Plate 2 000 000 / T75 in 10 ml regular MEMA

HPRT mutant expression datasheet - Day 7

Date: 14-May-01
Experiment: V79, HTdR, 50% cluster, Roger's exp # 3

Sample #	Coulter count			Coulter bckgr.	Coulter Mode (µl)	# of cells per ml	Total # of cells x 10^6 (in 5 ml)	Cell susp. volume for 1000000 cells (ml)
1	2486	2494	2319	4	500	971600	4.9	1.03
2								
3	3707	3712	3810	4	500	1495600	7.5	0.67
4	3070	3236	3239	4	500	1271067	6.4	0.79
5	3140	3161	3048	4	500	1244933	6.2	0.80
6	3254	3223	3238	4	500	1293733	6.5	0.77
7	2847	2849	2832	4	500	1135467	5.7	0.88
8	2182	2146	2177	4	500	865733	4.3	1.16
9	2243	2091	2165	4	500	864933	4.3	1.16
10	2004	1930	1969	4	500	785467	3.9	1.27

Protocol:
1. Wash cells 1x with 10 ml PBS (no Ca++, no Mg++)
2. Add 2 ml trypsinize/ T75 & keep at RT for 3-4 min.
3. Add 10 ml medium (MEMA) & resuspend the cells.
4. Transfere cell suspension in 15 ml conical tube.
5. Spin the cells down: 4-5 min, 2K rpm, RT.
6. Aspirate supernatant.
7. Resuspend cells in 5-7 ml regular MEMA medium.
8. Siringe cells 3x with 21 gauge.
7. Count the cells: 100 µl cell susp. + 20 ml Isotone
8. Plate 2 000 000 / T75 in 10 ml regular MEMA

HPRT mutant expresslon datasheet - Day 9

Date: 16-May-01
Experiment: V79, HTdR, 50% cluster (Roger's exp # 3)

Sample #	Coulter count			Coulter bckgr.	Coulter Mode (µl)	# of cells per ml	Total # of cells x 10^6 (in 5 ml)	Cell susp. volume for 1000000 cells (ml)
1	6330	6426	6698	5	500	2591867	13.0	0.39
2								
3	5781	5650	5363	5	500	2237200	11.2	0.45
4	6516	6725	6880	5	500	2680800	13.4	0.37
5	7150	7274	7095	5	500	2867200	14.3	0.35
6	6426	6325	6203	5	500	2525200	12.6	0.40
7	6768	6687	6843	5	500	2704400	13.5	0.37
8	6977	6993	6768	5	500	2763067	13.8	0.36
9	6693	6816	6789	5	500	2704400	13.5	0.37
10	7150	7464	7241	5	500	2912000	14.6	0.34

Note : Cells for sample # 2 was plated from sample # 1

Protocol:
1. Wash cells 1x with 10 ml PBS (no Ca++, no Mg++)
2. Add 2 ml trypsinize/ T75 & keep at RT for 3-4 min.
3. Add 10 ml medium (MEMA) & resuspend the cells.
4. Transfere cells suspension in 15 ml conical tube.
5. Spin the cells down: 4-5 min, 2K rpm, RT.
6. Aspirate supernatant.
7. Resuspend cells in 5-7 ml regular MEMA medium.
8. Siringe cells 3x with 21 gauge.
7. Count the cells: 100 µl cell susp. + 20 ml Isotone
8. Plate 2 000 000 / T75 in 10 ml regular MEMA

HPRT mutant expression datasheet - Day 11

Date: 18-May-01
Experiment: V79, HTdR, 50% cluster (Roger's exp # 3)

Sample #	Coulter count			Coulter bckgr.	Coulter Mode (µl)	# of cells per ml	Total # of cells x 10^6 (in 4 ml)	Volume of cell susp. For 10(6) cells (in ml)
1	8537	8249	8526	4	500	3373333	13.5	0.30
2	8326	8454	8210	4	500	3330400	13.3	0.30
3	8210	8365	8121	4	500	3291200	13.2	0.30
4	8448	8393	8443	4	500	3369600	13.5	0.30
5	7721	7978	7764	4	500	3126800	12.5	0.32
6	8554	8889	8856	4	500	3504933	14.0	0.29
7	7578	7742	7578	4	500	3051467	12.2	0.33
8	8559	8497	8371	4	500	3388667	13.6	0.30
9	8138	8326	8309	4	500	3301467	13.2	0.30
10	7247	7036	7074	4	500	2846000	11.4	0.35

Protocol:
1. Wash cells 1x with 10 ml PBS (no Ca++, no Mg++)
2. Add 2 ml trypsinize/ T75 & keep at RT for 3-4 min.
3. Add 10 ml medium (MEMA) & resuspend the cells.
4. Transfere cells suspension in 15 ml conical tube.
5. Spin the cells down: 4-5 min, 2K rpm, RT.
6. Aspirate supernatant.
7. Resuspend cells in 5-7 ml regular MEMA medium.
8. Siringe cells 3x with 21 gauge.
7. Count the cells: 100 µl cell susp. + 20 ml Isotone
8. Plate 2 000 000 / T75 in 10 ml regular MEMA

HPRT mutant expression datasheet - Day 13

Date: 20-May-01
Experiment: V79, HTdR, 50 % cluster, (Roger's exp. # 3)

Sample #	Coulter count			Coulter bckgr.	Coulter Mode (µl)	# of cells per ml	Total # of cells x 10^6 (in 4 ml)	Cell susp. volume for 1000000 cells (ml)
1	10709	10861	10791	23	500	4305600	17.2	0.23
2	9465	9403	9510	23	500	3774533	15.1	0.26
3	9522	9493	9664	23	500	3814667	15.3	0.26
4	9836	9973	10089	23	500	3977200	15.9	0.25
5	10072	9853	9939	23	500	3972667	15.9	0.25
6	10100	10117	10267	23	500	4055333	16.2	0.25
7	10072	10383	10204	23	500	4078667	16.3	0.25
8	8985	8957	8766	23	500	3551867	14.2	0.28
9	9193	9222	9193	23	500	3671867	14.7	0.27
10	8811	8759	8626	23	500	3483600	13.9	0.29

Protocol:
1. Wash cells 1x with 10 ml PBS (no Ca++, no Mg++)
2. Add 2 ml trypsinize/ T75 & keep at RT for 3-4 min.
3. Add 10 ml medium (MEMA) & harvest the cells.
4. Transfere cells suspension in 15 ml conical tube.
5. Spin the cells down: 4-5 min, 2K rpm, RT.
6. Aspirate supernatant.
7. Resuspend cells in 5-7 ml regular MEMA medium.
8. Siringe cells 3x with 21 gauge.
7. Count the cells: 100 µl cell susp. + 20 ml Isotone
8. Plate 2 000 000 / T75 in 10 ml regular MEMA

Challenge

HPRT mutant selection

Date: 21-May-01
Experiment: V79, HTdR, 50 % cluster, Roger's exp. #3

Sample #	Coulter count H			Coulter bckgr.	Coulter Mode (µl)	# of cells per ml	Cell susp. volume for 200 000 cells (ml)
1	4045	4068	4157	5	500	1634000	0.122
2	3408	3532	3463	10	500	1383067	0.145
3	4809	4918	4724	5	500	1924800	0.104
4	4955	4289	4451	5	500	1824000	0.110
5	4404	4404	4672	5	500	1795333	0.111
6	5520	5468	5692	5	500	2222000	0.090
7	5676	5750	5682	5	500	2279067	0.088
8	3131	3271	3201	10	500	1276400	0.157
9	3326	3438	3417	10	500	1353467	0.148
10	3347	3522	3587	10	500	1390133	0.144

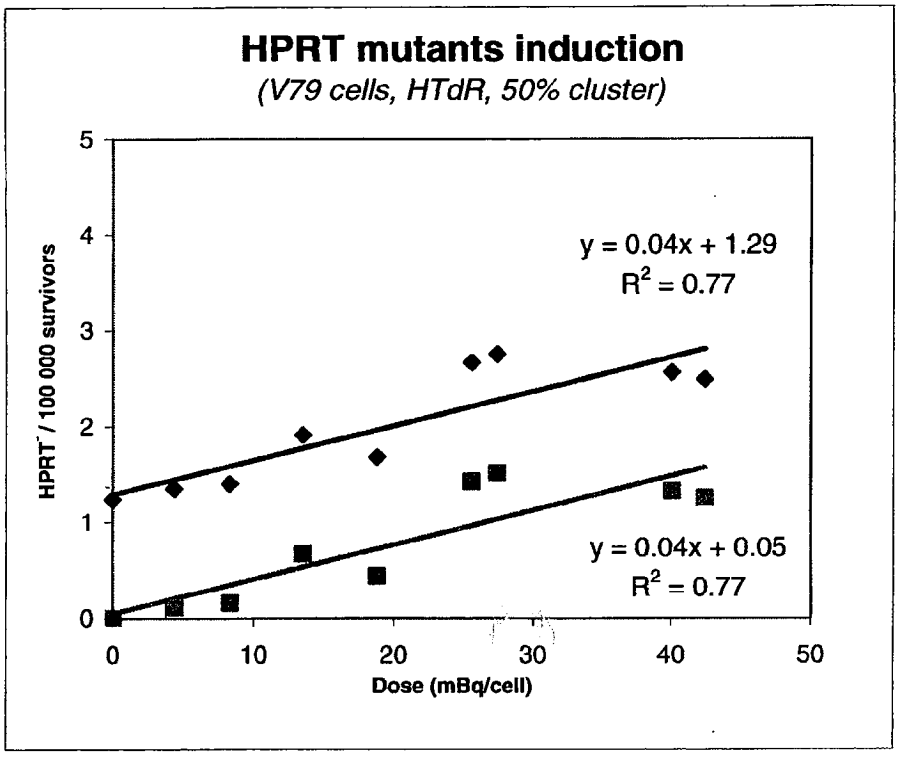
Protocol:
1. Wash T75 1-2x with PBS (no Ca++, no Mg++)
2. Trypsinize the cells (2 ml trypsin /T75, 2-3 min, RT)
3. Add 10 ml wash medium (wash MEMA) / T75 flask
4. Resuspend cells and transfere them in 15 ml conical tube.
5. Spin the cells down: 4-5 min, 2K rpm)
6. Aspirate supernatant, click tube to disperse pelet
7. Add 5 ml regular medium (MEMB/FCS10%) and resuspend the cells
8. Siringe the cells using 5 ml singe & 21G needle.
9. Count the cells using coulter couter: 100 µl cell susp. + 20 ml Isotone.
10. Plate 2 x 10(5) cells / P100 dish in 7 ml MEMA/FCS10% x 10 dishes/dose point
11. Transfere dishes into incubator and let the cells to attach (2-4 hrs)
12. Take 2 x 10(5) cells suspension, make serial dilution (2 x 10x) to obtain 200 cells/ml.
13. Plate 200 cells / P 60 x 3 dishes / dose point for Plating Efficiency.
14. Repaet staep 11 and 12 for each sampling point.
15. After 2-4 hrs add 3 ml MEMA + 6-TO (60 µg 6-TO) into each P 100.
    (Final concentartion for 6-TO = 6 µg/ml) 6-T0 Stock sol. = 60 µg/ 3 ml MEMA)
16. Keep the dishes in standard culture condition for 8-10 days.
17. Wash HPRT- colonies 1-2 times with PBS, fix them with MetOH and stain.
18. Count colonies.

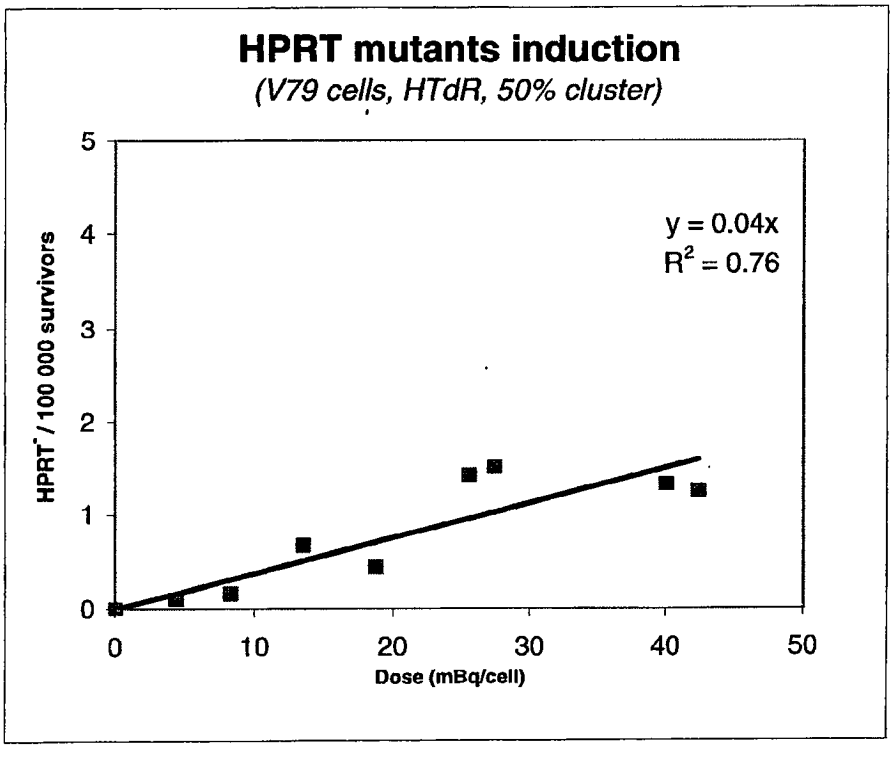
Table 1. Plating efficiency for HPRT challenge (Roger's exp. # 3)

Set	HTdR (mBq/cell)	Number of cells originally plated	Survival Numer of colonies	Avg. # of col./ plate	Abs PE	Abs PE Avg.	+/- Std
1.1	0	200	167	143	0.84	0.89	0.10
		200	175		0.88		
		200	191		0.96		
1.2	0	144	109		0.76	0.75	
		144	94		0.65		
		144	121		0.84		
3		200	141	141	0.71	0.71	0.01
		200	138		0.69		
		200	144		0.72		
4		200	118	127	0.59	0.63	0.08
		200	117		0.59		
		200	145		0.73		
5		200	188	181	0.94	0.91	0.03
		200	179		0.90		
		200	176		0.88		
6		200	164	165	0.82	0.82	0.03
		200	160		0.80		
		200	170		0.85		
7		200	166	161	0.83	0.80	0.03
		200	155		0.78		
		200	161		0.81		
8		200	166	171	0.83	0.86	0.05
		200	164		0.82		
		200	183		0.92		
9		200	165	173	0.83	0.87	0.07
		200	189		0.95		
		200	166		0.83		
10		200	157	160	0.79	0.80	0.02
		200	163		0.82		
		200	159		0.80		

Table 2. HPRT mutant frequency for V79 cells exposed to HTdR in 50% cluster model.

Sample #	Dose (mBq/cell)	# of cells (x10E5)	HPRT mutants per plate										SUM	Total # of cells plated	PE for challenge (in Tab.1)	HPRT per plated cell	mutants per survived cell	per 100000 survivors	
			1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th							
													12						
1.1	*0*	*2*	*5*	*2*	*2*	*2*	*1*							*12*	*1000000*	*0.89*	*0.000012*	*0.0000135*	*1.5*	*0.0*
1.2	*0*	*1.44*	*4*	*2*	*3*	*1*	*4*							*14*	*720000*	*0.75*	*0.000019*	*0.0000259*		
1.1+1.2	0	1.72	5	2	2	2	1	4	2	3	1	4		26	1720000	0.82	0.000015	0.0000184	1.2	0.0
3	4	2	1	5	5	5	3	3	4	5	0	7		38	2000000	0.71	0.000019	0.0000268	1.3	0.1
4	8	2	4	3	5	5	6	1	3	5	8			40	1800000	0.63	0.000022	0.0000353	1.4	0.2
5	14	2	8	3	2	4	2	5	8	3	4	3		42	2000000	0.91	0.000021	0.0000231	1.9	0.7
6	19	2	4	3	6	3	4	7	2	5	2	5		41	2000000	0.82	0.000021	0.0000250	1.7	0.4
7	26	2	6	10	10	3	3	8						40	1200000	0.8	0.000033	0.0000417	2.7	1.4
8	27	2	10	4	10	3	9	5	3	8	4	8		64	2000000	0.86	0.000032	0.0000372	2.8	1.5
9	40	2	7	11	7	7	2	4	2	9	7	3		59	2000000	0.87	0.000030	0.0000339	2.6	1.3
10	42	2	2	10	5	8	5	7	5	7	9	0		58	2000000	0.86	0.000029	0.0000337	2.5	1.3


HPRT mutants induction
(V79 cells, HTdR, 50% cluster)
HPRT⁻ / 100 000 survivors
y = 0.04x + 1.29
R² = 0.77
y = 0.04x + 0.05
R² = 0.77
0
1
2
3
4
5
0
10
20
30
40
50
Dose (mBq/cell)


HPRT mutants induction
(V79 cells, HTdR, 50% cluster)
HPRT⁻ / 100 000 survivors
y = 0.04x
R² = 0.76
0
1
2
3
4
5
0
10
20
30
40
50
Dose (mBq/cell)