V79 COLONY FORMING ASSAY

Experiment Name : ^{3}HTdR toxicity (cluster, 100% labeling) **Exp. # :** 5

Investigator: R. Howell **Date:** 7/16/01

Serum/Lot #s: Gibco Lot# 1023288 FCS

1. Set the rocker-roller at 37°C incubator with 5% CO_2, set the Coulter Counter, wash cells (from one 225 cm^2 flasks, subcultured 1:2 the day before) with PBS-PS, trypsinize cells with 2 ml trypsin 3 min at 37°C, resuspend in 10 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 ~4,000,000 cells/ml in MEMB [Actual count : 4.05×10^6 cells/ml)

   10135
10089
10152 } $10125 \times 400 = 4.05 \times 10^6$ cells/ml
3. Transfer 1 ml of cell suspension into 10 14 ml tubes (Falcon polypropylene 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:** 2:15 pm 7/16/01
5. Prepare MEMB containing radioactivity in hood

   160 µl ^{3}HTdR (Stock : 1 µCi/µl on 7/5/01) + ml MEMB

   Manufacturer: NEN NET-027Z Lot #: Calibration: 7/5/01
6. After 3-4 h, remove tubes from roller and add MEMB with radioactivity according to Table below.

Tube #	^{3}HTdR µCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{3}HTdR [40 µCi/ml] (ml)
1	0	1.0	1.0	0
2	0	1.0	1.0	0
3	1	1.0	0.95	0.05
4	2	1.0	0.90	0.10
5	3	1.0	0.85	0.15
6	4	1.0	0.8	0.20
7	5	1.0	0.75	0.25
8	10	1.0	0.5	0.5
9	15	1.0	0.25	0.75
10	20	1.0	0	1.0
			8ml	3ml

Make 4ml
160µl H-3
+ 3.84 ml MEMB

7. Return test tubes to roller for 12-14 h. **Date/Time:** 07/16/01; 6-30 pm
8. Next day, while tubes are in roller label 10 gamma-tubes (13 X 100 mm VWR glass tube)
9. After ~12-14 h incubation period, remove all tubes and centrifuge at 2000 rpm ~~at 4°C for~~ 10 min (~~*precooled centrifuge*~~). **Date/Time:**
10. Remove buckets from centrifuge and carefully remove 150 µl of supernatant from tubes containing radioactivity and place in pre-labeled gamma-tubes.
11. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
12. Centrifuge tubes for 10 min at 2000 rpm
13. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
14. Centrifuge tubes for 10 min at 2000 rpm
17. Decant supernatant, click tubes, vortex, resuspend in 10 ml **MEMA**.
18. Centrifuge tubes for 10 min at 2000 rpm.
22. Decant supernatant completely, click tubes, vortex.
23. Transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 µl) using 200 µl pipette tip.
24. Again add 200 µl MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 µl)
25. Centrifuge tubes for 5 min at 1000 rpm, 4°C
26. Transfer tubes at 10.5°C for 72 h. **Date/Time:** 10:30am 7/17/01
27. Transfer 30 µl supernatant in three sets of 7 ml scintillation vials and add 6 ml liquid scintillation cocktail (Ecoscint) from 150 ul supernatant removed earlier and count them for radioactivity **Date/Time:** 10:30am 7/17/01

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 pipette

Date/Time: 7/20/01

29. Again add 200 µl 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 60 mm tissue culture 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. Transer $2x10^6$ cells to T75 flask for mutant expression.
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 7 ml scintillation vial containing 6 ml cocktail (Ecolume)
41. Incubate tissue culture 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.

For hprt expression

TABLE-3 100% cluster, H1dR, V79 cells

Expt. # : 5 July 20, 2001 **Date/Time :** ~12:00 am

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count - Bckgr x 400] - Bckgr. Total in 2ml	pCi/cell ~~[uCi/ml x 10^6 Cells/ml]~~ 2×10^6 cells (in 1)
1	4827 4878 4587	1 896 800	3 793 600	1.05
2	5348 5384 5312	2 130 400	4 260 800	0.94
3	4752 4858 4672	1 894 133	3 788 267	1.06
4	5120 4944 4934	1990 933	3 981 867	1.00
5	5457 5625 5562	2 210 400	4 420 800	0.91
6	5265 5084 ~~+77~~	~~2 061 000~~ ~~2 001 733~~	4 122 000	0.97
7	4929 4878 4753	1932 533	3 865 067	1.04
8	5115 4965 5094	2 014 400	4 028 800	0.95
9	4615 4711 4583	1, 839 733	3 679 466	1, 08
10	4662 4596 4647	1845 200	3 690 400	1,08

Bckgr - 22.
Mode - 500 pt

7/23/2001

Exp. 5

Replating of cells for expression. Trypsinize, syring, coulter count 100ul, transfer 2×10^6 cells to new flasks. T75 flasks

Vol (ml)		ml cells	Coulter counts	# of cells/ml.	total $\times 10^6$	2×10^6 cells
6	1	6	8660 8632	3,46	20,74	0,58
6	2	6	8221 8643	3,37	20,23	0,59
6	3	6	8321 8571	3,38	20,26	0,59
6	4	6	7231 7155	2,88	17.25	0.69
6	5	6	7769 7770	3,11	18,63	0.64
	6	10.8	3920, 3990, 3970	3960	1.58×10^6	1.26
	7	10,5	3793, 3876, 3873		1.56×10^6	1.28ml
	8	7.7	4388, 4288, 4305	4328	1.73×10^6	1.16ml
	9	contaminated	~~4388, 4288, 4305~~	~~4328~~	~~1.73×10^6~~	~~1.16ml~~
	10	7.8	4728, 5001, 4888	4872	1.95×10^6/ml	1.03ml

NOTE:

① Sample 1-5 - Beligor - 4
Mode - 500

② Would not be surprise if any of 1-5 get contamination, because of syringe piston

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Academic Connections

TABLE-3

Expt. # : 5 **Date/Time :** 7/26/01

only manom. vol. 100ul

Tube #	Coulter count for 100 ul cell suspension Manometer vol. 100ul	Avg. count	Cells/ml [Avg. count x 400] x 5	~~pCi/cell~~ ~~[uCi/ml x 10^6 Cells/ml]~~ ml cells for 2×10^6
1	1533, 1634, 1624	1597	3.19×10^6/ml	0.63
2	1642, 1653, 1705	1667	3.33×10^6/ml	0.60
3	1246, 1154, 1201	1200	2.40×10^6/ml	0.83
4	1699, 1627, 1667	1664	3.33×10^6	0.60
5	991, 994, 905	963	1.93×10^6	1.04
6	1257, 1323, 1224	1268	2.54×10^6	0.79
7	1397, 1350, 1405	1384	2.77×10^6	0.72
8	1485, 1383, 1367		2.82×10^6	0.71
9				
10	1381, 1331, 1364		2.75×10^6	0.73

Replating of cells for expression. Remove media from T75, wash 1X with PBS-PS, add 2 ml trypsin, set at room temp > 5 min. Resuspend in 8 ml wash MEMA, transfer to 12 ml tube, centrifuge 2000 rpm, decant, resuspend in 5 ml MEMA. Syringe 5X, 100ul to Coulter, coulter manometer volume 100ul. Transfer 2×10^6 cells to T150 flask containing 20 ml MEMA.

Split for mutation expression on 7/30/01

TABLE-3

Expt. # : 5 **Date/Time :** 7/30/01

Tube #	Coulter count for 100 ul cell suspension 500 µl manometer	Avg. count	Cells/ml [Avg. count x 400]	pCi/cell [uCi/ml x 10^6 Cells/ml] 2 x 10^6 cells
1	6039, 5960, 6050	6016	2.41 x 10^6/ml	0.83ml
2	7639, 7858, 7775	7757	3.10 x 10^6	0.65ml
3	contaminated			
4				0.75
5				0.75
6				0.75
7				0.75
8				0.75
9				
10	7333, 7150			0.75

Seed into T75 flasks

7/31/01 Colony Counting for Survival

Tube	Dilution	Colony Counts
1	200	108 114 114
2	200	85 81
3	200	63 68 83
4	200	94 84 73
5	200	66 108 67
6	200	61 93 73
7	200	56 55 52
8	200	47 38 37
9	200	46 38 54
10	200	40 30 30

Ex. 5

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 MON 23 JUL 2001 13:22
SAMPLE 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	9.00	66.67	1.00	1.42	82.0		1M
2	**- 2	1	8.00	70.71	1.00	3.00	82.0		
3	**- 3	1	7.00	75.59	1.00	4.57	83.0		
4	**- 4	1	5.00	89.44	1.00	6.13	81.0		2M
5	**- 5	1	16.00	50.00	1.00	7.69	81.0		
6	**- 6	1	8.00	70.71	1.00	9.26	82.0		
7	**- 7	1	29785.71	1.99	0.35	10.16	82.0		3M
8	**- 8	1	26352.50	1.95	0.40	11.17	81.0		
9	**- 9	1	28608.57	2.00	0.35	12.07	83.0		
10	**-10	1	57790.00	1.86	0.20	12.82	82.0		4M
11	**-11	1	55970.00	1.89	0.20	13.57	83.0		
12	**-12	1	57645.00	1.86	0.20	14.32	83.0		
13	**-13	1	85580.00	1.77	0.15	15.02	84.0		5M
14	**-14	1	83353.33	1.79	0.15	15.72	83.0		
15	**-15	1	82146.66	1.80	0.15	16.43	82.0		
16	**-16	1	115919.99	1.52	0.15	17.13	83.0		6M
17	**-17	1	116026.66	1.52	0.15	17.83	83.0		
18	**-18	1	117066.66	1.51	0.15	18.54	83.0		
[redacted]	**- 1	1	142806.66	1.37	0.15	19.29	82.0		7M
20	**- 2	1	134140.00	1.41	0.15	20.01	81.0		
21	**- 3	1	142500.00	1.37	0.15	20.72	82.0		
22	**- 4	1	346220.00	0.88	0.15	21.44	81.0		8M
23	**- 5	1	330266.66	0.90	0.15	22.16	82.0		
24	**- 6	1	336800.00	0.89	0.15	22.89	80.0		
25	**- 7	1	427873.31	0.79	0.15	23.63	82.0		9M
26	**- 8	1	409359.97	0.81	0.15	24.35	82.0		
27	**- 9	1	416326.66	0.80	0.15	25.09	82.0		
28	**-10	1	525273.31	0.71	0.15	25.83	82.0		10M
29	**-11	1	548100.00	0.70	0.15	26.58	83.0		
30	**-12	1	548013.31	0.70	0.15	27.32	83.0		
31	**-13	1	7.00	75.59	1.00	28.88	102.0		1C
32	**-14	1	9.00	66.67	1.00	30.44	101.0		
33	**-15	1	17.00	48.51	1.00	32.01	99.0		
34	**-16	1	7.00	75.59	1.00	33.57	101.0		2C
35	**-17	1	6.00	81.65	1.00	35.13	102.0		
36	**-18	1	7.00	75.59	1.00	36.71	102.0		
37	**- 1	1	7.00	75.59	1.00	38.32	74.0		3C
38	**- 2	1	2515.00	3.99	1.00	39.89	101.0		
39	**- 3	1	2487.00	4.01	1.00	41.47	100.0		
40	**- 4	1	5132.00	2.79	1.00	43.03	100.0		4C
41	**- 5	1	4865.00	2.87	1.00	44.61	102.0		
42	**- 6	1	4606.00	2.95	1.00	46.18	100.0		
43	**- 7	1	9053.00	2.10	1.00	47.76	101.0		5C
[redacted]	**- 8	1	8805.00	2.13	1.00	49.32	100.0		
[redacted]	**- 9	1	8504.00	2.17	1.00	50.90	101.0		
46	**-10	1	11281.11	1.98	0.90	52.37	101.0		

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#		ERR
[redacted]	**-11	1	11345.55	1.98	0.90	53.83	101.0	6C	
[redacted]	**-12	1	10826.32	1.97	0.95	55.36	102.0		
49	**-13					55.83		7C	101
50	**-14	1	13424.00	1.99	0.75	57.15	102.0		
51	**-15	1	13333.33	2.00	0.75	58.47	101.0		
52	**-16	1	41116.00	1.97	0.25	59.32	103.0	8C	
53	**-17	1	39310.00	1.84	0.30	60.17	101.0		
54	**-18	1	36616.66	1.91	0.30	61.04	102.0		
55	**- 1	1	27582.50	1.90	0.40	62.10	94.0	9C	
56	**- 2	1	20047.27	1.90	0.55	63.21	95.0		
57	**- 3	1	21732.00	1.92	0.50	64.27	96.0		
58	**- 4					64.73		10C	101
59	**- 5	1	50000.00	1.79	0.25	65.52	102.0		
60	**- 6	1	53260.00	1.94	0.20	66.28	101.0		

Control std. 29323 cpm

6-TG.

1. (2.5 mg + 10 ml MEMA) x 2 sample

Stock - 5 mg / 20 ml

2. Dilute powder in each bottle (10 ml)
3. Sirinye each one through filter (0.2)
4. Collect all 6-TG into 50 ml conical tube
5. It is your stock, sterile 6-TG solution.

Final concentration = 5 µg/ml

a) 10 ml / P100 ⟹ 10 ml × 5 µg ⟹ 50 µg/P100 (10 ml)

b) you have already 7 ml in each P100

c) 3 ml + 50 µg × # of P100's → X

Example:

~~10 samples~~

Need 250 ml @ $\frac{50}{3}$ mg/ml = 4167 mg

Stock = $\frac{5\text{ mg}}{20\text{ ml}}$ = 0.25 $\frac{\text{mg}}{\text{ml}}$ = $\frac{250\text{ µg}}{\text{ml}}$

Need 16.7 ml stock + 233.3 mg MEMA

B007445

MCA #1 - Canberra S100 -

Tag Number:	1028	Readout:	Sat 14 Jul 2001 @ 15:04:45
Report Group:	Full	Acquire Started:	Sat 14 Jul 2001 @ 14:48:03
Channel Size:	4096		

Elapsed Live Time:	10.00 min.
Elapsed True Time:	10.03 min.
Dead Time:	0.27 %

ADC:	Can1510,LLD=0.04,4k
Detector:	Can CAM300 PIPS
Geometry:	Am-241 disk against PIPS
Sample Description:	Am-241 disk (Tracerlab)

MCA Mode:	PHA+
Preset Conditions:	Live Time = 10.00 min.

Area Statistics:	Background Channels = 4
	% Error Sigma = 1.65

$$\frac{x\left(5.13\times10^{6}\ \frac{\text{cells}}{\text{ml}}\right)}{4.5+x} = 2\times10^{6}/\text{ml}$$

$$5.13\times10^{6}\,x = 9\times10^{6} + 2\times10^{6}x$$

$$3.13\times10^{6}\,x = 9\times10^{6} = 2.87$$

8/2/01

Exp. 5

Processing for mutant selection

Coulter counts (100 µl manometer, 100 µl cells)

					ml cells to add to 4.5 ml to get 2×10^6/ml
1)	2503	2631	2542	$2559\times400\times5 = 5.12\times10^6$/ml	2.9
2)	2411	2478	2399	$2429\times400\times5 = 4.86\times10^6$/ml	3.1
4)	~~5269~~ 2616	2806	2650	$2691\times400\times5 = 5.38\times10^6$/ml	2.7
5)	2692	2730	2659	$2694\times400\times5 = 5.38\times10^6$/ml	2.7
6)	2224	2289	2213	$2242\times400\times5 = 4.48\times10^6$/ml	3.6
7)	2206	2335	2294	$2278\times400\times5 = 4.56\times10^6$/ml	3.5
8)	2497	2317	2410	$2379\times400\times5 = 4.76\times10^6$/ml	3.3
10)	2087	2138	2051	$2092\times400\times5 = 4.18\times10^6$/ml	4.1

Insufficient volume of stock. dilute with 4 ml instead of 4.5

Preload: 80 100 mm dishes w/ 7 ml MEMA and put in incubator
24 60 mm dishes w/ 4 ml MEM "
40 12 ml round bottom with 4.5 ml wash MEMA

Procedure:

Trypsinize flasks, ~~resusp~~ with 2 ml trypsin after 10 ml PBS PS wash. Add 10 ml wash MEMA to flask & transfer to conical 15 ml tube. Centrifuge 4-5 min 2000 rpm. Decant, resusp in 5 ml MEMB. Syringe 5X with 21g, counter count 100 µl, transfer aliquot to 4.5 ml wash MEMA so final conc. is 2×10^6 cells/ml. Transfer 10×100 µl aliquots to 100 mm dishes. Perform 4 serial dilutions 0.5 → 4.5 ml and transfer 3×1 ml of final dilution to P60's.

$$\frac{x(4.18\times10^6)}{4+x} = 2\times10^6/\text{ml}$$

$$x = \frac{8\times10^6}{2.18\times10^6} = 3.7$$

8/31/01

Exp. 5

Mutant Colonies

1) 3, 2, 2, 1, 3, 1, 1, 2, 1, 4
2) 3, 2, 5, 4, 8, 8, 4, 5, 9
4) 6, 12, 9, 7, 8, 10, 6, 7, 9, 10
5) 7, 6, 9, 6, 5, 6, 9, 6, 6, 4
6) 8, 8, 3, 4, 6, 2, 5, 5, 4, 4
7) 7, 7, 10, 9, 6, 14, 5, 8, 9, 7
8) 15, 4, 8, 5, 8, 9, 7, 9, 10, 9
10) 9, 3, 8, 12, 8, 8, 9, 10, 6, 6

Exp 5 Selection 5/12
Stain 8/13

Plating Efficiency

1) 191, 193, 199
2) 272, 208, ~~250~~ 241
4) 271, 281, 290
5) 234, 223, 275
6) 221, 238, 225
7) 260, 239, 230
8) 208, 225, 211
10) 245, 248, 227

Exp 5 Plating Effic. 8/2
Stain 8/9

Parameters

Date	7/16/01		
Experiment No.	5		
Investigator	R. Howell		
Cell Line	V79 Frozen 10/00		
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	H3TdR		
Manufacturer/Lot	NEN NET-027Z Lot 3106-431		
Original Calibration Date/Time	7/5/01 0:00	Original Activity Concentration (MBq/ml)	37
Present Calibration Date/Time	7/16/01 0:00	Time Elapsed Since Original Calibration (d)	11
Fraction of Cells Labeled	1	Present Activity Concentration (MBq/ml)	36.94
Liquid Scintillation Cocktail	Ecolume		
Volume of LSC Cocktail (ml)	6		
Volume/Type Counting Vial	7 ml		
Model of Counter	Beckman 5500		
Counting Efficiency	0.65		
Activity Added (Date/Time)	7/16/01 18:30		
Cells Washed (Date/Time)	7/17/01 10:30	Time Elapsed Between Add and Wash (hr)	14.00
Medium Tubes Counted (Date/Time)	7/23/01 13:22	Time Elapsed Between Add and Count (hr)	163.00
Cell Tubes Counted (Date/Time)	7/23/01 13:22	Time Elapsed Between Wash and Count (hr)	163.00
Vol. Supernatant Counted (µl)	30		
Vol. Suspension Counted Cell Activity (µl)	200		
Vol. Suspension Coulter (µl)	100		
Coulter Manometer Volume (µl)	500		
Average Coulter Background Counts	22		
Coulter Calibration Parameter	400		
Hemocytometer Counting (Yes or No)?	No		

Background

Coulter 1	Coulter 2	Coulter 3
22	22	22

Parameters

Date	7/16/01		
Experiment No.	5		
Investigator	R. Howell		
Cell Line	V79 Frozen 10/00		
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	H3TdR		
Manufacturer/Lot	NEN NET-027Z Lot 3106-431		
Original Calibration Date/Time	7/5/01 0:00	Original Activity Concentration (MBq/ml)	37
Present Calibration Date/Time	7/16/01 0:00	Time Elapsed Since Original Calibration (d)	11
Fraction of Cells Labeled	1	Present Activity Concentration (MBq/ml)	36.94
Liquid Scintillation Cocktail	Ecolume		
Volume of LSC Cocktail (ml)	6		
Volume/Type Counting Vial	7 ml		
Model of Counter	Beckman 5500		
Counting Efficiency	0.65		
Activity Added (Date/Time)	7/16/01 18:30		
Cells Washed (Date/Time)	7/17/01 10:30	Time Elapsed Between Add and Wash (hr)	14.00
Medium Tubes Counted (Date/Time)	7/23/01 13:22	Time Elapsed Between Add and Count (hr)	163.00
Cell Tubes Counted (Date/Time)	7/23/01 13:22	Time Elapsed Between Wash and Count (hr)	163.00
Vol. Supernatant Counted (µl)	30		
Vol. Suspension Counted Cell Activity (µl)	200		
Vol. Suspension Coulter (µl)	100		
Coulter Manometer Volume (µl)	500		
Average Coulter Background Counts	22		
Coulter Calibration Parameter	400		
Hemocytometer Counting (Yes or No)?	No		

Background		
Coulter 1	Coulter 2	Coulter 3
22	22	22

MediumActivity

Experiment: 5
Date: 7/16/01

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	*9*	*8*	*7*	*9*	*0*	*0*	*0*	*0*	*0*
2	*5*	*16*	*8*		*0*	*0*	*0*	*0*	*0*
3	28785	26352	28608	27915	27906	42933	0.6446	0.6453	23.8764
4	57790	55970	57645	57135	57126	87886	1.3196	1.3210	48.8769
5	85580	83353	82146	83693	83684	128745	1.9331	1.9351	71.5998
6	115919	116026	117066	116337	116328	178966	2.6872	2.6900	99.5298
7	142806	134140	142500	139815	139807	215087	3.2295	3.2329	119.6178
8	346220	330266	336800	337762	337753	519620	7.8021	7.8103	288.9800
9	427873	409359	416326	417853	417844	642837	9.6522	9.6623	357.5052
10	525273	548100	548013	540462	540453	831466	12.4845	12.4975	462.4091

CellSuspension

Experiment: 5
Date: 07/16/01

Tube #	Suspension count (CPM) 1st	2nd	3rd	CPM Average	CPM corrected for control	DPM CPM/(y e)	A_t µCi/ml on counting	A_o µCi/ml after uptake	A_o kBq/ml after uptake
1	*7*	*9*	*17*	*9*	*0*	*0*	*0.00000*	*0*	*0.0000*
2	*7*	*6*	*7*		*0*	*0*	*0.00000*	*0*	*0.0000*
3	2515	2487		2501	2492	3834	0.00864	0.00864	0.3198
4	5132	4865	4606	4868	4859	7475	0.01684	0.01685	0.6236
5	9053	8805	8504	8787	8779	13505	0.03042	0.03045	1.1266
6	11281	11345	10826	11151	11142	17141	0.03861	0.03865	1.4299
7	13424	13333		13379	13370	20569	0.04633	0.04637	1.7159
8	41116	39310	36616	39014	39005	60008	0.13515	0.13529	5.0059
9	27582	20047	21732	23120	23112	35556	0.08008	0.08017	2.9661
10	50000	53260		51630	51621	79417	0.17887	0.17905	6.6250

CoulterSurvival

riment: 5
e/Time: 7/16/01

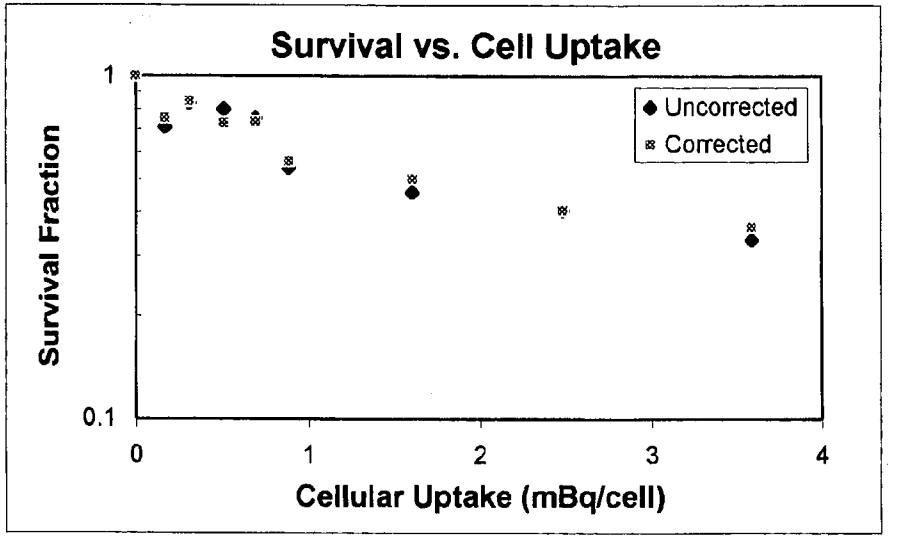
Tube #	Coulter count 1st	2nd	3rd	Average	Cells/ml	Hemocytometer Count in Grid 1st	2nd	3rd	4th
1	*4827*	*4878*	*4587*	*4764*	*1896800*				
2	*5348*	*5384*	*5312*	*5348*	*2130400*				
3	4752	4898	4672	4774	1900800				
4	5120	4944	4934	4999	1990933				
5	5457	5625	5562	5548	2210400				
6	5265	5084		5175	2061000				
7	4929	4878	4753	4853	1932533				
8	5115	4965	5094	5058	2014400				
9	4615	4711	4583	4636	1845733				
10	4662	4596	4647	4635	1845200				

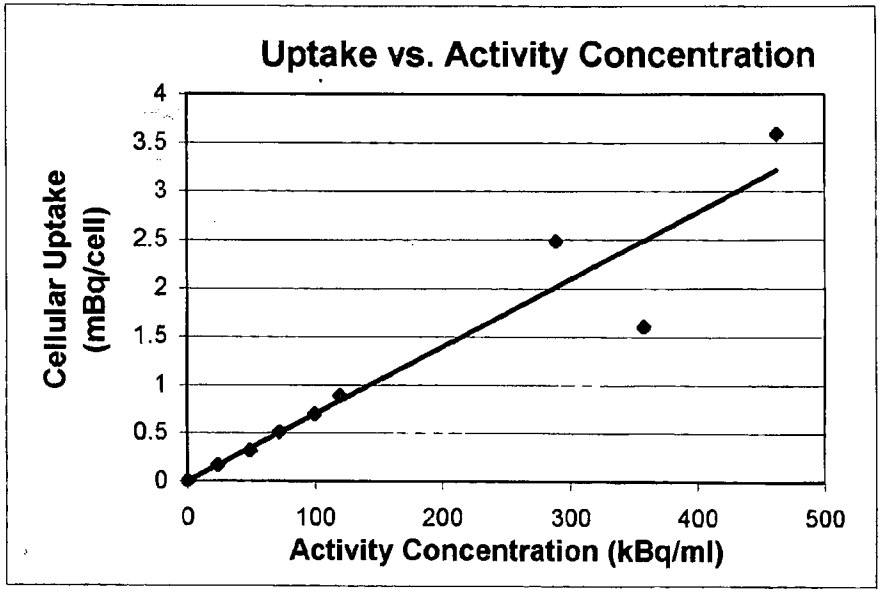
Tube #	Predicted # Cells Seeded	Actual # Cells Seeded	Colony count 1st	2nd	3rd	Average	PE (%)	SF Uncorrected	SF Corrected
1	200	*190*	*108*	*114*	*114*	*100*	*49.861*	*1.00*	*1.0000*
2	200	*213*	*85*	*81*					
3	200	190	63	68	83	71	37.528	0.7105	0.7527
4	200	199	94	84	73	84	42.024	0.8333	0.8428
5	200	221	66	108	67	80	36.343	0.8001	0.7289
6	200	206	61	93	73	76	36.714	0.7537	0.7363
7	200	193	56	55	52	54	28.115	0.5412	0.5639
8	200	201	47	38	37	41	20.188	0.4050	0.4049
9	200	185	46	38	54	46	24.922	0.4582	0.4998
10	200	185	40	30	30	33	18.065	0.3320	0.3623

Summary

Experiment:
Date/Time: 7/16/01

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	23.876	0.168	0.7105	0.7527
4	48.877	0.313	0.8333	0.8428
5	71.600	0.510	0.8001	0.7289
6	99.530	0.694	0.7537	0.7363
7	119.618	0.888	0.5412	0.5639
8	288.980	2.485	0.4050	0.4049
9	357.505	1.607	0.4582	0.4998
10	462.409	3.590	0.3320	0.3623

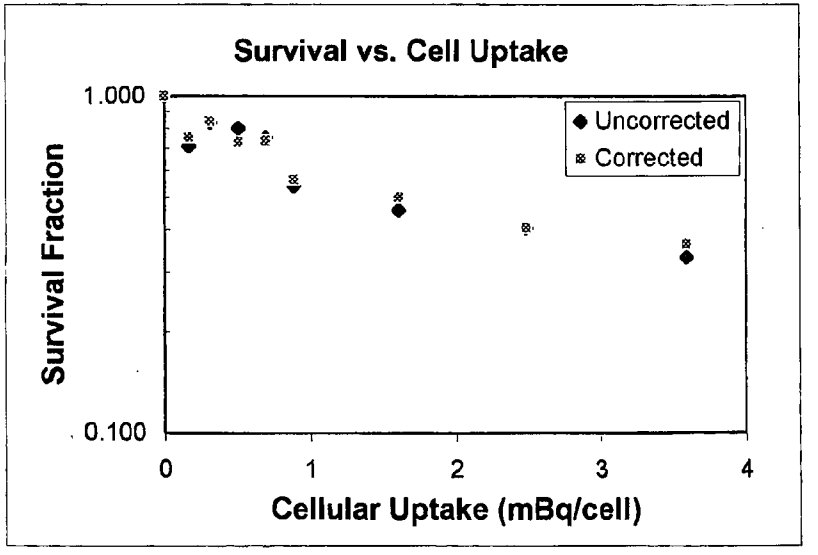


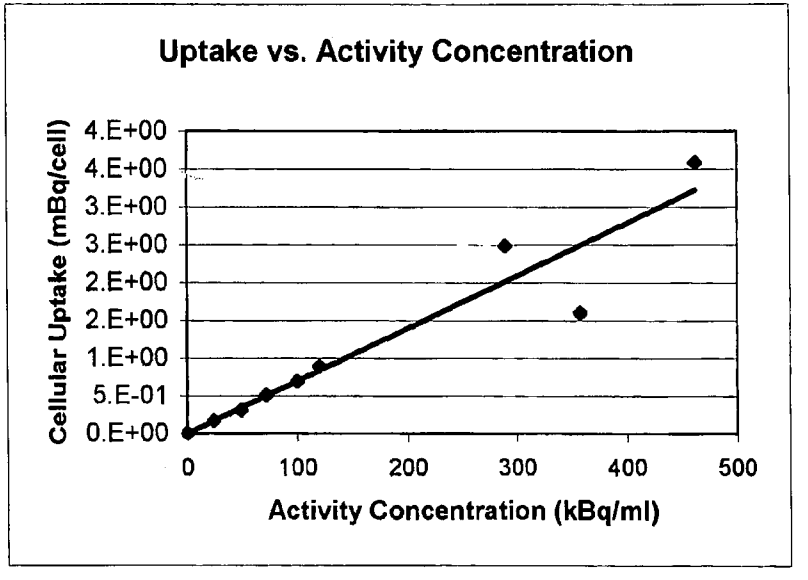


B007455

Experiment:
Date/Time: 7/16/01

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	23.876	0.168	0.7105	0.7527
4	48.877	0.313	0.8333	0.8428
5	71.600	0.510	0.8001	0.7289
6	99.530	0.694	0.7537	0.7363
7	119.618	0.888	0.5412	0.5639
8	288.980	2.485	0.4050	0.4049
9	357.505	1.607	0.4582	0.4998
10	462.409	3.590	0.3320	0.3623





Challenge

HPRT mutant selection

Date: 20-Aug-01
Experiment: V79, HTdR, 100 % cluster, Roger's exp. #5

Sample #	Coulter count H			Coulter bckgr.	Coulter Mode (µl)	# of cells per ml	Cell susp. volume for 200 000 cells (ml)
1	2503	2631	2542	5	100	1021467	0.196
2	2411	2478	2399	5	100	969733	0.206
3		NO SAMPLE		5			
4	2616	2806	2650	5	100		
5	2692	2730	2659	5	100	1075467	0.186
6	2224	2289	2213	5	100	894800	0.224
7	2206	2335	2294	5	100	909333	0.220
8	2497	2317	2410	5	100	961200	0.208
9				5	100	#DIV/0!	#DIV/0!
10	2087	2138	2051	5	100	834800	0.240

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. #5)

Set	HTdR (mBq/cell)	Number of cells originally plated	Survival Numer of colonies	Avg. # of col./ plate	Abs PE	Abs PE Avg.	+/- Std
1	0	200	191	194	0.96	0.97	0.02
		200	193		0.97		
		200	199		1.00		
2	0	200	272	240	1.36	1.20	0.16
		200	208		1.04		
		200	241		1.21		
3	0.1685	200		#DIV/0!	0.00	0.00	0.00
		200			0.00		
		200			0.00		
4	0.313	200	271	281	1.36	1.40	0.05
		200	281		1.41		
		200	290		1.45		
5	0.5095	200	234	244	1.17	1.22	0.14
		200	223		1.12		
		200	275		1.38		
6	0.694	200	221	228	1.11	1.14	0.04
		200	238		1.19		
		200	225		1.13		
7	0.888	200	260	243	1.30	1.22	0.08
		200	239		1.20		
		200	230		1.15		
8	2.485	200	208	215	1.04	1.07	0.05
		200	225		1.13		
		200	211		1.06		
9	1.607	200		#DIV/0!	0.00	0.00	0.00
		200			0.00		
		200					
10	3.5905	200	245	240	1.23	1.20	0.06
		200	248		1.24		
		200	227		1.14		

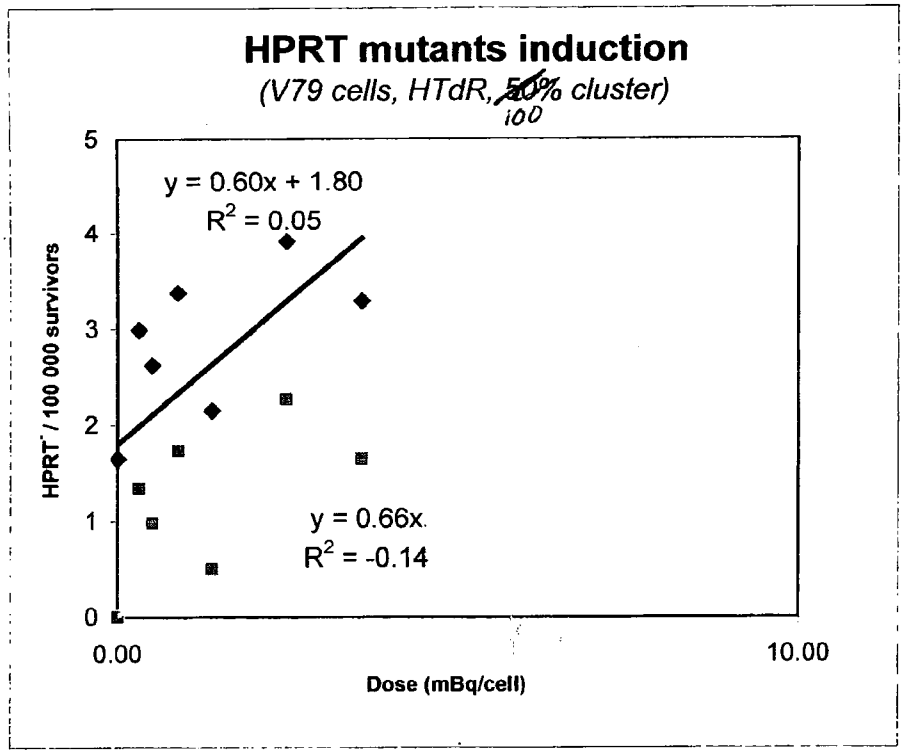
Table 2. HPRT mutant frequency for V79 cells exposed to HTdR in 100% cluster model (Roger's exp #5)

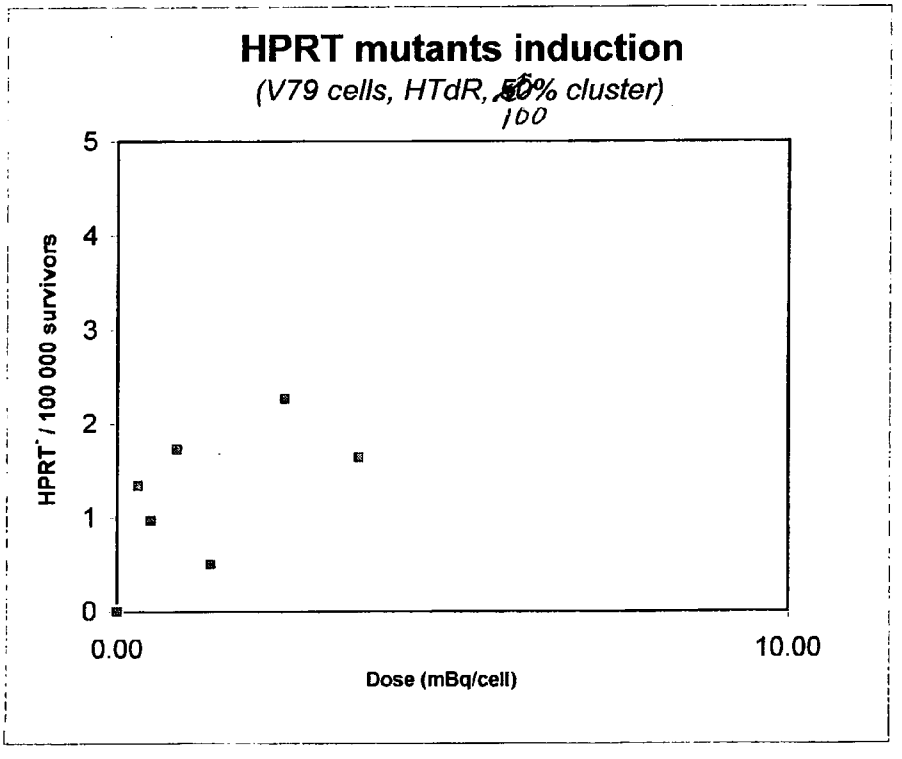
Sample #	Dose (mBq/cell)	# of cells ($x10^5$)	HPRT mutants per plate 1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	SUM	No of dishes plated	Total # of cells plated	PE for challenge (in Tab.1)	HPRT mutants per plated cell	per survived cell	per 100000 survivors	
1	*0.00*	*2*	*3*	*2*	*2*	*1*	*3*	*1*	*1*	*2*	*1*	*4*	*20*	*10*	*2000000*	*0.97*	*0.000010*	*0.000010*	*1.0*	
2	*0.00*	*2*	*3*	*2*	*5*	*4*	*8*	*8*	*4*	*5*	*9*		*48*	*9*	*1800000*	*1.20*	*0.000027*	*0.000022*	*2.2*	
1+ 2	0.00	2											68	19	*3800000*	1.09	0.000018	0.000016	1.6	0.0
3															*0*	0.00	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
4	0.31	2	6	12	9	7	8	10	6	7	9	10	84	10	*2000000*	1.40	0.000042	0.000030	3.0	1.3
5	0.51	2	7	6	9	6	5	6	9	6	6	4	64	10	*2000000*	1.22	0.000032	0.000026	2.6	1.0
6	1.39	2	8	8	3	4	6	2	5	5	4	4	49	10	*2000000*	1.14	0.000025	0.000021	2.1	0.5
7	0.89	2	7	7	10	9	6	14	5	8	9	7	82	10	*2000000*	1.22	0.000041	0.000034	3.4	1.7
8	2.49	2	15	4	8	5	8	9	7	9	10	9	84	10	*2000000*	1.07	0.000042	0.000039	3.9	2.3
9															*0*	0.00	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
10	3.59	2	9	3	8	12	8	8	9	10	6	6	79	10	*2000000*	1.20	0.000040	0.000033	3.3	1.6

Statistic

t-test
(p)

#DIV/0!	
#DIV/0!	p<0.05
#DIV/0!	
#DIV/0!	p<0.05
#DIV/0!	p<0.05
#DIV/0!	p<0.01
#DIV/0!	p<0.01
#DIV/0!	p<0.01


HPRT mutants induction
(V79 cells, HTdR, 50% cluster)
100
y = 0.60x + 1.80
R2 = 0.05
y = 0.66x
R2 = -0.14
HPRT- / 100 000 survivors
0
1
2
3
4
5
0.00
10.00
Dose (mBq/cell)


HPRT mutants induction
(V79 cells, HTdR, 50% cluster)
100
HPRT- / 100 000 survivors
0
1
2
3
4
5
0.00
10.00
Dose (mBq/cell)