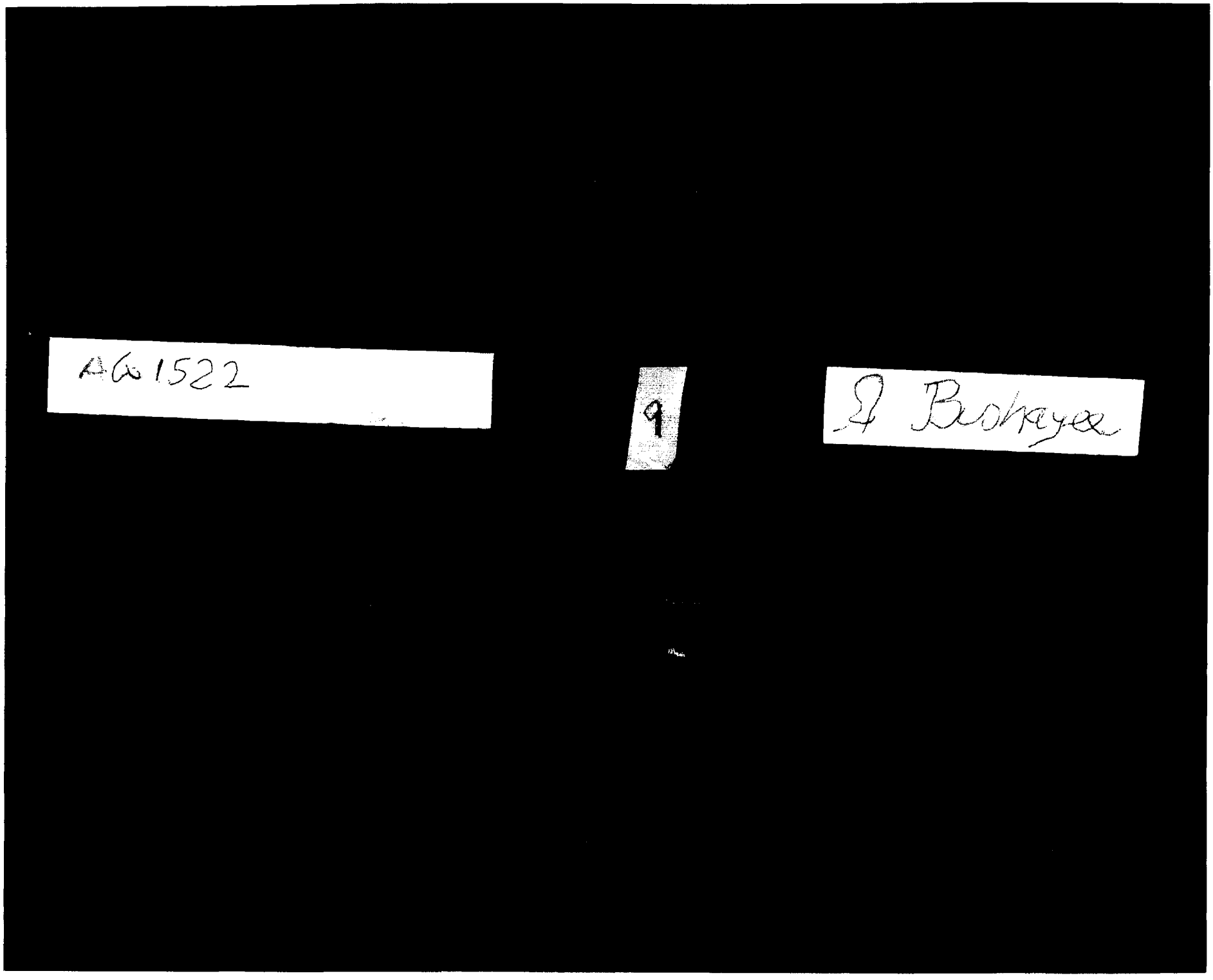
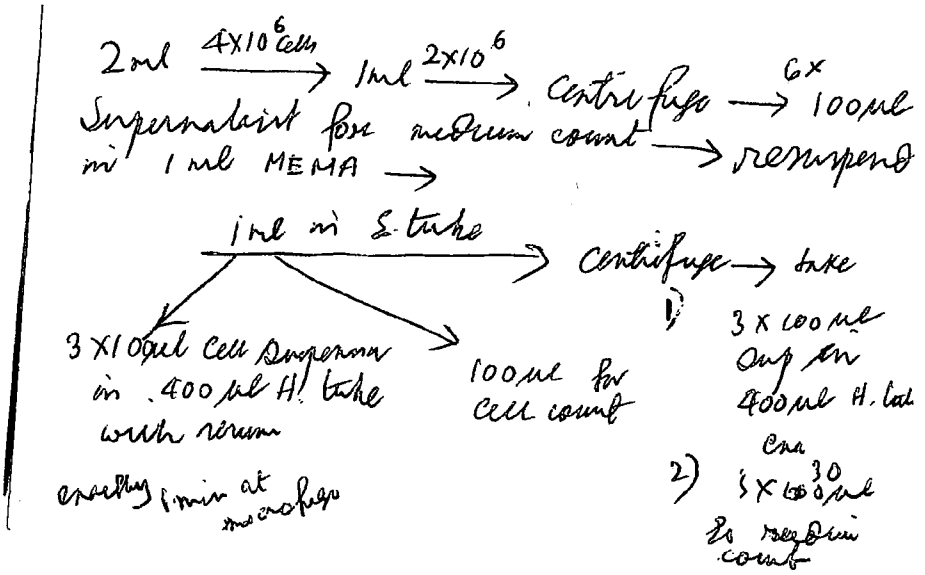



2ml 4X10^6 cells
1ml 2X10^6
centrifuge
6X
100µl
Supernatant for medium count
in 1ml MEMA
resuspend
1ml in E-tube
centrifuge
take
3X10µl cell Suspension in 400µl H. tube with serum
100µl for cell count
1) 3X100µl cup on 400µl H. tube
2) 3X30µl for medium count

AG1522 COLONY FORMING ASSAY

Experiment Name : ^{3}HTdR toxicity (cluster, 100% labeling); **Exp. #** 1

Experiment performed by: A. Bishayee **Date:** 06/07/01

1. Wash monolayer of cells (from 225 cm^2 flasks, cells were contact inhibited for 4 days, refed every two days) twice with PBS with 50 U/ml Penicillin and 50 µg/ml Streptomycin (PBS-PS) at 37°C, trypsinize cells with 4 ml trypsin for 4 min at 37°C, resuspend in 15 ml MEM (with 15% FBS (Hyclone, Cat# SH30071.02, Lot# AJD10234), 15 mM HEPES, 2 mM L-glutamine, 50 U/ml Penicillin and 50 µg/ml Streptomycin) at 37°C, pool, pass five times through 10 cc syringe with 21 gauge needle, perform cell count by transferring 100 µl in Coulter cup containing 20 ml Isotone II (Coulter balanced electrolyte solution).

2X 225 cm² flasks (from 6/6/01) P12
Total vol. = 36 ml
MS = 500 nl; Background = 13
Coulter count = 3036, 3059, 3065
Cell conc. (coulter counter) = 1,221,333 cells/ml
Cell conc. (hemocytometer) = 1,100,000 cells/ml
} 22×10⁶ cells/225 cm² flask

vol. containing 4.7×10^6 cells = $4.7\times10^6/1.22\times10^6$ = 3.8 ml

2. Plate 4.7×10^6 cells in 175 cm^2 flasks with 25 ml MEM at 37°C. Transfer the flasks in the incubator for 20 h. **Date/Time:** 06/17/01 ; 6-30 pm
3. After 20 h, prepare stock radioactivity solution in MEM with 0.375% BSA and 1% FBS (MEM-BSA) in hood

900 µl ^{3}HTdR (Stock : 10 µCi/µl on) + 8.1 ml MEMA-BSA
Manufacturer: Perkin-Elmer Lot #: 310427 Calibration: 05/30/01

4. Wash monolayer twice with 10 ml PBS-PS at 37°C. **Date/Time:**
5. Add as follows to get different activity concentration in each flask:

Flask #	^{3}HTdR (µCi/ml)	MEMA-BSA (ml)	^{3}HTdR [100 µCi/ml] (ml)
1	0	1.0	0
2	0	1.0	0
3	1	19.8	0.2
4	2	19.6	0.4
5	5	19	1
6	10	18	2
7	20	16	4

6. Transfer the flasks in the incubator for 20 h. **Date/Time:** 06/18/01; 3-30 pm
7. After 20 h, carefully aspirate the medium with radioactivity. Transfer aliquots in gamma tubes.
   Date/Time: 06/19/01; 11-30 a.m
8. Wash monolayer five times with 10 ml PBS-PS at room temperature.
9. Trypsinize cells with 2 ml trypsin (room temperature), resuspend in 15 ml MEM (room temperature), transfer cell suspension to 50 ml conical centrifuge tubes, pass five times through 10 cc syringe with 21 gauge needle, perform cell count, transfer cell suspension equivalent to $2x10^6$ cells in 14 ml tubes (Falcon plastic test tube, 17x100 mm). Make up the volume 10 ml with MEM in each tube. Transfer 3x100 µl cells for radioactivity count.
10. Centrifuge tubes for 10 min at 2000 rpm at room temperature
11. Decant supernatant, click tubes, vortex
12. Transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 µl) using 200 µl pipette tip.
13. Again add 200 µl MEM in 14 ml tube, vortex and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 µl)
14. Centrifuge tubes for 5 min at 1000 rpm at room temperature
15. Transfer tubes at 10.5°C for 72 h. **Date/Time:** 06/19/01; 1-30 pm
16. Transfer 30 µl medium containing radioactivity removed earlier (Step 7) in three sets of 7 ml scintillation vials and add **5 ml** liquid scintillation cocktail (Ecolume) and count them for radioactivity **Date/Time:** 06/19/01; 5-00 pm
17. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 µl remaining MEM and transfer the content to14 ml tubes (Falcon plastic test tube, 17x100 mm, labeled both on cap and side) containing 10 ml MEM (room temperature) by using Pasteur pipette
    Date/Time: 06/22/01; 1-30 pm
18. Again add 200 µl MEM (room temperature) in microcentrifuge tubes, resuspend and transfer the cell suspensions in 14 ml tubes
19. Centrifuge the tubes for 10 min at 2000 rpm at room temperature
20. Labeling and preparation of dilution tubes and colony dishes
    - load 100 mm tissue culture dishes with 10 ml MEMA (room temperature)
    - load sterile tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4; 2.2, 2.3, 2.4, X.2, X.3, X.4, etc.
21. Decant supernatant, click tubes, vortex, resuspend in 2 ml MEMA, pass five times through 5 cc syringe with 21 gauge needle
22. Determine cell concentration by transferring 100 µl to Coulter cup
23. Vortex tube, transfer 0.5 ml into dilution tube X.4, vortex tube X.4 and transfer 0.5 ml to tube X.3, vortex tube X.3 and transfer 0.5 ml to tube X.2 and vortex.
24. Transfer 0.8 ml (approximately 800, 8000 or 80000 cells) from dilution tubes into dishes labeled X.2, X.3, X.4 (in triplicate). Only X.2 should be seeded for control.
25. Incubate tissue culture dishes for two weeks

26. Transfer 100 µl of cell suspension (in triplicate) to 7 ml scintillation vial containing **5 ml** cocktail (Ecolume)
27. Count vials for radioactivity **Date/Time :** 06/22/01; 4-30 pm
28. After two weeks, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
29. Count colonies.

Tube	Dilution	Colony Counts

~~2nd cell [illegible] counts~~ ~~50%, [illegible]~~

30 µl medium

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 TUE 19 JUN 2001 16:51
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
H#: 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: (
DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:Q 1.00000
HALF LIFE(DAYS):N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERF
1	**- 1	1	12.00	57.74	1.00	1.48	76.0	
2	**- 2	1	7.00	75.59	1.00	3.06	75.0	
3	**- 3	1	4.00	100.0	1.00	4.63	75.0	
4	**- 4	1	8.00	70.71	1.00	6.19	76.0	
5	**- 5	1	7.00	75.59	1.00	7.77	77.0	
6	**- 6	1	11.00	60.30	1.00	9.33	79.0	
7	**- 7	1	24213.33	1.92	0.45	10.35	78.0	
8	**- 8	1	23582.22	1.94	0.45	11.41	77.0	
9	**- 9	1	24266.67	1.91	0.45	12.47	76.0	
10	**-10	1	44388.00	1.90	0.25	13.27	76.0	
11	**-11	1	46832.00	1.85	0.25	14.07	75.0	
12	**-12	1	48704.00	1.81	0.25	14.93	76.0	
13	**-13	1	113519.99	1.53	0.15	15.63	76.0	
14	**-14	1	116713.33	1.51	0.15	16.33	75.0	
15	**-15	1	119679.99	1.49	0.15	17.03	77.0	
16	**-16	1	242413.33	1.05	0.15	17.76	78.0	
17	**-17	1	236419.98	1.06	0.15	18.48	76.0	
18	**-18	1	238486.66	1.06	0.15	19.19	76.0	
19	**- 1	1	451299.97	0.77	0.15	19.98	76.0	
20	**- 2	1	470759.97	0.75	0.15	20.72	74.0	
21	**- 3	1	422806.66	0.79	0.15	21.45	75.0	
22	**- 4	1	29145.71	1.98	0.35	22.41	-1.0	
37	28- 1	1	12.00	57.74	1.00	24.16	87.0	
38	28- 2	1	12.00	57.74	1.00	25.72	85.0	
39	28- 3	1	9.00	66.67	1.00	27.29	88.0	
40	28- 4	1	10.00	63.25	1.00	28.86	86.0	
41	28- 5	1	13.00	55.47	1.00	30.49	89.0	
42	28- 6	1	12.00	57.74	1.00	32.12	89.0	
43	28- 7	1	22264.44	2.00	0.45	33.12	87.0	
44	28- 8	1	24128.89	1.92	0.45	34.13	88.0	
45	28- 9	1	25285.00	1.99	0.40	35.08	88.0	
46	28-10	1	43796.00	1.91	0.25	35.95	89.0	
47	28-11	1	46132.00	1.86	0.25	36.75	90.0	
48	28-12	1	44844.00	1.89	0.25	37.56	88.0	
49	28-13	1	45792.00	1.87	0.25	38.37	83.0	
50	28-14	1	64405.00	1.76	0.20	39.12	89.0	
51	28-15	1	54780.00	1.91	0.20	39.87	87.0	
52	28-16	1	60545.00	1.82	0.20	40.63	87.0	
53	28-17	1	57425.00	1.87	0.20	41.38	84.0	
54	28-18	1	68080.00	1.98	0.15	42.09	89.0	
55	**- 1	1	141286.66	1.37	0.15	42.85	89.0	
56	**- 2	1	83326.66	1.79	0.15	43.55	85.0	
57	**- 3	1	116933.33	1.51	0.15	44.25	87.0	
58	**- 4	1	103986.66	1.60	0.15	44.97	87.0	
59	**- 5	1	106686.66	1.58	0.15	45.67	89.0	
60	**- 6	1	115913.33	1.52	0.15	46.37	87.0	

1C 2C 3C 4C 5C 6C 7C 8C

30μl medium

(For checking, not used)

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 WED 20 JUN 2001 14:06
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
H#: 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:0 1.00000
HALF LIFE(DAYS):N

SAM	POS	CH		CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**- 1	1	1M	9.00	66.67	1.00	1.42	77.0	
2	**- 2	1		13.00	55.47	1.00	3.00	76.0	
3	**- 3	1		13.00	55.47	1.00	4.58	76.0	
4	**- 4	1	2M	14.00	53.45	1.00	6.14	77.0	
5	**- 5	1		11.00	60.30	1.00	7.71	78.0	
6	**- 6	1		13.00	55.47	1.00	9.28	80.0	
7	**- 7	1	3M	24311.11	1.91	0.45	10.29	78.0	
8	**- 8	1		24142.22	1.92	0.45	11.34	77.0	
9	**- 9	1		24313.33	1.91	0.45	12.35	77.0	
10	**-10	1	4M	45784.00	1.87	0.25	13.15	77.0	
11	**-11	1		48472.00	1.82	0.25	13.95	78.0	
12	**-12	1		47808.00	1.83	0.25	14.75	77.0	
13	**-13	1	5M	113519.99	1.53	0.15	15.45	78.0	
14	**-14	1		118226.66	1.50	0.15	16.16	76.0	
15	**-15	1		122439.99	1.48	0.15	16.88	79.0	
16	**-16	1	6M	244406.66	1.04	0.15	17.59	79.0	
17	**-17	1		239673.33	1.05	0.15	18.31	77.0	
18	**-18	1		243219.98	1.05	0.15	19.03	78.0	
19	**- 1	1	7M	451473.31	0.77	0.15	19.81	75.0	
20	**- 2	1		478746.66	0.75	0.15	20.55	75.0	
21	**- 3	1		442273.31	0.78	0.15	21.28	74.0	
37	29- 1	1		8.00	70.71	1.00	23.04	84.0	
38	29- 2	1		12.00	57.74	1.00	24.62	84.0	
39	29- 3	1		7.00	75.59	1.00	26.18	86.0	
40	29- 4	1		181.00	14.87	1.00	27.76	83.0	
41	29- 5	1		15.00	51.64	1.00	29.33	82.0	
42	29- 6	1		8.00	70.71	1.00	30.89	84.0	
43	29- 7	1		1072.00	6.11	1.00	32.46	86.0	
44	29- 8	1		901.00	6.66	1.00	34.03	84.0	
45	29- 9	1		1039.00	6.20	1.00	35.59	84.0	
46	29-10	1		1224.00	5.72	1.00	37.17	81.0	
47	29-11	1		1858.00	4.64	1.00	38.74	87.0	
48	29-12	1		10.00	63.25	1.00	40.37	72.0	
49	29-13	1		3608.00	3.33	1.00	41.94	94.0	
50	29-14	1		4157.00	3.10	1.00	43.52	89.0	
51	29-15	1		4061.00	3.14	1.00	45.08	88.0	
52	29-16	1		5108.00	2.80	1.00	46.67	84.0	
53	29-17	1		6050.00	2.57	1.00	48.23	86.0	
54	29-18	1		3978.00	3.17	1.00	49.81	80.0	
55	**- 1	1		5418.00	2.72	1.00	51.43	82.0	
56	**- 2	1		6710.00	2.44	1.00	53.01	85.0	
57	**- 3	1		4082.00	3.13	1.00	54.58	78.0	

RACK MOTION ERROR - ABORTING CURRENT USER

100µl Cells

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 TUE 19 JUN 2001 14:05
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
HV: 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	13.00	55.47	1.00	1.42	84.0	
2	**- 2	1	26.00	39.22	1.00	2.99	82.0	
3	**- 3	1	13.00	55.47	1.00	4.57	85.0	
4	**- 4	1	72.00	23.57	1.00	6.13	83.0	
5	**- 5	1	38.00	32.44	1.00	7.69	82.0	
6	**- 6	1	6.00	81.65	1.00	9.26	84.0	
7	**- 7	1	1431.00	5.29	1.00	10.82	87.0	
8	**- 8	1	1291.00	5.57	1.00	12.38	83.0	
9	**- 9	1	1160.00	5.87	1.00	13.95	82.0	
10	**-10	1	1732.00	4.81	1.00	15.53	83.0	
11	**-11	1	2633.00	3.90	1.00	17.09	87.0	
12	**-12	1	6.00	81.65	1.00	18.67	72.0	
13	**-13	1	5644.00	2.66	1.00	20.24	94.0	
14	**-14	1	5430.00	2.71	1.00	21.81	87.0	
15	**-15	1	5397.00	2.72	1.00	23.37	88.0	
16	**-16	1	6867.00	2.41	1.00	24.96	83.0	
17	**-17	1	8804.00	2.13	1.00	26.52	86.0	
18	**-18	1	4600.00	2.95	1.00	28.10	80.0	
19	**- 1	1	6128.00	2.55	1.00	29.72	81.0	
20	**- 2	1	9220.00	2.08	1.00	31.30	86.0	
21	**- 3	1	5621.00	2.67	1.00	32.87	78.0	
22	**- 4	1	29014.29	1.98	0.35	33.82	0.0	

1c, 2c, 3c, 4c, 5c, 6c, 7c

→ sample not added

→ Handed

100 µl cells

A.R.

(for checking, not used)

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 TUE 19 JUN 2001 16:16
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
H#: 1 AQC:N QCF:N RCM:N
CHANNEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKB 2SIG: 0.00 LSR: 0
DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:Q 1.00000
HALF LIFE(DAYS):N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	29- 1	1	9.00	66.67	1.00	1.42	84.0	
2	29- 2	1	16.00	50.00	1.00	3.00	83.0	
3	29- 3	1	11.00	60.30	1.00	4.63	85.0	
4	29- 4	1	10.00	63.25	1.00	6.19	82.0	
5	29- 5	1	9.00	66.67	1.00	7.76	81.0	
6	29- 6	1	9.00	66.67	1.00	9.32	84.0	
7	29- 7	1	1408.00	5.33	1.00	10.93	85.0	
8	29- 8	1	1241.00	5.68	1.00	12.50	83.0	
9	29- 9	1	1108.00	6.01	1.00	14.08	82.0	
10	29-10	1	1542.00	5.09	1.00	15.66	79.0	
11	29-11	1	2463.00	4.03	1.00	17.28	86.0	
12	29-12	1	5.00	89.44	1.00	18.85	71.0	
13	29-13	1	5202.00	2.77	1.00	20.43	93.0	
14	29-14	1	5194.00	2.78	1.00	22.00	88.0	
15	29-15	1	5033.00	2.82	1.00	23.57	87.0	
16	29-16	1	6304.00	2.52	1.00	25.14	84.0	
17	29-17	1	8036.00	2.23	1.00	26.72	86.0	
18	29-18	1	4278.00	3.06	1.00	28.29	80.0	
19	**- 1	1	5569.00	2.68	1.00	29.91	81.0	
20	**- 2	1	8558.00	2.16	1.00	31.47	86.0	
21	**- 3	1	4899.00	2.86	1.00	33.04	78.0	
22	**- 4	1	29025.71	1.98	0.35	34.00	-1.0	

1c
2c
3c
4c
5c
6c
7c

Experiment: AG1522/H-3TdR-/100%
Date: 06/17/01

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	*13*	*26*	*13*	*28*	*0*	*0*	*0.00000*	*0*	*0.0000*
2	*72*	*38*	*6*		*0*	*0*	*0.00000*	*0*	*0.0000*
3	1431	1291	1160	1294	1266	1948	0.00877	0.00877	0.3246
4	1732	2633	2183	2183	2155	3315	0.01493	0.01493	0.5525
5	5644	5430	5397	5490	5462	8404	0.03785	0.03786	1.4006
6	6867	8804	4600	6757	6729	10352	0.04663	0.04663	1.7254
7	6128	9220	5621	6990	6962	10710	0.04824	0.04825	1.7851
8	0	0	0	0	-28	-43	-0.00019	-0.00019	-0.0072
9	0	0	0	0	-28	-43	-0.00019	-0.00019	-0.0072
10	0	0	0	0	-28	-43	-0.00019	-0.00019	-0.0072

CoulterSurvival

riment: AG1522/H-3TdR-/100%
e/Time: ######

Tube #	Coulter count 1st	2nd	3rd	Average	Cells/ml	Hemocytometer Count in Grid 1st	2nd	3rd	4th
1	*715*	*713*	*693*	*707*	*277600*				
2	*725*	*697*	*677*	*700*	*274667*				
3	647	571	503	574	224267				
4	533	669	677	626	245333				
5	535	499	510	515	200667				
6	514	494	515	508	197867				
7	510	532	530	524	204400				
8				#DIV/0!	#DIV/0!				
9				#DIV/0!	#DIV/0!				
10				#DIV/0!	#DIV/0!				

Tube #	Predicted # Cells Seeded	Actual # Cells Seeded	Colony count 1st	2nd	3rd	Average	PE (%)	SF Uncorrected	SF Corrected
1		*#DIV/0!*				*#DIV/0!*	*#DIV/0!*	*#DIV/0!*	*#DIV/0!*
2		*#DIV/0!*							
3		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
4		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
5		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
6		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
7		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
8		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
9		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
10		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Before making the clusters

Bdigr - 13

Mode - 500µl

6/19/01

	# of cells / ml	Total in ml	Total ≈ ×10^6	2×10^6 cells ↓ ml.
1, ~~800~~, 715, 713, 693	277600	14	~~4,112~~ 3,886,400	7.2
2. 725, 697, 677	274667	15	4,120,005	7.3
3, 647, 571, 503	224267	13/14	2,915,471 / 2,989,707	8.9
4. 533, 669, 677	245333	14	3,434,667	8.2
5. 535, 499, 510	200667	16	2,809,333	10.0
6. 514, 494, 515	198000	17	3,366,000	10.1
7. 510, 532, 530	204400	14	2,861,600	9.8

Micronucleus assay

	Vol used for 150,000 cells (ml)
1	0.54
2	0.55
3	0.67
4	0.61
5	0.74
6	0.76
7	0.73

① 150,000 cells were plated in 2× 60mm lisme culture dish with 2ml MEM

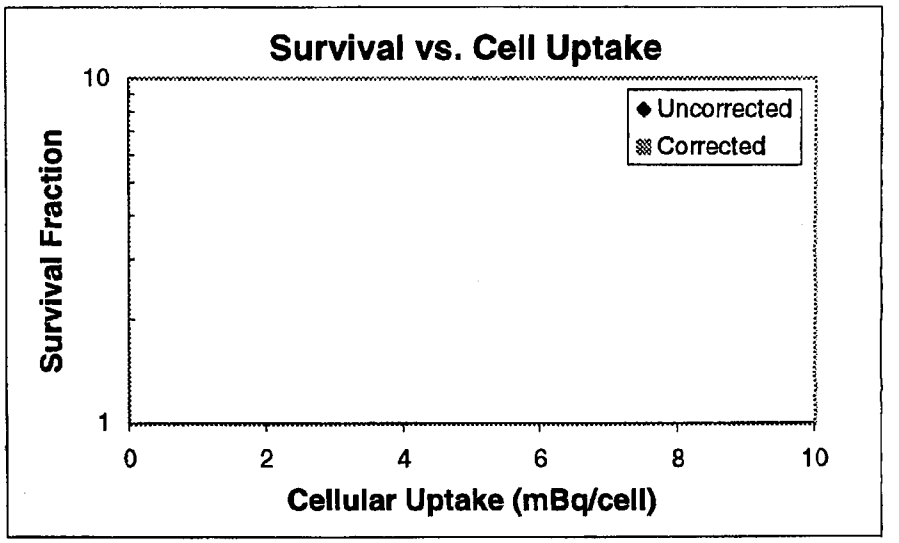
② After 5 hr, 2 ml MEM with 4µg/ml cytochalasin B was added in each dish (final conc. ~ 2µg/ml)

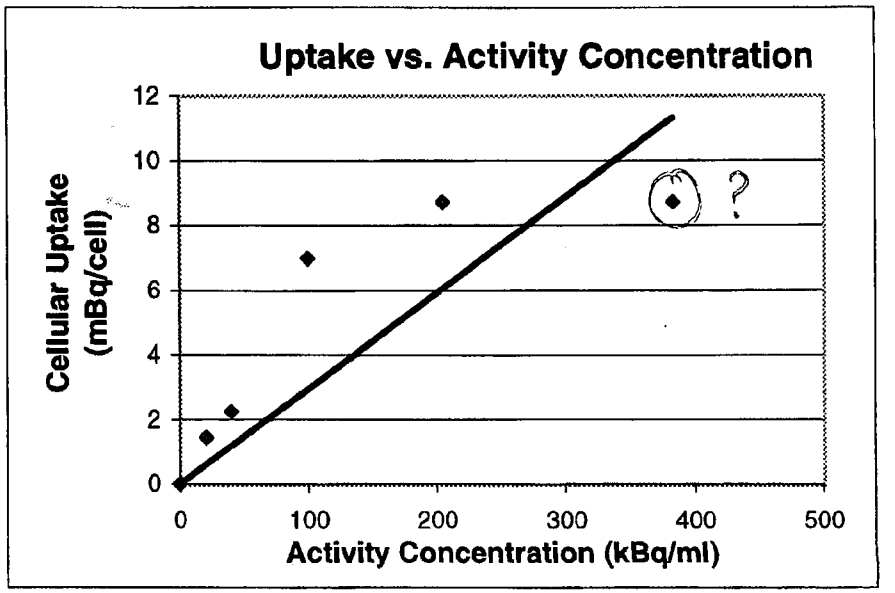
③ cells plated 1:30 pm

Summary

Experiment:
Date/Time: 6/17/2001

Tube #	Activity Conc. (kBq/ml)	Activity/Cell (mBq/cell)	Survival Uncorrected	Survival Corrected
1	*0.000*	*0.000*	*#DIV/0!*	*#DIV/0!*
2	*0.000*	*0.000*		
3	20.527	1.447	#DIV/0!	#DIV/0!
4	39.864	2.252	#DIV/0!	#DIV/0!
5	99.699	6.980	#DIV/0!	#DIV/0!
6	204.391	8.720	#DIV/0!	#DIV/0!
7	383.208	8.733	#DIV/0!	#DIV/0!
8	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
9	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
10	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!



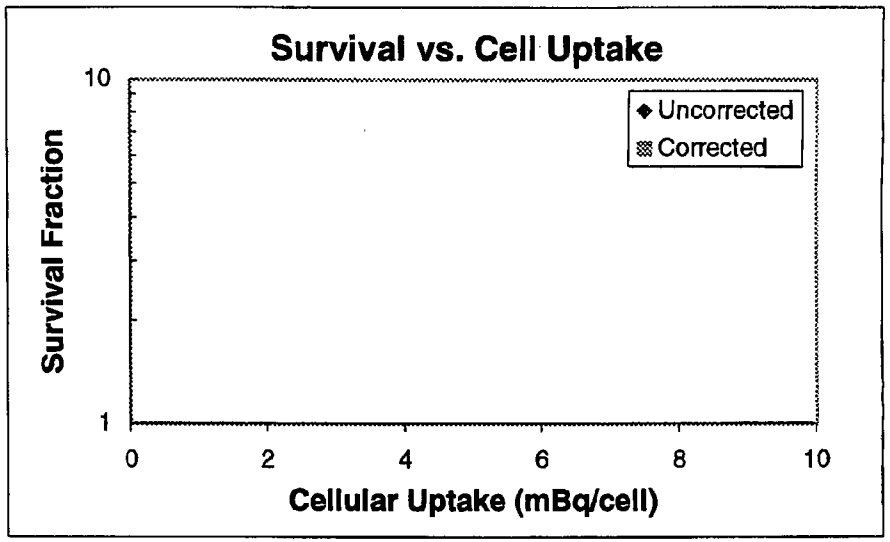


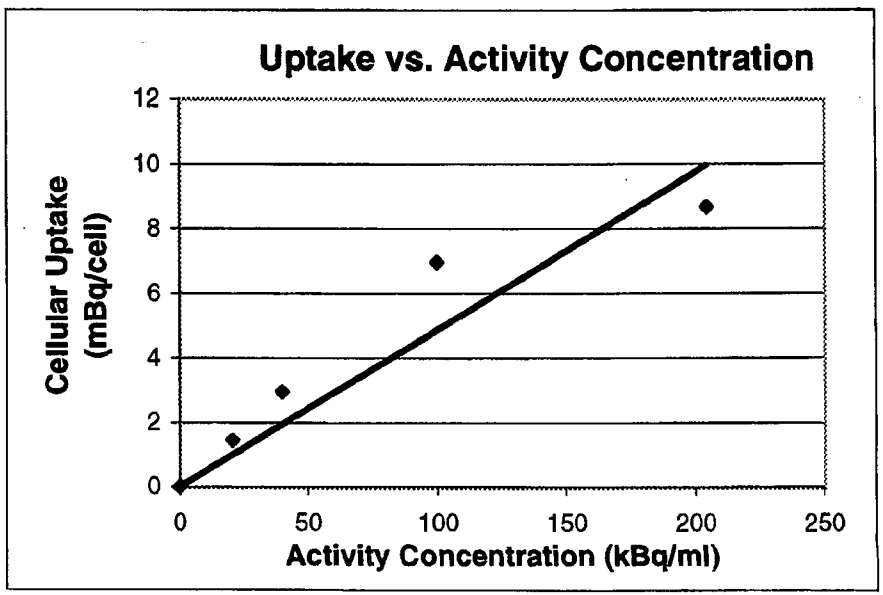
B002129

Summary

Experiment:
Date/Time: 6/17/2001

Tube #	Activity Conc. (kBq/ml)	Activity/Cell (mBq/cell)	Survival Uncorrected	Survival Corrected
1	*0.000*	*0.000*	*#DIV/0!*	*#DIV/0!*
2	*0.000*	*0.000*		
3	20.527	1.448	#DIV/0!	#DIV/0!
4	39.864	2.952	#DIV/0!	#DIV/0!
5	99.699	6.983	#DIV/0!	#DIV/0!
6	204.391	8.695	#DIV/0!	#DIV/0!
7	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
8	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
9	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
10	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!





B002130

AGC 1522 — 100μl cells (after dismantling the clusters)

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 FRI 22 JUN 2001 16:29
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
[redacted] 1 AQC:N QCF:N RCM:N
C[redacted]NEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
DA[redacted] 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	1c	10.00	63.25	1.00	1.42	87.0	
2	**- 2	1		6.00	81.65	1.00	3.06	84.0	
3	**- 3	1		6.00	81.65	1.00	4.62	86.0	
4	**- 4	1	2c	8.00	70.71	1.00	6.23	87.0	
5	**- 5	1		6.00	81.65	1.00	7.85	88.0	
6	**- 6	1		13.00	55.47	1.00	9.46	85.0	
7	**- 7	1	3c	6069.00	2.57	1.00	11.09	90.0	
8	**- 8	1		5623.00	2.67	1.00	12.72	91.0	
9	**- 9	1		3659.00	3.31	1.00	14.34	87.0	
10	**-10	1	4c	8507.00	2.17	1.00	15.91	89.0	
11	**-11	1		8395.00	2.18	1.00	17.54	88.0	
12	**-12	1		8130.00	2.22	1.00	19.11	87.0	
13	**-13	1	5c	18845.46	1.96	0.55	20.26	84.0	
14	**-14	1		13566.67	1.98	0.75	21.63	79.0	
15	**-15	1		13561.33	1.98	0.75	22.95	85.0	
16	**-16	1	6c	38053.33	1.87	0.30	23.80	87.0	
17	**-17	1		34776.66	1.96	0.30	24.65	89.0	
18	**-18	1		20616.00	1.97	0.50	25.71	81.0	
19	**- 1	1	7c	49004.00	1.81	0.25	26.62	84.0	
[redacted]	**- 2	1		46564.00	1.85	0.25	27.42	83.0	
[redacted]	**- 3	1		47732.00	1.83	0.25	28.27	84.0	
22	**- 4	1		29522.86	1.97	0.35	29.23	-1.0	→ Standard

AG1522 100 µl cells (after dismantling the cluster) ANUPAM PAGE: 1

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 TUE 26 JUN 2001 16:02
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
H#: 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:0 1.00000
HALF LIFE(DAYS):N

SAM	POS	CH		CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**- 1	1	1c	24.00	40.82	1.00	1.48	87.0	
2	**- 2	1		21.00	43.64	1.00	3.05	86.0	
3	**- 3	1		5.00	89.44	1.00	4.63	86.0	
4	**- 4	1	2c	7.00	75.59	1.00	6.19	89.0	
5	**- 5	1		9.00	66.67	1.00	7.76	88.0	
6	**- 6	1		11.00	60.30	1.00	9.38	87.0	
7	**- 7	1	3c	3615.00	3.33	1.00	11.00	91.0	
8	**- 8	1		3307.00	3.48	1.00	12.57	93.0	
9	**- 9	1		1962.00	4.52	1.00	14.19	88.0	
10	**-10	1	4c	4825.00	2.88	1.00	15.76	89.0	
11	**-11	1		4088.00	3.13	1.00	17.33	90.0	
12	**-12	1		3806.00	3.24	1.00	18.89	90.0	
13	**-13	1	5c	8233.00	2.20	1.00	20.47	85.0	
14	**-14	1		6975.00	2.39	1.00	22.04	81.0	
15	**-15	1		7831.00	2.26	1.00	23.61	86.0	
16	**-16	1	16c	17288.33	1.96	0.60	24.77	86.0	
17	**-17	1		18150.00	1.92	0.60	25.94	89.0	
18	**-18	1		12843.75	1.97	0.80	27.30	85.0	
19	**- 1	1	17c	26047.50	1.96	0.40	28.36	84.0	
20	**- 2	1		27175.00	1.92	0.40	29.37	84.0	
21	**- 3	1		28577.14	2.00	0.35	30.32	87.0	
22	**- 4	1		29300.00	1.97	0.35	31.28	0.0	
23	**- 5	1		59870.00	1.83	0.20	32.08	2.0	

0.0 – old Standard
2.0 – New Standard

Counted in Radiation Safety's New Scintilation Counter

100µl Cells

E = 0·63

ID = HIS HODEL 25 JUN 2001 15:19

USER: 6 COMMENT:

PRESET TIME	:	1.00							
DATA CALC	:	CPM	H#	:YES	SAMPLE REPEATS:	1	PRINTER	:	STD
COUNT BLANK	:	NO	IC#	: NO	REPLICATES :	1	RS232	:	OFF
TWO PHASE	:	NO	AQC	: NO	CYCLE REPEATS :	1			
SCINTILLATOR:		LIQUID	LUMEX:	NO	LOW SAMPLE REJ:	0			
LOW LEVEL	:	NO	HALF LIFE CORRECTION DATE:			none			

ISOTOPE 1: 3H %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

SAM NO	POS	TIME MIN	H#	3H CPM	3H %ERROR	LUMEX %	ELAPSED TIME
1	**-1	1.00	85.1	7.00	75.59	2.50	1.50
2	**-2	1.00	83.9	4.00	100.00	2.22	3.10
3	**-3	1.00	82.7	5.00	89.44	0.90	4.70
4	**-4	1.00	85.4	3.00	115.47	1.48	6.30
5	**-5	1.00	86.1	8.00	70.71	1.05	7.93
6	**-6	1.00	85.0	4.00	100.00	0.95	9.52
7	**-7	1.00	88.8	3851.00	3.22	0.01	11.24
8	**-8	1.00	89.6	3579.00	3.34	0.01	12.84
9	**-9	1.00	85.7	2189.00	4.27	0.01	14.45
10	**-10	1.00	87.7	5237.01	2.76	0.01	16.06
11	**-11	1.00	87.8	4500.01	2.98	0.01	17.58
12	**-12	1.00	86.3	4113.01	3.12	0.01	19.18
13	**-13	1.00	82.8	9499.02	2.05	0.01	20.81
14	**-14	1.00	78.3	7583.02	2.30	0.01	22.41
15	**-15	1.00	83.8	8907.02	2.12	0.01	24.04
16	**-16	1.00	85.0	18742.05	1.46	0.01	25.67
17	**-17	1.00	86.8	19503.06	1.43	0.01	27.30
18	**-18	1.00	80.6	14697.04	1.65	0.01	29.02
19	**-1	1.00	82.3	29684.10	1.16	0.01	30.78
20	**-2	1.00	82.0	30659.10	1.14	0.01	32.40
21	**-3	1.00	83.4	31518.11	1.13	0.01	34.04
22	**-4	1.00	-0.7	61850.23	0.80	0.00	35.79

No Std.

B002133

CellSuspension

Experiment: AG1522/H-3TdR-/100%
Date: 06/17/01

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	*10*	*6*	*6*	*8*	*0*	*0*	*0.00000*	*0*	*0.0000*
2	*8*	*6*	*13*		*0*	*0*	*0.00000*	*0*	*0.0000*
3	6069	5623	3659	5117	5109	7860	0.03540	0.03542	1.3106
4	8507	8395	8130	8344	8336	12824	0.05777	0.05780	2.1384
5	18845	13566	13561	15324	15316	23563	0.10614	0.10619	3.9291
6	38053	34776	20616	31148	31140	47908	0.21580	0.21591	7.9886
7	49004	46564	47732	47767	47759	73475	0.33097	0.33113	12.2518
8				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
9				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
10				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

AG1522 Coulter count after breaking the clusters. 6/22/01

MS = 500 µl

Background = 13

1. 2250, 2358, 2232
2. 2217, 2136, 2093
3. 2806, 2883, 2796
4. 3084, 3175, 3054
5. 1948, 1948, 1914
6. 1901, 1858, 1764
7. 1856, 1786, 1726

CoulterSurvival

riment: AG1522/H-3TdR-/100%
e/Time: ######

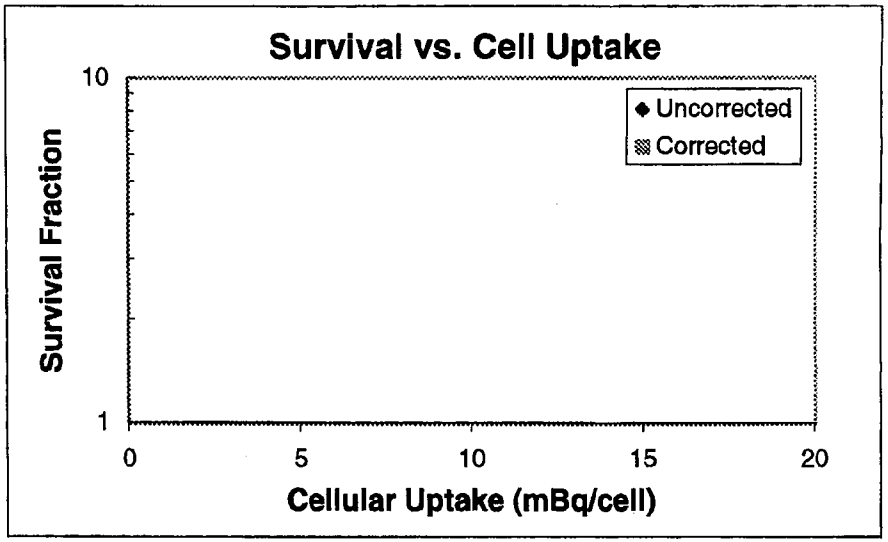
Tube #	Coulter count 1st	2nd	3rd	Average	Cells/ml	Hemocytometer Count in Grid <1st	2nd	3rd	4th
						# of cells plated			
1	*2250*	*2358*	*2232*	*2280*	*906800*	→ 725			
2	*2217*	*2136*	*2083*	*2145*	*852933*	→ 683			
3	2806	2883	2796	2828	1126133	→ 900			
4	3084	3175	3054	3104	1236533	→ 988			
5	1948	1948	1914	1937	769467	→ 615			
6	1901	1858	1764	1841	731200	→ 584			
7	1856	1786	1726	1789	710533	→ 568			
8				#DIV/0!	#DIV/0!				
9				#DIV/0!	#DIV/0!				
10				#DIV/0!	#DIV/0!				

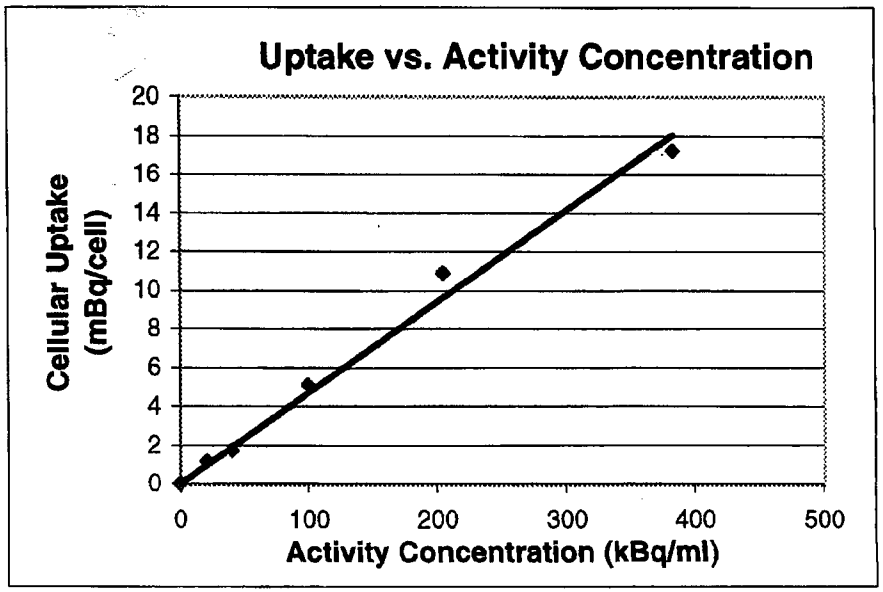
Tube #	Predicted # Cells Seeded	Actual # Cells Seeded	Colony count 1st	2nd	3rd	Average	PE (%)	SF Uncorrected	SF Corrected
1		*#DIV/0!*				*#DIV/0!*	*#DIV/0!*	*#DIV/0!*	*#DIV/0!*
2		*#DIV/0!*							
3		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
4		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
5		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
6		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
7		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
8		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
9		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
10		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Summary

Experiment:
Date/Time: 6/17/2001

Tube #	Activity Conc. (kBq/ml)	Activity/Cell (mBq/cell)	Survival Uncorrected	Survival Corrected
1	*0.000*	*0.000*	*#DIV/0!*	*#DIV/0!*
2	*0.000*	*0.000*		
3	20.527	1.164	#DIV/0!	#DIV/0!
4	39.864	1.729	#DIV/0!	#DIV/0!
5	99.699	5.106	#DIV/0!	#DIV/0!
6	204.391	10.925	#DIV/0!	#DIV/0!
7	383.208	17.243	#DIV/0!	#DIV/0!
8	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
9	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
10	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

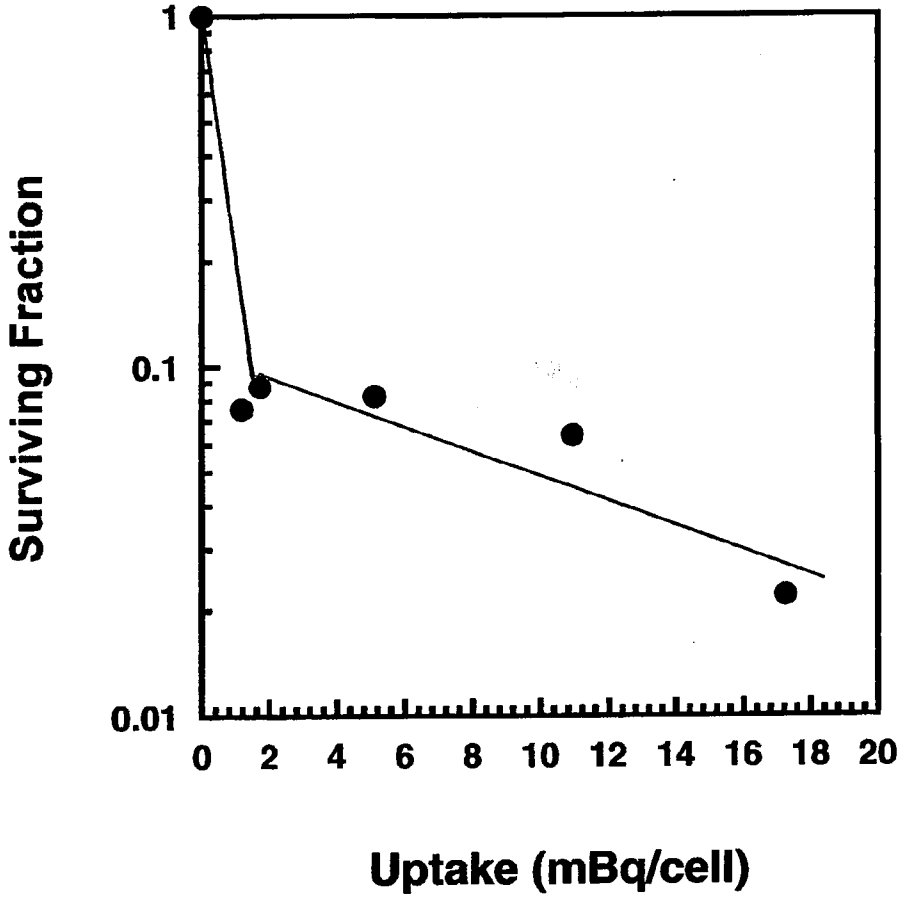




Colony count for AG1522

Tube	Colony		SF
1.2	191, 160, 181	174.83	1 = 0.75
2.2	178, 154, 185		
3.3	124, 127, 146	132.33	0.07569
4.3	154, 157, 148	153.0	0.08751
5.3	142, 142, 148	144	0.08236
6.3	106, 92, 135	111	0.06348
7.3	47, 40, 32	39	0.022

AG1522: 100% LABELING WITH H-3TdR



Study of GJIC in AG1522 Cells by Flow Cytometry Analysis

Experiment Name : Dye transfer study with Calcein AM in AG1522 cells **Exp. # :** 1

Experiment performed by : A. Bhattacharjee **Date:** 06/05/01

1. Take 3 x 175 cm^2 flask with AG 1522 cells. Approximately 4.7×10^6 cells were plated per 175 cm^2 flask 40 h before from a 175 cm^2 flask containing contact inhibited cells for four days.
2. Wash monolayer of cells for five times with 10 ml warm PBS-PS (37°C).
3. Add 5 µl of stock CMTMR (5 mM) and 20 µl of 5 mM Calcein AM per 10 ml warm PBS-PS (37°C). Prepare 30 ml. (Final concentration: CMTMR 2.5 µM and Calcein: 20 µM)
4. Add 10 ml of above dye solution for each 175 cm^2 flask
5. Incubate flasks at 37°C for 15-20 min.
6. Aspirate the dye solution. Wash monolayer twice with 10 ml warm PBS-PS (37°C) in each time.
7. Add 20 ml warm MEMA (37°C).
8. Incubate flasks at 37°C for 30 min.
9. Aspirate the medium. Wash monolayer twice with 10 ml warm PBS-PS (37°C).
10. Trypsinize cells with 4 ml of warm trypsin (37°C) per 175 cm^2 flask for 4 min, add 10 ml of MEMA (37°C), and transfer the cell suspension to 50 ml tube.
11. Syringe the cell suspension five times with 5 cc syringe with 21 G needle, and perform cell count.
12. Take another 175 cm^2 flask with AG1522 cells that were contact inhibited for 6 days.
13. Trypsinize cells with 4 ml of warm trypsin (37°C) per 175 cm^2 flask for 4 min, add 10 ml of MEMA (37°C), and transfer the cell suspension to 50 ml tube.
14. Syringe the cell suspension five times with 5 cc syringe with 21 G needle, and perform cell count.
15. Transfer cell suspension containing 2×10^6 cells from each 50 ml tube to a 14 ml 17x100 tube. Raise volume to a total of 10 ml MEMA at room temperature.
16. Centrifuge the tube at 2000 rpm for 10 min at room temperature
17. Decant the supernatant, transfer cells to a 400 µl microcentrifuge tube, wash 14 ml tube with 200 µl MEMA (at room temperature) and transfer to the microcentrifuge tube.
18. Centrifuge the microcentrifuge tube at 1000 rpm for 5 min at room temperature.
19. Transfer the microcentrifuge tubes with clusters to 10.5°C. 06/05/01 ; 4-30pm
20. Add different time intervals (e.g., following 15 min, 30 min, 1 h, 2h, 3h, 1 day, 2 day, and 3 day etc.), take out one microcentrifuge tube.

AK

21. Transfer cells to a 14ml centrifuge tube containing 10 ml PBS-PS (ice cold).
22. Centrifuge tubes for 10 min at 2000 rpm in precooled centrifuge at 4°C and decant the supernatant
23. Resuspend cells in 1.8 ml of PBS-PS (ice cold).
24. Slowly add one Pasteur pipette full of cold 95% EtOH while vortexing the sample vigorously to avoid clumping of cells during the fixation.
25. Add two more Pasteur pipette full of cold 95% EtOH that gives a final concentration of approximately 70% EtOH.
26. Store fixed sample in the refrigerator. They are stable for at least one month.
27. Analyze all samples using a flow cytometer.

- Take out all samples from refrigerator
- Centrifuge tubes at 2000 rpm for 5 min at room temperature
- Decant supernatant
- Resuspend pellet in 1 ml of PBS
- Syringe with 1 ml syringe
- Transfer cell suspension in Falcon 12x75 mm polystyrene tube
- Take the tubes to FACS machine for analysis.

1X175 cm² from 5/25/01 6/3/01
Plated on 5/28/01 & 06/01/01
Suspended in 10 ml HCMA

3807, 3813, 3878

3832

1,533,066 cells/ml

X 10 ml

Plate 4,660,000 cells / 175 cm²

Vol. = $\frac{4,660,000}{1,533,066}$

= 3.0 ml

Plate 3.0 ml X 3

	100%	1×10^6 cells ←	leave 3d
	0%	2×10^6 cells	
3d	50%	1×10^6 (100%) + (1×10^6) 0%	leave 3d
1h	50%	" "	
3h	50%	" "	
1d	50%	" "	

From Sonia

AM 540 nm
CMTMR – 5mM 566 nm

Thy labelling

- cells on confluency for 4 days (refeed every 2 days) → trypsinize → 5-30 pm
→ plate 1-2.10^5 c / T75 in full. media (15mL) MF_{15}
MEM + 15% FBS

1-30 pm after 20 hrs : rinse cells 2x PBS
- add 15mL MBF (MEM + 0.375% BSA + 1% FBS)
- add 3 Thy.

9:30 a.m. after 20 hrs, rinse 7x w/ 5mL PBS
- add PBS 10mL + 5 ul CMTMR 5mM *PBS → warm (2.5 μM)
40 ul calcein AM 5mM

Stock = 5mM

- 2 min 37°C / 15-20 min
- rinse 2x PBS
- add warm MBF (15mL) MF_{15}
- 37°C / 30 min
- rinse w/ PBS 2-3x and trypsinize (2mL) 37°C / 2 min
- neutralize w/ MBF and mix appropriately

hyperconf. cells.

- cells on confluency for 6 days
- trypsinize and remix appropriately either on MBF or MF

Cyto added 6:30 / 6/19

0 0.5 10

B002143

Dye (-)ve cells

MS = 500 µl ; Background = 21

Cell count: 2467, 2603, 2496, 2438

Cell conc. = 994533 cells/ml

Total Volume = 26 ml

Vol. containing 1×10^6 cells = 1.0 ml

" " 2×10^6 Cells = 2.0 ml

Dye (+)ve cells:

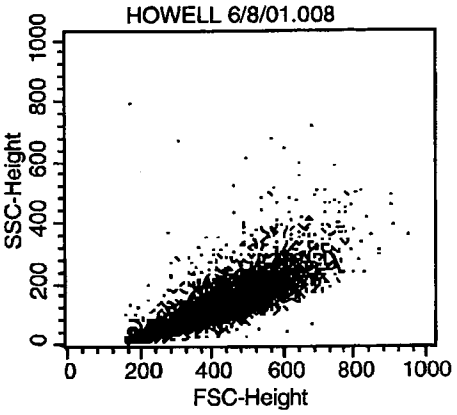
MS = 500 µl ;

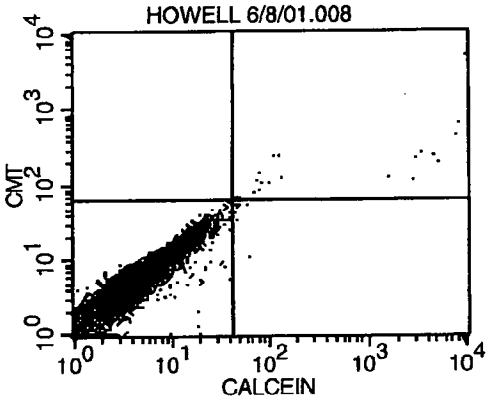
429, 342, 320, 309

Cell conc. = 129466 cells/ml

Total Volume = 42 ml

Vol containing 1×10^6 cells = 7.7 ml



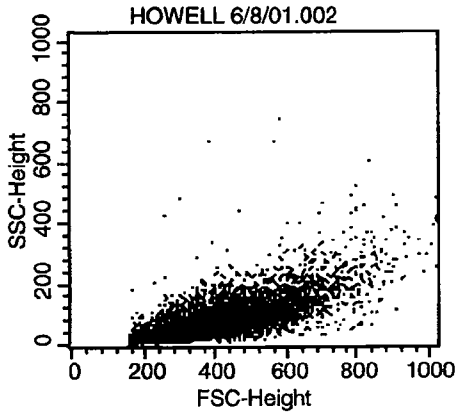


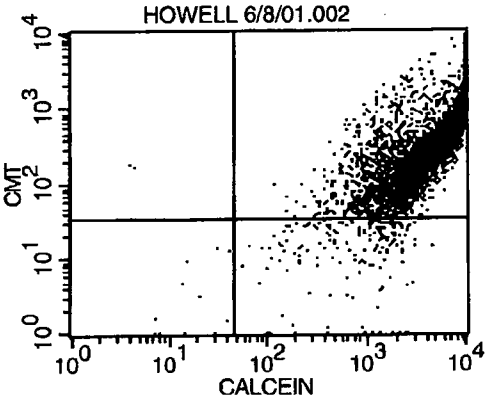
Quadrant Statistics

File: HOWELL 6/8/01.008
Sample ID: CELLS ALONE NEW SETT
Tube:
Acquisition Date: 8-Jun-1
Gated Events: 10000
X Parameter: FL1-H CALCEIN (Log)
Quad Location: 40, 61

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: No Gate
Total Events: 10000
Y Parameter: FL2-H CMT (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	23	0.23	0.23	2078.37	361.90	378.19	157.16
LL	9968	99.68	99.68	5.32	4.44	6.68	5.56
LR	9	0.09	0.09	47.65	47.26	43.38	39.68



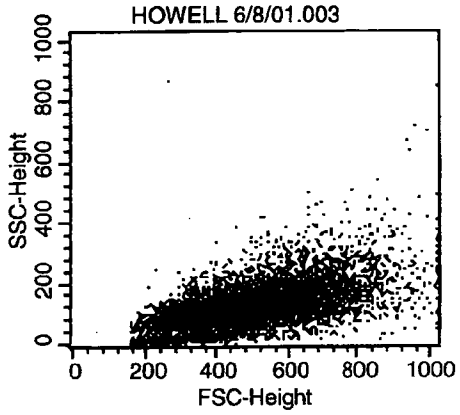


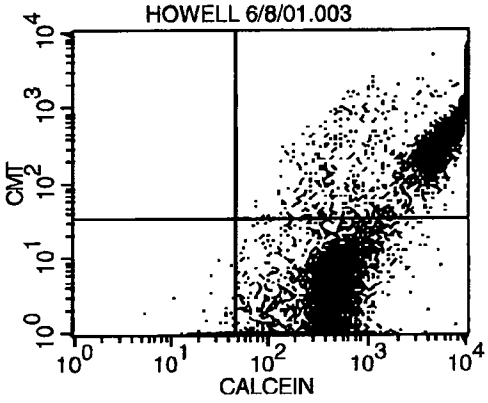
Quadrant Statistics

File: HOWELL 6/8/01.002
Sample ID: 100% R&G
Tube:
Acquisition Date: 8-Jun-1
Gated Events: 10000
X Parameter: FL1-H CALCEIN (Log)
Quad Location: 45, 33

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: No Gate
Total Events: 10000
Y Parameter: FL2-H CMT (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	2	0.02	0.02	4.09	4.09	169.38	169.24
UR	9787	97.87	97.87	6363.22	5420.03	956.67	489.34
LL	11	0.11	0.11	19.94	16.49	4.50	2.68
LR	200	2.00	2.00	1142.01	803.31	18.03	13.37



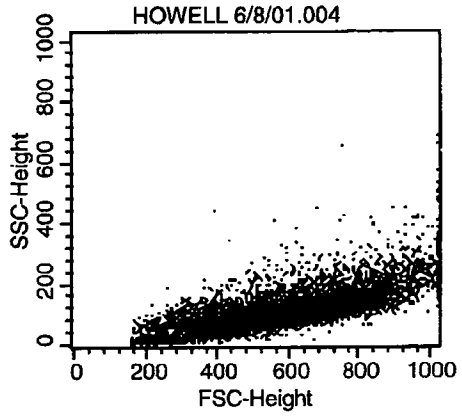


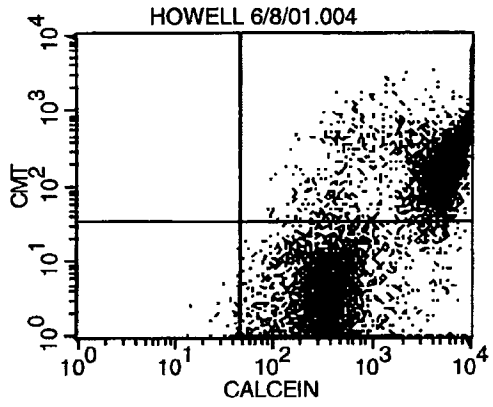
Quadrant Statistics

File: HOWELL 6/8/01.003
Sample ID: 50% R&G 1 HOUR
Tube:
Acquisition Date: 8-Jun-1
Gated Events: 10000
X Parameter: FL1-H CALCEIN (Log)
Quad Location: 45, 33

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: No Gate
Total Events: 10000
Y Parameter: FL2-H CMT (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	4301	43.01	43.01	5937.13	4876.39	602.70	380.23
LL	92	0.92	0.92	30.42	28.64	1.40	1.21
LR	5607	56.07	56.07	488.26	405.97	5.41	3.53



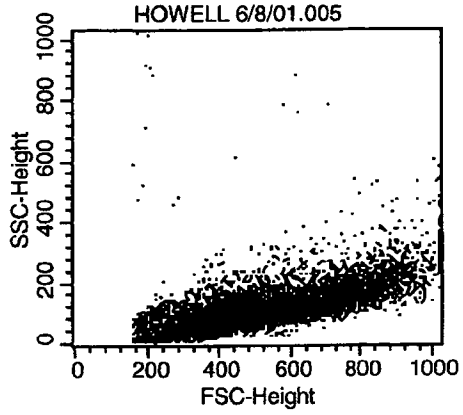


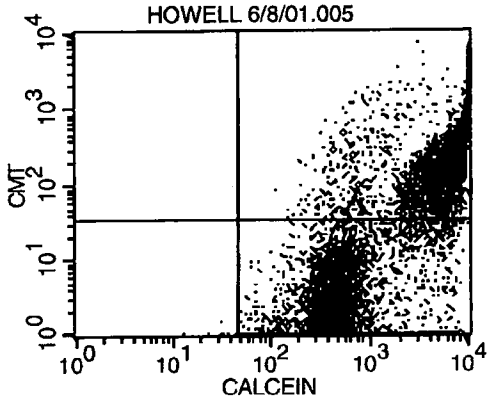
Quadrant Statistics

File: HOWELL 6/8/01.004
Sample ID: 50% R&G 1DAY
Tube:
Acquisition Date: 8-Jun-1
Gated Events: 10000
X Parameter: FL1-H CALCEIN (Log)
Quad Location: 45, 33

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: No Gate
Total Events: 10000
Y Parameter: FL2-H CMT (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	4337	43.37	43.37	6044.90	4968.79	478.68	247.38
LL	42	0.42	0.42	37.01	35.74	3.03	2.00
LR	5621	56.21	56.21	541.18	376.62	5.04	3.06



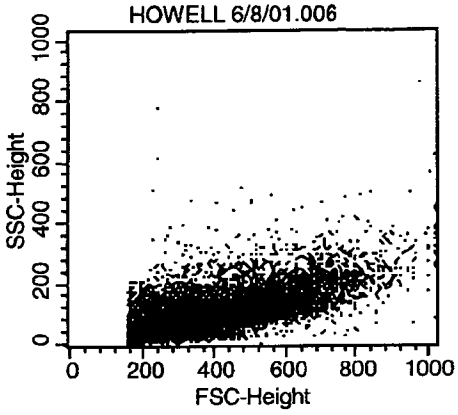


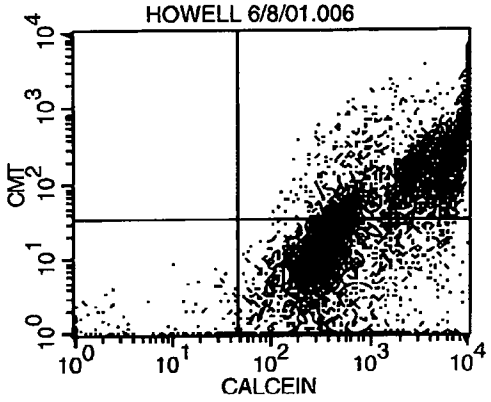
Quadrant Statistics

File: HOWELL 6/8/01.005
Sample ID: 50% R&G 2DAY
Tube:
Acquisition Date: 8-Jun-1
Gated Events: 10000
X Parameter: FL1-H CALCEIN (Log)
Quad Location: 45, 33

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: No Gate
Total Events: 10000
Y Parameter: FL2-H CMT (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	4240	42.40	42.40	6377.48	5320.47	552.29	236.72
LL	11	0.11	0.11	32.09	30.48	1.36	1.19
LR	5749	57.49	57.49	781.47	500.09	4.57	2.52



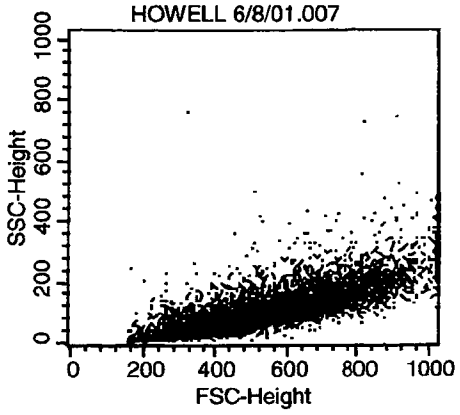


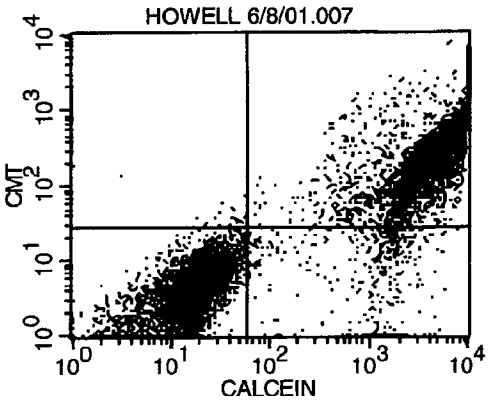
Quadrant Statistics

File: HOWELL 6/8/01.006
Sample ID: 50% R&G 3 DAY
Tube:
Acquisition Date: 8-Jun-1
Gated Events: 10000
X Parameter: FL1-H CALCEIN (Log)
Quad Location: 45, 33

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: No Gate
Total Events: 10000
Y Parameter: FL2-H CMT (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	4628	46.28	46.28	4994.57	3307.48	516.95	203.05
LL	129	1.29	1.29	19.34	10.36	1.75	1.47
LR	5243	52.43	52.43	550.08	368.69	13.04	9.24



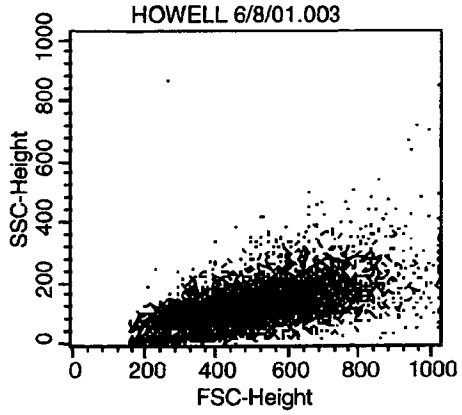


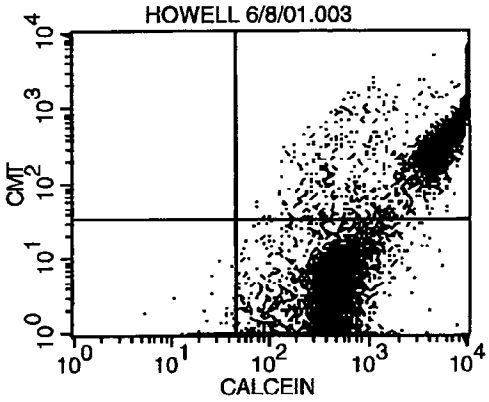
Quadrant Statistics

File: HOWELL 6/8/01.007
Sample ID: ADD BACK
Tube:
Acquisition Date: 8-Jun-1
Gated Events: 10000
X Parameter: FL1-H CALCEIN (Log)
Quad Location: 57, 26

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: No Gate
Total Events: 10000
Y Parameter: FL2-H CMT (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	28	0.28	0.28	36.31	29.71	45.67	40.07
UR	4375	43.75	43.75	4925.61	3958.20	511.72	248.47
LL	5159	51.59	51.59	17.83	15.20	4.72	3.77
LR	438	4.38	4.38	1403.71	905.95	11.54	7.51



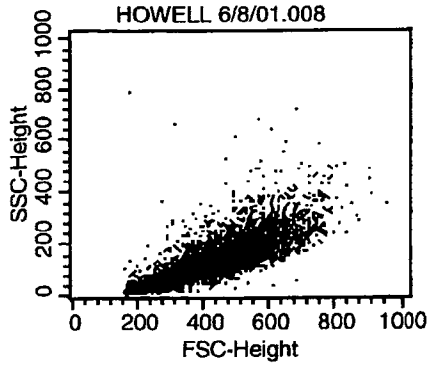


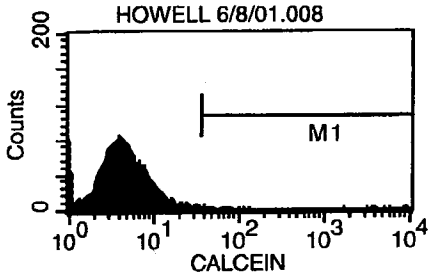
Quadrant Statistics

File: HOWELL 6/8/01.003
Sample ID: 50% R&G 1 HOUR
Tube:
Acquisition Date: 8-Jun-1
Gated Events: 10000
X Parameter: FL1-H CALCEIN (Log)
Quad Location: 45, 33

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: No Gate
Total Events: 10000
Y Parameter: FL2-H CMT (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	4301	43.01	43.01	5937.13	4876.39	602.70	380.23
LL	92	0.92	0.92	30.42	28.64	1.40	1.21
LR	5607	56.07	56.07	488.26	405.97	5.41	3.53



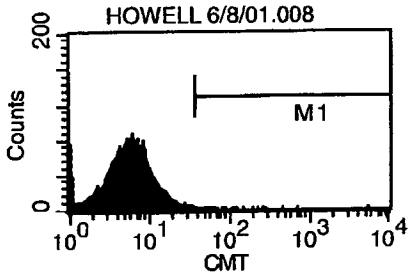


Histogram Statistics

File: HOWELL 6/8/01.008
Sample ID: CELLS ALONE NEW SETT
Tube:
Acquisition Date: 8-Jun-1
Gated Events: 10000
X Parameter: FL1-H CALCEIN (Log)

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: No Gate
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	Median
All	1, 9910	10000	100.00	100.00	10.13	4.50	4.29
M1	36, 9910	41	0.41	0.41	1184.79	141.40	57.25

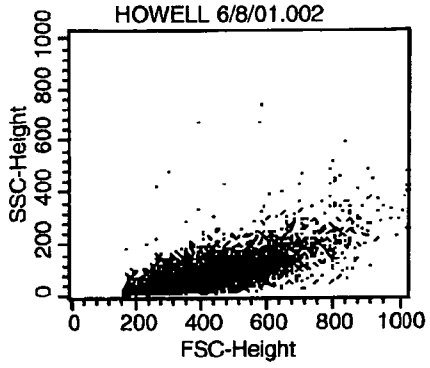


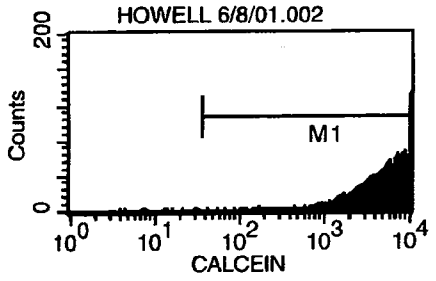
Histogram Statistics

File: HOWELL 6/8/01.008
Sample ID: CELLS ALONE NEW SETT
Tube:
Acquisition Date: 8-Jun-1
Gated Events: 10000
X Parameter: FL2-H CMT (Log)

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: No Gate
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	Median
All	1, 9910	10000	100.00	100.00	7.57	5.61	5.57
M1	38, 9910	51	0.51	0.51	195.73	79.46	56.74



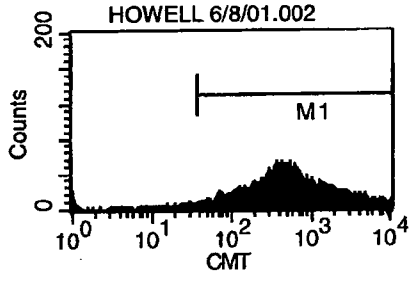


Histogram Statistics

File: HOWELL 6/8/01.002
Sample ID: 100% R&G
Tube:
Acquisition Date: 8-Jun-1
Gated Events: 10000
X Parameter: FL1-H CALCEIN (Log)

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: No Gate
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	Median
All	1, 9910	10000	100.00	100.00	6250.55	5176.38	6378.04
M1	36, 9910	9989	99.89	99.89	6257.42	5211.61	6378.04

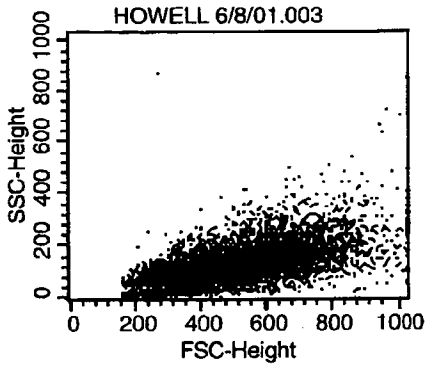


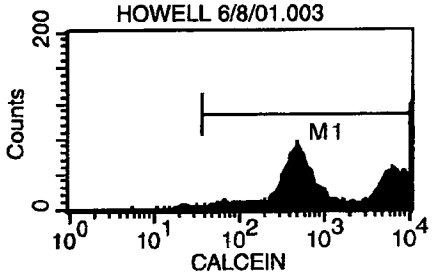
Histogram Statistics

File: HOWELL 6/8/01.002
Sample ID: 100% R&G
Tube:
Acquisition Date: 8-Jun-1
Gated Events: 10000
X Parameter: FL2-H CMT (Log)

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: No Gate
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	Median
All	1, 9910	10000	100.00	100.00	936.69	452.64	441.09
M1	38, 9910	9760	97.60	97.60	959.24	493.06	453.16



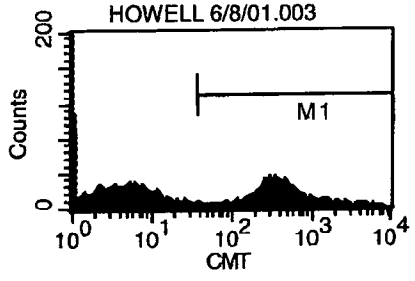


Histogram Statistics

File: HOWELL 6/8/01.003
Sample ID: 50% R&G 1 HOUR
Tube:
Acquisition Date: 8-Jun-1
Gated Events: 10000
X Parameter: FL1-H CALCEIN (Log)

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: No Gate
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	Median
All	1, 9910	10000	100.00	100.00	2827.61	1154.09	664.15
M1	36, 9910	9942	99.42	99.42	2843.96	1180.62	667.14

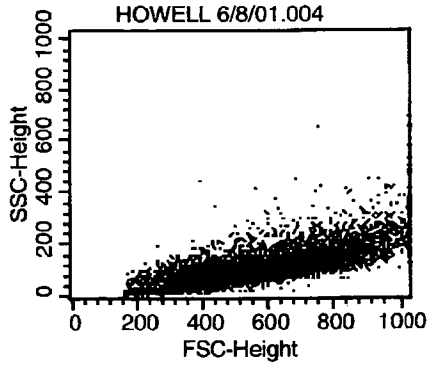


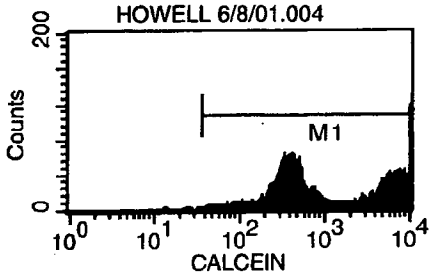
Histogram Statistics

File: HOWELL 6/8/01.003
Sample ID: 50% R&G 1 HOUR
Tube:
Acquisition Date: 8-Jun-1
Gated Events: 10000
X Parameter: FL2-H CMT (Log)

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: No Gate
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	Median
All	1, 9910	10000	100.00	100.00	262.27	26.14	10.94
M1	38, 9910	4266	42.66	42.66	607.35	387.74	366.82



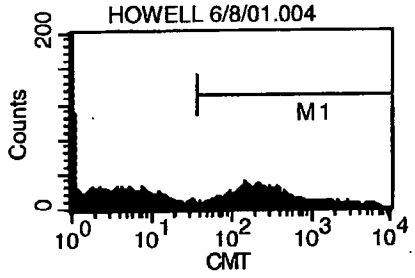


Histogram Statistics

File: HOWELL 6/8/01.004
Sample ID: 50% R&G 1DAY
Tube:
Acquisition Date: 8-Jun-1
Gated Events: 10000
X Parameter: FL1-H CALCEIN (Log)

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: No Gate
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	Median
All	1, 9910	10000	100.00	100.00	2926.02	1141.57	609.76
M1	36, 9910	9987	99.87	99.87	2929.80	1147.22	615.27

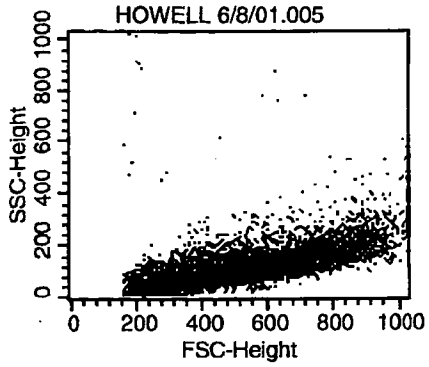


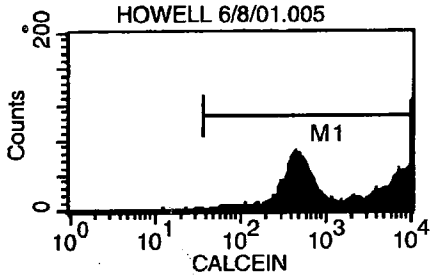
Histogram Statistics

File: HOWELL 6/8/01.004
Sample ID: 50% R&G 1DAY
Tube:
Acquisition Date: 8-Jun-1
Gated Events: 10000
X Parameter: FL2-H CMT (Log)

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: No Gate
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	Median
All	1, 9910	10000	100.00	100.00	210.45	20.51	11.14
M1	38, 9910	4294	42.94	42.94	483.12	252.25	224.68



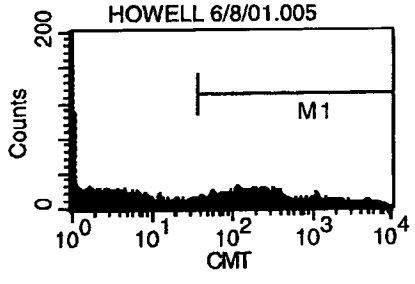


Histogram Statistics

File: HOWELL 6/8/01.005
Sample ID: 50% R&G 2DAY
Tube:
Acquisition Date: 8-Jun-1
Gated Events: 10000
X Parameter: FL1-H CALCEIN (Log)

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: No Gate
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	Median
All	1, 9910	10000	100.00	100.00	3153.35	1358.69	791.48
M1	36, 9910	9992	99.92	99.92	3155.86	1362.96	791.48

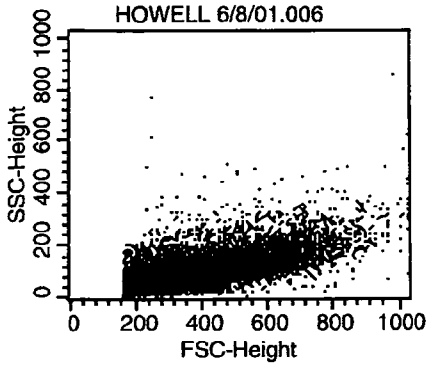


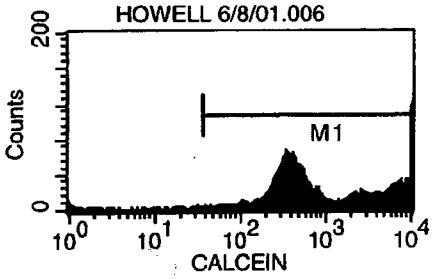
Histogram Statistics

File: HOWELL 6/8/01.005
Sample ID: 50% R&G 2DAY
Tube:
Acquisition Date: 8-Jun-1
Gated Events: 10000
X Parameter: FL2-H CMT (Log)

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: No Gate
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	Median
All	1, 9910	10000	100.00	100.00	236.80	17.27	9.39
M1	38, 9910	4175	41.75	41.75	560.34	243.82	203.51



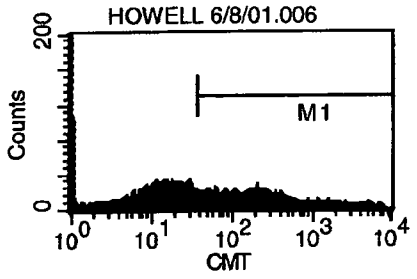


Histogram Statistics

File: HOWELL 6/8/01.006
Sample ID: 50% R&G 3 DAY
Tube:
Acquisition Date: 8-Jun-1
Gated Events: 10000
X Parameter: FL1-H CALCEIN (Log)

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: No Gate
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	Median
All	1, 9910	10000	100.00	100.00	2600.14	971.91	562.34
M1	36, 9910	9903	99.03	99.03	2625.49	1020.60	572.55

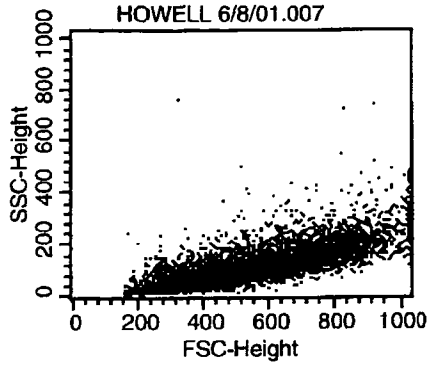


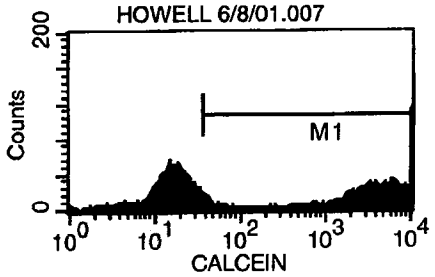
Histogram Statistics

File: HOWELL 6/8/01.006
Sample ID: 50% R&G 3 DAY
Tube:
Acquisition Date: 8-Jun-1
Gated Events: 10000
X Parameter: FL2-H CMT (Log)

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: No Gate
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	Median
All	1, 9910	10000	100.00	100.00	246.11	37.70	28.13
M1	38, 9910	4424	44.24	44.24	539.17	220.15	189.38



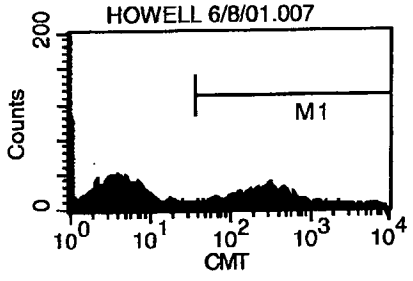


Histogram Statistics

File: HOWELL 6/8/01.007
Sample ID: ADD BACK
Tube:
Acquisition Date: 8-Jun-1
Gated Events: 10000
X Parameter: FL1-H CALCEIN (Log)

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: No Gate
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	Median
All	1, 9910	10000	100.00	100.00	2225.74	207.61	39.95
M1	36, 9910	5116	51.16	51.16	4334.93	2668.62	3751.62

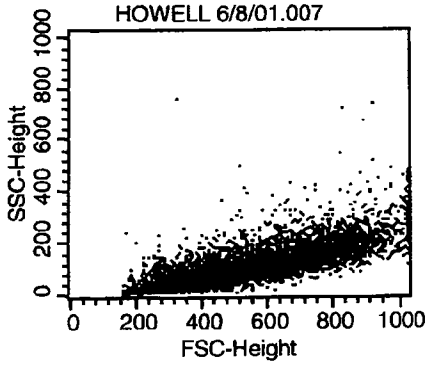


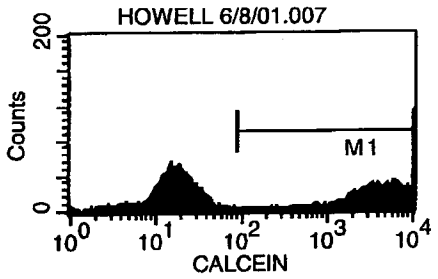
Histogram Statistics

File: HOWELL 6/8/01.007
Sample ID: ADD BACK
Tube:
Acquisition Date: 8-Jun-1
Gated Events: 10000
X Parameter: FL2-H CMT (Log)

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: No Gate
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	Median
All	1, 9910	10000	100.00	100.00	226.95	24.43	10.09
M1	38, 9910	4260	42.60	42.60	524.77	263.06	249.16



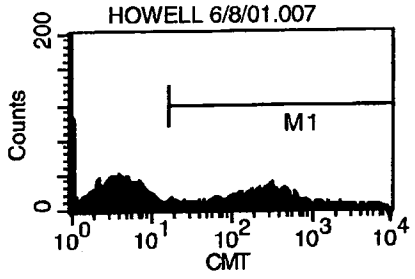


Histogram Statistics

File: HOWELL 6/8/01.007
Sample ID: ADD BACK
Tube:
Acquisition Date: 8-Jun-1
Gated Events: 10000
X Parameter: FL1-H CALCEIN (Log)

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: No Gate
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	Median
All	1, 9910	10000	100.00	100.00	2225.74	207.61	39.95
M1	87, 9910	4763	47.63	47.63	4652.72	3606.45	4067.94

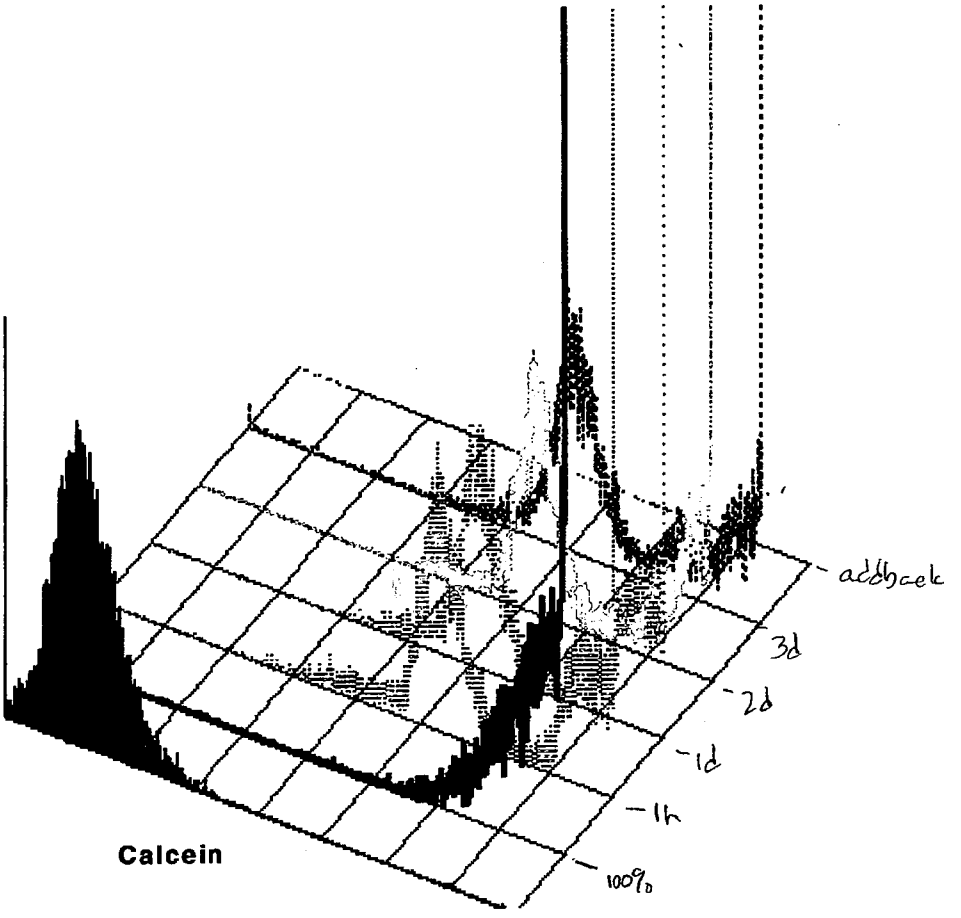


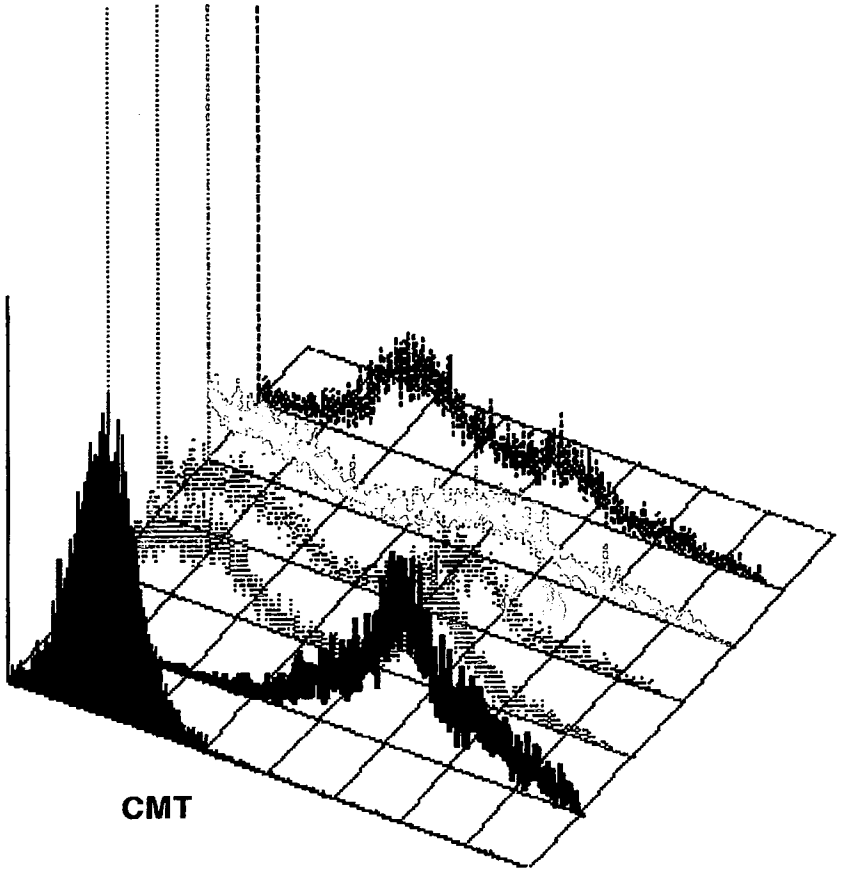
Histogram Statistics

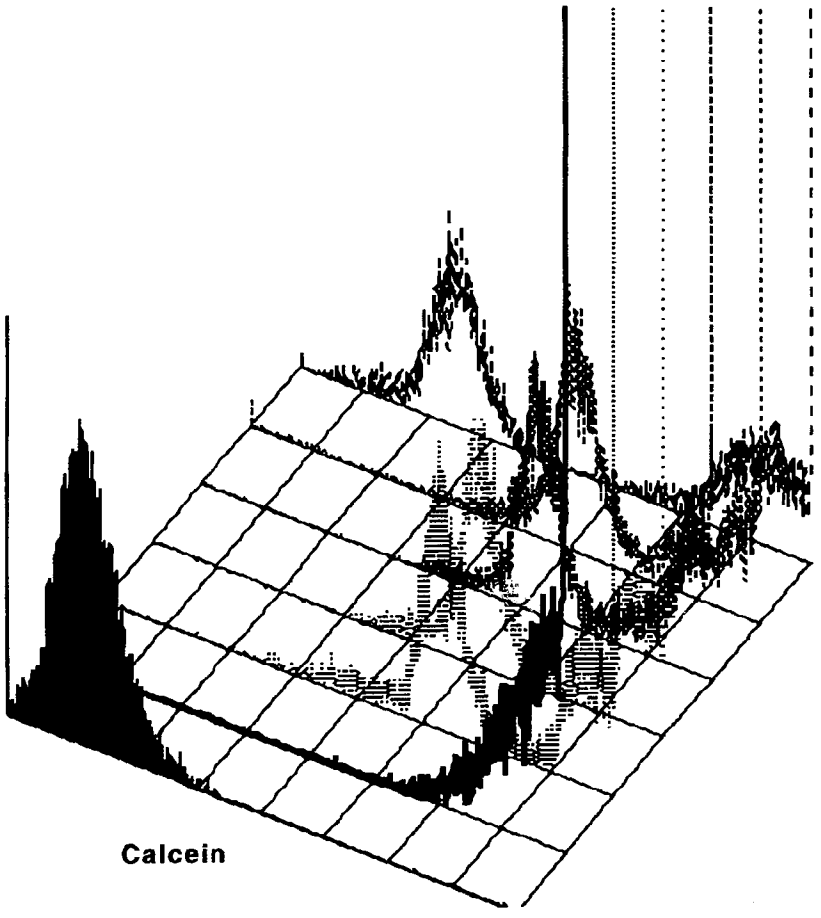
File: HOWELL 6/8/01.007
Sample ID: ADD BACK
Tube:
Acquisition Date: 8-Jun-1
Gated Events: 10000
X Parameter: FL2-H CMT (Log)

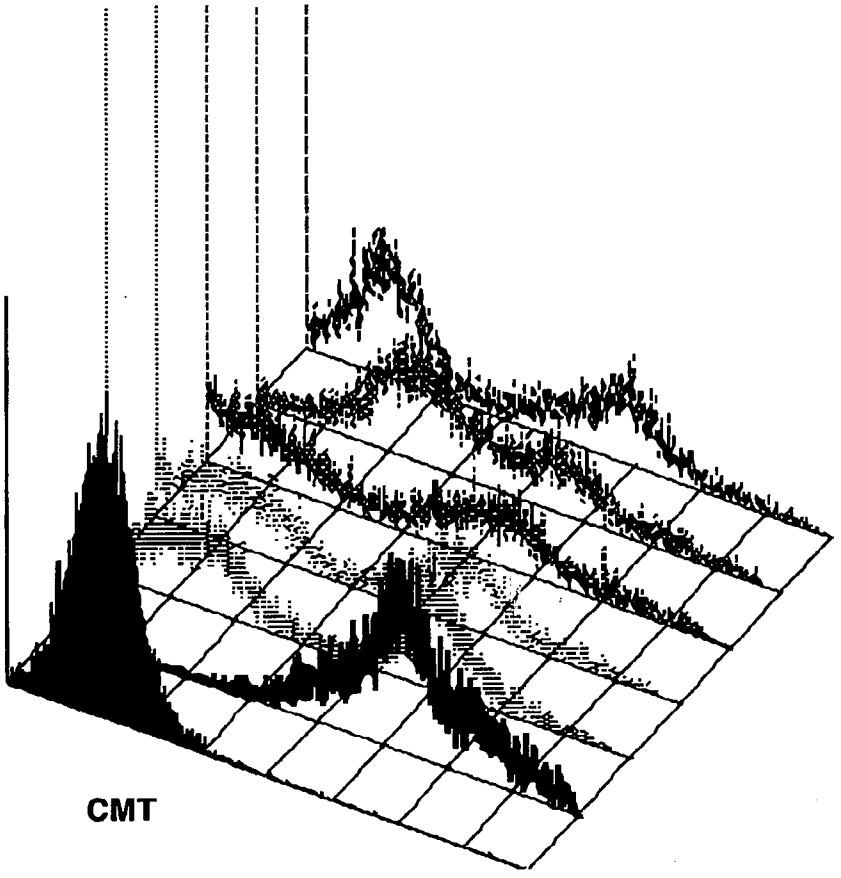
Log Data Units: Linear Values
Patient ID:
Panel:
Gate: No Gate
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	Median
All	1, 9910	10000	100.00	100.00	226.95	24.43	10.09
M1	17, 9910	4617	46.17	46.17	486.15	219.04	224.68


Calcein
100%
1h
1d
2d
3d
add back


CMT


Calcein


CMT

05/25/01 ; 6-00 pm

1. Received two very confluent flasks of 1522 cells (2X 75 cm^2), p. 14 from Sonia.
2. Trypsinazine, resuspend in MEM @ 15% FBS, 10 mM HEPES and poded cells
3. Transfer cell suspension equally in 3X 175cm^2 flasks

05/28/01 ; 6-00 pm

4. Change the medium for one 175 cm^2 flask
5. Trypsinize cells and pooled cells from 2X175 cm^2 flask.
6. Perform cell count

MS = 500 µl ; Background = 8
Cell count (100µl) = 1887, 1880, 1920
Cell conc = 758266 Cells/ml
Total # of cells = 758266 X 24 = 18,198,400 cells

7. Place 3,500,000 cells (~4.6 ml) in 4X 175cm^2 flasks

Uptake of 3HTOR by AG 1522 cells

05/01/01

1. AG 1522 cells were collected from 4×175-cm² flasks. Cells were contact inhibited for ~~same~~ 4-5 days but refed with medium ~ 20h before
2. Dilute 4,000,000 cells/ml of MEMB
3. Aliquot 1 ml into 11 17×100 mm Falcon test tube
4. ~~Put~~ Roll tubes in a rocker-roller for 3-4h

   Date/Time: 05/01/01; 4-00pm
5. After that add 1 ml of 10 μci/ml ^{3}HTOR in MEMB for 7 tubes and 1 ml of MEMB for 4 tubes.
6. Return the tubes in roller (Total Volume 2 ml) in 1 ml MEMA
7. Dilute the stock cell and plate ~1000 cells (actually 1069) in triplicate in P100 with 10 ml MEMA with 10 mM HEPES

   Date/Time: 05/01/01; 8-00pm
8. Next day, (after ~ 14h), take one tube with radioactivity from the roller

   Date/Time: 05/02/01; 10-00pm
9. Syringe with 5 ml Syringe C 21G needle five times.
10. Place 100 ml of cell suspension ~~from~~ in a Micro-centrifuge tube with 300 ml FBS. (Total Volume ~400 ml) and perform cell count by hand counting 100 μl in coulter counter
11. centrifuge tubes in minicentrifuge 3200 exactly for 1 min
12. Freeze ~~the~~ one microcentrifuge tube at a time by by immersing the tube in a a bucket with liquid nitrogen, cut the bottom tip of the tube using Guilotine, collect the tip in 12×75 mm glass tube, transfer the tip in 6 ml Scintillation Vial. and
13. Add 6 ml of Ecolume

13. Centrifuge the original 17×100 mm tube for 5 min at 2000 rpm.

14. Transfer 100 µl of supernatant in Microcentrifuge tube in triplicate containing 300 µl Serum and proces as described before

15 Transfer 30 µl of supernatant in triplicate in 6 ml of scintilation vial and add 6 ml of Ecolume

16 Count all scintillation vials.

Note: The experiment was terminated after 22 h timepoint due to severe aggregation of cells in tubes while they are in roller.

1238, 1289, 1253

2,520,000 cells/ml

22 ml x 2,000,000 cells/ml

= 44,000,000

Vol $\frac{44,000,000}{2,520,000}$

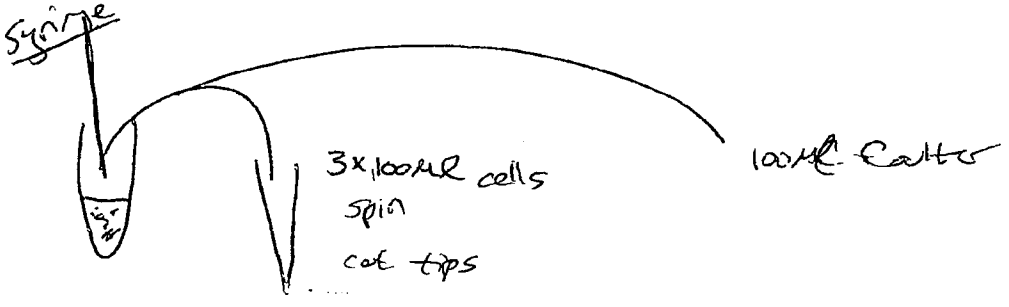
= 17.5 cells

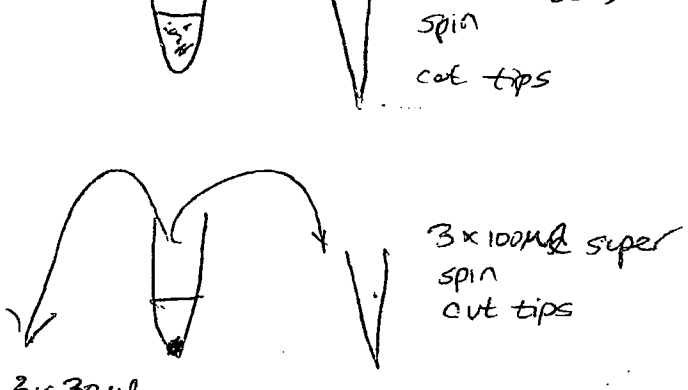
4.5 MEM

22.00

Summary of Experimental design

0h	8-00 pm	05/01/01	→ Addition of radioactivity 5 μci/ml
14h	10-00 am	05/02/01	→ Medium count, uptake study ~~Study/min, count~~
18h	2-00 pm	05/02/01	→ ~~Medium count~~, uptake study ~~200 cells/p100~~
✓ 24h	5-00 pm	05/02/01	→ Medium count, uptake study, preparation of the cluster, put at 0.5°C for 3 days
✓ 38h	9-00 a.m	05/03/01	
42h	1-00 pm	05/03/01	
✓ 48h	7 pm	5/03/01	
✓ 62h	9 am	5/04/01	





Preparation of radioactivity

05/01/01

Prepare 10 μCi/ml prepare 8 ml

≃ 80 μCi required

① Take 80 μl of stock
② Add 7.92 ml of MEMβ

Cell count at different time intervals: MS = 500 ml

14 hr ① 1313, 1256, 1256 (Back = 30)
18 hr ② 1383, 1398, 1462 (Back = 24)
24 hr ③ 801, 773, 783 (Back = 17)

05/01/01

MS = 500nL : Background = 9, 5,

Trypsinize AG1522 cells from 4 x 175 cm² flasks
pool cells, centrifuge, resuspend in 12 mL
~~3874, 3903, 3869~~ MEMB, Syringe
~~3882~~

~~Cell conc. = 15, 52,800/mL~~

~~Cell # = 31,056,000~~

Cell count =

10610, 10692, 10768

Avg. count 10690

Cell conc. = 4,276,000 cells/mL X 12 = 51,312,000 cells/4 flask

↓ 1:4

1,069,000 cells/mL

↓ 1:100

1069000

↓ 1:10

10690 cells/mL

~1069 cells were plated in triplicate in p100
Colonies were counted following 2 weeks.

After 14h

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 WED 02 MAY 2001 11:32
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:0 1.00000
HALF LIFE(DAYS):N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**- 1	1	107506.66	1.57	0.15	0.56	77.0	
2	**- 2	1	114666.66	1.52	0.15	1.27	78.0	
3	**- 3	1	133290.00	1.73	0.10	1.98	82.0	
4	**- 4	1	1415.00	5.32	1.00	3.61	76.0	
5	**- 5	1	963.00	6.44	1.00	5.18	77.0	
6	**- 6	1	1371.00	5.40	1.00	6.80	76.0	
7	**- 7	1	185.00	14.70	1.00	8.36	76.0	
8	**- 8	1	324.00	11.11	1.00	9.98	80.0	
9	**- 9	1	413.00	9.84	1.00	11.60	80.0	
10	**-10	1	30077.14	1.95	0.35	12.56	1.0	

30 µl medium

100 µl cells through screen

100 µl medium through screen

→ Standard

After 18h

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 WED 02 MAY 2001 14:10
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
H#: 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:0 1.00000
HALF LIFE(DAYS):N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR	
1	29- 1	1	357.00	10.59	1.00	1.42	80.0		100 µl medium through screen
2	29- 2	1	153.00	16.17	1.00	3.00	79.0		
3	29- 3	1	398.00	10.03	1.00	4.63	79.0		
4	29- 4	1	1571.00	5.05	1.00	6.23	78.0		100 µl cells through screen
5	29- 5	1	1443.00	5.26	1.00	7.81	76.0		
6	29- 6	1	1296.00	5.56	1.00	9.38	77.0		
7	29- 7	1	106360.00	1.94	0.10	10.09	79.0		30 µl medium
8	29- 8	1	98186.66	1.65	0.15	10.85	78.0		
9	29- 9	1	129986.66	1.43	0.15	11.55	81.0		
10	29-10	1	29731.43	1.96	0.35	12.52	0.0		→ Standard

After ~ 22h

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 WED 02 MAY 2001 17:57
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
H#: 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

	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**- 1	1	135660.00	1.40	0.15	0.57	82.0	} 30 µl medium
2	**- 2	1	141846.66	1.37	0.15	1.28	82.0	
3	**- 3	1	148960.00	1.34	0.15	1.99	82.0	
4	**- 4	1	2026.00	4.44	1.00	3.57	78.0	} 100 µl cells through screen
5	**- 5	1	636.00	7.93	1.00	5.13	80.0	
6	**- 6	1	1967.00	4.51	1.00	6.72	82.0	
7	**- 7	1	449.00	9.44	1.00	8.34	83.0	} 100 µl medium through screen
8	**- 8	1	274.00	12.08	1.00	9.91	82.0	
9	**- 9	1	214.00	13.67	1.00	11.54	82.0	
10	**-10	1	29762.86	1.96	0.35	12.50	0.0	→ Std

AG1522 COLONY FORMING ASSAY

Experiment Name: Effect of temperature on survival **Exp. # :** 1

Experiment performed by: A. Bishayee **Date:** 2/2/01

1. Set the rocker-roller at 37°C incubator with 5% CO_2, set the Coulter Counter, wash confluent monolayer of cells (from 175 cm^2 flask, subcultured 7 days prior to the experiment) two times with PBS, trypsinize cells with 2 ml of trypsin-EDTA, resuspend in 8 ml MEMB, pass five times through 5 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 2 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:** 02/02/01 ; 1-00 pm
5. After ~3-4 h incubation period, remove tubes and centrifuge at 2000 rpm at 4°C for 10 min (***precooled centrifuge***). **Date/Time:** 02/02/01 ; 4-00 pm
6. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
7. Centrifuge tubes for 10 min at 2000 rpm, 4°C
8. Decant supernatant, click tubes, vortex, resuspend cells from one tube in in 2 ml wash MEMA, perform cell count, dilute and seed 800 cells in triplicate in 100x20 mm Falcon tissue culture dish with 10 ml MEMA with 15% FBS.
9. For other tubes, transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips
10. Again add 200 ul MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
11. Centrifuge tubes for 5 min at 1000 rpm, 4°C
12. Transfer tubes at 8.5, 10.5, 12 or 15°C for 72 h. **Date/Time:** 02/02/01 ; 6-00 pm
13. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the content to 14 ml tubes (Falcon plastic test tube, 17x100 mm) containing 10 ml wash MEMA by using pasteur pipet **Date/Time:** 02/05/01; 3-00 pm
14. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 14 ml tubes
15. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***
16. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
17. Centrifuge tubes for 10 min at 2000 rpm, 4°C
18. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 5 cc syringe with 21 gauge needle

19. Determine cell concentration by transfering 100 µl to Coulter cup
20. Dilute and seed 800 cells in triplicate in 100x20 mm Falcon tissue culture dish with 10 ml MEMA with 15% FBS
21. After 2 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
22. Count colonies.

TABLE-1

Expt # : 1 **Date :** 02/19/01

Temp. (°C)	Colony 1	Colony 2	Colony 3	Avg Colony	SF	P/E
37	115	123	109	116.6	–	0.15
8.5	105	96	90	97	0.832	0.12
10.5	119	100	105	108	0.926	0.14
12	118	99	90	102	0.875	0.13
15	71	107	91	89	0.763	0.11

TABLE-2

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

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					
4					
5					
6					
7					
8					
9					
10					

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
Tube #	^{125}IUdR µCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{125}IUdR [2.0 uCi/ml]		
1	0	1.0	1.0	0		
2	0	1.0	1.0	0		
3	0.005	1.0	0.995	0.005		
4	0.01	1.0	0.99	0.01		
5	0.05	1.0	0.95	0.05		
6	0.1	1.0	0.9	0.1		
7	0.2	1.0	0.8	0.2		
8	0.5	1.0	0.5	0.5		
9	0.75	1.0	0.25	0.75		
10	1.0	1.0	0	1.0		

Expt # : **Date :**

TABLE-4

Effect of temperature on the survival of AG1522 cells from multicellular clusters