

3

V79/100

V79 COLONY FORMING & ASSAY

Experiment Name : 100 % cluster, 3HTdR);
Investigator: M. Lenarczyk
May 3, 2001

Exp.
Date:

1. Set the rocker-roller at 37°C incubator with 5% CO₂, set the Coulter Counter, wash cells (from two 150 cm² flasks, sub-cultured 20x10(6) cells/flask 24h before) with PBS, trypsinize cells, each re-suspend in 9 ml MEMB, pass 5x through 10 cc syringe with 21 gauge needle, count the cells using Coulter counter (100 ul cell suspension / 20 ml Isotone)
2. Dilute to ~4,000,000 cells/ml in MEMB [Actual count : 4 200 000 cells/ml]
[10499 + 10517 + 10534 / 3] - 2] x 100 $\xrightarrow{\uparrow}$
3. Transfer 1 ml of cell suspension into ten 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% CO₂

Date/Time: May 3,

2001/ 18:20

5. Prepare MEMB containing radioactivity in hood

60 μ l 3 HTdR (Stock : μ Ci/ μ l on) + 5 ml MEMB

6. After 3-4 h, remove test tubes from roller and add MEMB with or without radioactivity according to Table below.

Date/Time: May 3, 2001 / 20:15

Tube #	3 HTdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ 3 HTdR (ml) [12 uCi/ml]
1	0	1.0	1.0	0
2	0	1.0	1.0	0
3	1	1.0	0.830	0.170
4	1.5	1.0	0.750	0.250
5	2.0	1.0	0.670	0.330
6	2.5	1.0	0.680	0.420
7	3.0	1.0	0.500	0.500
8	4.0	1.0	0.330	0.670
9	5.0	1.0	0.170	0.830
10	6.0	1.0	0	1

7. Return test tubes to roller for 12 h

Date/Time: May 3, 2001 / 20:20

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: May 4, 2001 / 10:10 am

10. Remove buckets from centrifuge and carefully remove 150 µl of supernatant and place in pre-labeled gamma-tube.
11. Decant supernatant, click tubes, vortex, re-suspend in 10 ml wash MEMA
12. Centrifuge tubes for 10 min at 2000 rpm, 4°C
13. Decant supernatant, click tubes, vortex, re-suspend in 10 ml wash MEMA
14. Centrifuge tubes for 10 min at 2000 rpm, 4°C
15. Decant supernatant, click tubes, vortex, re-suspend in 10 ml wash MEMA
16. Centrifuge tubes for 10 min at 2000 rpm, 4°C
17. Decant supernatant, click tubes, vortex, re-suspend in 7 ml of MEMA
18. Centrifuge tubes for 10 min at 2000 rpm, 4°C
19. Decant supernatant, click tubes, vortex, transfer the cell suspension in polypropylene micro-centrifuge tubes with attached caps (Helena Plastics, 400 µl) using 200 µl pipet tips
20. Again add 200 µl ice cold MEMA, re-suspend and transfer the cell suspensions in the same polypropylene micro-centrifuge tubes (Total volume ~400 µl)
21. Centrifuge tubes for 5 min at 1000 rpm, 4°C
22. Transfer tubes at 10°C for 72 h.

Date/Time: MAY 4, 2001 / 11:55 am

23. Transfer 30 µl supernatant in three sets of 20 ml scintillation vials containing 6 ml liquid scintillation cocktail (EcoLume) from 150 µl supernatant removed earlier (Step 10) and count them for radioactivity

Date/Time: MAY 4, 2001 /

24. After 72 h, carefully remove the supernatant from the top, re-suspend pellet in 200 µl 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: MAY 7, 2001 / 13:00

- ✓ 25. Again add 200 µl wash MEMA in micro-centrifuge tubes, re-suspend and transfer the cell suspensions in 12 ml tubes
- ✓ 26. Centrifuge the tubes for 10 min at 2000 rpm, 4°C (precooled centrifuge)
- ✓ 27. 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.

- ✓ 28. Decant supernatant, click tubes, vortex, re-suspend in 10 ml wash MEMA
- ✓ 29. Centrifuge tubes for 10 min at 2000 rpm, 4°C
- ✓ 30. Decant supernatant, click tubes, vortex, re-suspend in 10 ml wash MEMA
- ✓ 31. Centrifuge tubes for 10 min at 2000 rpm, 4°C
32. Decant supernatant, click tubes, vortex, re-suspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
- ✓ 33. Determine cell concentration by Coulter counting (100 µl cell suspension + 20 ml Isotone II)
- ✓ 34. 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.
- ✓ 35. 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.
- ✓ 36. Transfer 200 µl of cell suspension (in triplicate) to 20 ml scintillation vial containing 6 ml cocktail (EcoLume)
37. Incubate P60's for 1 week
38. Count vials for radioactivity **Date/Time :**
39. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
40. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.

41. For HGPRT⁻ - plate 2×10^6 cells / T75ml + 15ml MEMA
 0 day - May 7, 2001
 3 day May 10, 2001
 * On May 11 all the T75 flasks were contaminated. Why?

TABLE-3

Expt. #: V79, 100%, H3

Date/Time: MAY 3 / 2001 - start

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [pCi/ml x 10⁶ Cells/ml]
1	6479, 6522, 6564		2605467	0.39
2	7117, 6864, 6859		2775467	0.37
3	6336, 6410, 6373		2546000	0.40
4	7404, 7377, 7432		2958533	0.34
5	7595, 7546, 7263		2984000	0.34
6	6399, 6607, 6325		2574267	0.39
7	5950, 6039, 5761		2363467	0.41
8	6655, 6821, 6607		2674533	0.38
9	5587 5416 5598		2210267	0.45
10	7052 7258 7144		2857333	0.36

For
Mn
↓
plate
0.15ml
clear.
per
P60'

Bolegr - 8-11
Mode - 500ul

↑
Volume for
10⁶ cells

TABLE-4

Expt # :

Date :

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF

Medium count (1st)

Medium count (1st)

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 FRI 04 MAY 2001 15:57
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
 H#: 1 ADC: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	5.00	89.44	1.00	1.48	77.0	
2	**	2	9.00	66.67	1.00	3.12	78.0	
3	**	3	11.00	60.30	1.00	4.68	79.0	
4	**	4	13.00	55.47	1.00	6.30	83.0	
5	**	5	10.00	63.25	1.00	7.86	79.0	
6	**	6	9.00	66.67	1.00	9.43	80.0	
7	**	7	19516.36	1.93	0.55	10.53	80.0	
8	**	8	18031.67	1.92	0.60	11.69	78.0	1945
9	**	9	20814.00	1.96	0.50	12.80	79.0	
10	**	10	27027.50	1.92	0.40	13.76	80.0	
11	**	11	27402.50	1.91	0.40	14.78	79.0	27760
12	**	12	28791.43	1.99	0.35	15.73	80.0	
13	**	13	31642.86	1.90	0.35	16.63	79.0	
14	**	14	34666.66	1.96	0.30	17.49	79.0	34481
15	**	15	37136.66	1.89	0.30	18.35	80.0	
16	**	16	45684.00	1.87	0.25	19.22	80.0	
17	**	17	53830.00	1.93	0.20	19.97	78.0	49099
18	**	18	47784.00	1.83	0.25	20.78	76.0	
19	**	1	53825.00	1.93	0.20	21.59	74.0	
20	**	2	47648.00	1.83	0.25	22.39	80.0	59011
21	**	3	60560.00	1.82	0.20	23.14	76.0	
22	**	4	83953.33	1.78	0.15	23.84	76.0	
23	**	5	83053.33	1.79	0.15	24.54	77.0	79649
24	**	6	72093.33	1.92	0.15	25.24	74.0	
25	**	7	99273.33	1.64	0.15	25.94	74.0	
26	**	8	112750.00	1.88	0.10	26.66	77.0	107576
27	**	9	110706.66	1.55	0.15	27.36	77.0	
28	**	10	111130.00	1.90	0.10	28.08	75.0	
29	**	11	117413.33	1.51	0.15	28.78	74.0	121418
30	**	12	135713.33	1.40	0.15	29.49	78.0	
31	**	13	29774.29	1.96	0.35	30.44	0.0	
36	**	18	27.00	38.49	1.00	32.07	72.0	

\bar{x}
 8.33 }
 10.67 } → 9.49
 1945
 27760
 34481
 49099
 59011
 79649
 107576
 121418

SONIA

[] - standard DPM - 98200
 H-3

326 Cyt B

$$10 \times 3 = 30 \text{ ml} \\ 35 \text{ ml}$$

$$3 \mu\text{g/ml} \\ \times 35 \\ x = \boxed{105 \mu\text{g}}$$

$$\text{Stock} - 2 \mu\text{g/ml} \\ 105 \mu\text{g} - \gamma \gamma = \boxed{52.5 \mu\text{g}}$$

52.5 μg (of 2 $\mu\text{g/ml}$) + 35 ml HEPES

↓ mix

add 1 ml into each P60
(final concn. Cytoc. - B = 3 $\mu\text{g/ml}$)

↓ incubate overnight

Wash 2-3 x with PBS

↓

fix MeOH

↓

keep for analysis (staining).

Medium count (2nd)

(better than 1st?)

USER: 6 IO:H3 HOWELL PRESET TIME: 1.00 MON 07 MAY 2001 16:55
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N R5232:N
H#: 1 ABC:N BCF:N RCM:N
CHANNEL 1-LL: 0 UL: 400 ZSIGMA: 2.00 BKG SUB: 0.00 BKG ZSIG: 0.00 LSR: 0
DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000
HALF LIFE(DAYS):N

SAM	POS	CH	CPM	ZSIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	7.00	75.59	1.00	1.42	79.0	
2	**	2	14.00	53.45	1.00	3.06	79.0	
3	**	3	10.00	63.25	1.00	4.63	80.0	
4	**	4	9.00	66.67	1.00	6.18	84.0	
5	**	5	7.00	75.59	1.00	7.81	81.0	
6	**	6	10.00	63.25	1.00	9.38	81.0	
7	**	7	21080.00	1.95	0.50	10.44	81.0	
8	**	8	21150.00	1.94	0.50	11.50	82.0	
9	**	9	21824.00	1.91	0.50	12.61	81.0	
10	**	10	29814.29	1.96	0.35	13.51	81.0	
11	**	11	31682.86	1.90	0.35	14.42	81.0	
12	**	12	32317.14	1.88	0.35	15.38	81.0	
13	**	13	37050.00	1.90	0.30	16.28	81.0	
14	**	14	39913.33	1.83	0.30	17.13	81.0	
15	**	15	41980.00	1.95	0.25	17.94	81.0	
16	**	16	50450.00	1.99	0.20	18.74	82.0	
17	**	17	61230.00	1.81	0.20	19.49	80.0	
18	**	18	53895.00	1.93	0.20	20.24	78.0	
19	**	1	62655.00	1.79	0.20	21.04	77.0	
20	**	2	58785.00	1.84	0.20	21.79	83.0	
21	**	3	69060.00	1.97	0.15	22.49	77.0	
22	**	4	89513.33	1.73	0.15	23.19	75.0	
23	**	5	91533.33	1.71	0.15	23.89	79.0	
24	**	6	80480.00	1.82	0.15	24.59	77.0	
25	**	7	111973.33	1.54	0.15	25.29	77.0	
26	**	8	113726.66	1.53	0.15	25.99	77.0	
27	**	9	124366.66	1.46	0.15	26.70	78.0	
28	**	10	120866.66	1.49	0.15	27.40	76.0	
29	**	11	133246.66	1.41	0.15	28.11	75.0	
30	**	12	138546.66	1.39	0.15	28.82	78.0	
31	**	13	29408.00	1.90	0.38	29.85	1.0	
36	**	18	9.00	66.67	1.00	31.52	72.0	

Standard
43 - DM-98200

Cell count 1st.

Cell count 1st

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 MON 07 MAY 2001 17:26
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
 H#: 1 AQC:N BCF: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	6.00	81.65	1.00	1.42	87.0	
2	**	2	9.00	66.67	1.00	2.99	83.0	
3	**	3	10.00	63.25	1.00	4.56	78.0	
4	**	4	7.00	75.59	1.00	6.13	78.0	
5	**	5	13.00	55.47	1.00	7.74	83.0	
6	**	6	10.00	63.25	1.00	9.30	76.0	
7	**	7	2917.00	3.70	1.00	10.93	80.0	
8	**	8	2641.00	3.89	1.00	12.50	76.0	
9	**	9	1393.00	5.36	1.00	14.13	75.0	
10	**	10	2969.00	3.67	1.00	15.76	77.0	
11	**	11	3048.00	3.62	1.00	17.38	77.0	
12	**	12	3103.00	3.59	1.00	18.95	77.0	
13	**	13	4278.00	3.06	1.00	20.53	80.0	
14	**	14	3442.00	3.41	1.00	22.16	75.0	
15	**	15	4643.00	2.94	1.00	23.72	79.0	
16	**	16	5077.00	2.81	1.00	25.31	79.0	
17	**	17	4441.00	3.00	1.00	26.88	76.0	
18	**	18	5056.00	2.81	1.00	28.46	79.0	
19	**	1	11143.33	2.00	0.90	29.97	85.0	
20	**	2	12341.18	1.95	0.85	31.43	85.0	
21	**	3	10489.00	1.95	1.00	33.01	83.0	
22	**	4	14350.00	2.00	0.70	34.27	84.0	
23	**	5	15761.54	1.98	0.65	35.48	84.0	
24	**	6	15638.46	1.98	0.65	36.69	83.0	
25	**	7	18510.91	1.98	0.55	37.87	86.0	
26	**	8	11655.55	1.95	0.90	39.38	80.0	
27	**	9	11031.58	1.95	0.95	40.90	78.0	
28	**	10	13272.86	1.93	0.70	42.17	81.0	
29	**	11	12711.25	1.97	0.80	43.54	75.0	
30	**	12	13858.67	1.96	0.75	44.91	75.0	

ABOVE DATA ARE IN THE TABLE (Cell suspension - spreadsheet)

Cell count 2nd

Cell count 2nd

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 TUE 08 MAY 2001 09:10
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N R5232:N
 H#: 1 AEC:N BCF:N RCM:N
 CHANNEL 1-LL: 0 UL: 400 ZSIGMA: 2.00 BKG SUB: 0.00 BKG ZSIG: 0.00 LSR: 0
 DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:0 1.00000
 HALF LIFE(DAYS):N

SAM	POS	CH	CPM	ZSIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	3.00	115.5	1.00	1.42	86.0	
2	**	2	5.00	89.44	1.00	3.00	81.0	
3	**	3	11.00	60.30	1.00	4.57	79.0	
4	**	4	7.00	75.59	1.00	6.14	77.0	
5	**	5	11.00	60.30	1.00	7.77	81.0	
6	**	6	18.00	47.14	1.00	9.33	75.0	
7	**	7	2476.00	4.02	1.00	10.96	78.0	
8	**	8	2140.00	4.32	1.00	12.53	75.0	
9	**	9	1352.00	5.44	1.00	14.16	75.0	
10	**	10	2437.00	4.05	1.00	15.73	77.0	
11	**	11	2971.00	3.67	1.00	17.30	77.0	
12	**	12	2692.00	3.85	1.00	18.92	77.0	
13	**	13	3971.00	3.17	1.00	20.49	80.0	
14	**	14	2957.00	3.68	1.00	22.07	75.0	
15	**	15	4233.00	3.07	1.00	23.64	78.0	
16	**	16	4449.00	3.00	1.00	25.21	79.0	
17	**	17	3906.00	3.20	1.00	26.77	77.0	
18	**	18	4528.00	2.97	1.00	28.36	78.0	
19	**	1	9452.00	2.06	1.00	29.97	86.0	
20	**	2	10550.53	2.00	0.95	31.56	84.0	
21	**	3	8974.00	2.12	1.00	33.13	83.0	
22	**	4	12129.41	1.97	0.85	34.54	84.0	
23	**	5	13510.67	1.99	0.75	35.90	84.0	
24	**	6	13151.25	1.95	0.80	37.27	82.0	
25	**	7	15584.61	1.99	0.65	38.53	86.0	
26	**	8	10376.00	1.96	1.00	40.10	79.0	
27	**	9	10118.00	1.99	1.00	41.67	78.0	
28	**	10	13584.00	1.98	0.75	42.97	81.0	
29	**	11	10576.84	2.00	0.95	44.49	75.0	
30	**	12	12405.88	1.95	0.85	45.91	74.0	
31	**	13	30082.86	1.95	0.35	46.87	2.0	

Standard
 H3
 CPM=98200

Cell count - 3rd

Cell count 3rd

PAGE: 1

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 WED 07 MAY 2001 13:15
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
 H#: 1 AGC:N DCF: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: 0.100000
 HALF LIFE(DAYS):N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	7.00	75.59	1.00	1.42	86.0	
2	**	2	8.00	70.71	1.00	3.00	82.0	1
3	**	3	6.00	81.65	1.00	4.62	79.0	
4	**	4	1.00	200.0	1.00	6.23	77.0	
5	**	5	9.00	66.67	1.00	7.81	81.0	2
6	**	6	6.00	81.65	1.00	9.38	76.0	
7	**	7	2336.00	4.14	1.00	10.96	78.0	
8	**	8	2107.00	4.36	1.00	12.58	74.0	3
9	**	9	1608.00	4.99	1.00	14.20	75.0	
10	**	10	2801.00	3.78	1.00	15.83	77.0	
11	**	11	3071.00	3.61	1.00	17.39	77.0	4
12	**	12	2915.00	3.70	1.00	18.95	79.0	
13	**	13	3946.00	3.16	1.00	20.52	80.0	
14	**	14	3427.00	3.42	1.00	22.09	74.0	5
15	**	15	4249.00	3.07	1.00	23.67	77.0	
16	**	16	4626.00	2.94	1.00	25.24	79.0	
17	**	17	4490.00	2.98	1.00	26.80	77.0	6
18	**	18	4843.00	2.87	1.00	28.37	78.0	
19	**	1	8709.00	2.14	1.00	30.00	84.0	
20	**	2	9789.00	2.02	1.00	31.57	84.0	7
21	**	3	8282.00	2.20	1.00	33.15	83.0	
22	**	4	11174.44	1.99	0.90	34.62	84.0	
23	**	5	12597.50	1.99	0.80	36.04	83.0	8
24	**	6	11784.71	2.00	0.85	37.46	82.0	
25	**	7	14464.29	1.99	0.70	38.72	85.0	
26	**	8	10190.00	1.98	1.00	40.29	79.0	9
27	**	9	10148.00	1.99	1.00	41.86	79.0	
28	**	10	13646.67	1.98	0.75	43.22	81.0	
29	**	11	11627.78	1.96	0.90	44.73	78.0	10
30	**	12	12940.00	1.97	0.80	46.11	76.0	
32	**	14	29554.29	1.97	0.35	47.07	2.0	

Standard H3
 DPM-98200

Parameters

Date	5/3/01
Experiment No.	3-May-01
Investigator	M. Lenarczyk
Cell Line	V79
Modifier	None
Radionuclide	H-3
Half-life (days)	4500.45
Radiation Yield	1
Radiochemical	H-3 thymidine
Manufacturer/Lot	NEN/3106-421
Original Calibration Date/Time	4/16/01 12:00
Present Calibration Date/Time	5/3/01 20:15
Fraction of Cells Labeled	1
Liquid Scintillation Cocktail	EcoLume
Volume of LSC Cocktail (ml)	6
Volume/Type Counting Vial	7
Model of Counter	Beckman LS5000TD
Counting Efficiency	0.65
Activity Added (Date/Time)	5/3/01 20:15
Cells Washed (Date/Time)	5/4/01 10:10
Medium Tubes Counted (Date/Time)	5/4/01 16:00
Cell Tubes Counted (Date/Time)	5/7/01 17:30
Vol. Supernatant Counted (µl)	30
Vol. Suspension Counted Cell Activity (µl)	100
Vol. Suspension Coultur (µl)	100
Coultur Manometer Volume (µl)	500
Average Coultur Background Counts	8
Coultur Calibration Parameter	400
Hemocytometer Counting (Yes or No)?	

I-125=59.408, H-3=4500.45, Po-210=138.376, I-131=8.02
 I-125=1.47, H-3=1.0, Po-210=1.0, I-131=8.02

Original Activity Concentration (MBq/ml) 37
 Time Elapsed Since Original Calibration (d) 17
 Present Activity Concentration (MBq/ml) 36.90

Time Elapsed Between Add and Wash (hr) 14.00
 Time Elapsed Between Add and Count (hr) 19.75
 Time Elapsed Between Wash and Count (hr) 97.00

Background
 Coultur 1 8 Coultur 2 8 Coultur 3 8

MediumActivity

Experiment: 37014
Date: 5/3/2001

Tube #	1st	2nd	3rd	CPM Average	CPM corrected for control	DPM CPM/(y e)	At $\mu\text{Ci/ml on counting}$	Ao $\mu\text{Ci/ml at addition [At/e-0.6931/T]}$	Ao $\text{kBq/ml at addition}$
1	5	9	11	10	0	0	0	0	0
2	13	10	9		0	0	0	0	0
3	19516	18031	20814	19454	19444	29914	0.4492	0.4492	16.6211
4	27027	27402	28791	27740	27731	42662	0.6406	0.6407	23.7043
5	31642	34666	37136	34481	34472	53034	0.7963	0.7964	29.4668
6	45684	53830	47784	49099	49090	75523	1.1340	1.1341	41.9624
7	53825	47648	60560	54011	54002	83079	1.2474	1.2476	46.1610
8	83953	83053	72093	79700	79690	122600	1.8408	1.8411	68.1199
9	99273	112750	110706	107576	107567	165487	2.4848	2.4851	91.9491
10	111130	117413	135713	121419	121409	186783	2.8046	2.8049	103.7817

CellSuspension

Experiment: 37014
Date: 05/03/01

Tube #	Suspension count (CPM)			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
	1st	2nd	3rd						
1	6	9	10	9	0	0	0.00000	0	0.0000
2	7	13	10		0	0	0.00000	0	0.0000
3	2917	2641		2779	2770	4261	0.01919	0.01921	0.7107
4	2969	3048	3103	3040	3031	4663	0.02100	0.02102	0.7776
5	4278	3442	4643	4121	4112	6326	0.02850	0.02851	1.0550
6	5077	4441	5056	4858	4849	7460	0.03360	0.03362	1.2441
7	11143	12341	10489	11324	11315	17408	0.07841	0.07846	2.9031
8	14350	15761	15638	15250	15241	23447	0.10562	0.10568	3.9103
9	18510	11655	11031	13732	13723	21112	0.09510	0.09516	3.5209
10	15272	12911	13858	14014	14005	21545	0.09705	0.09711	3.5931

CoulterSurvival

Experiment: 37014
 Date/Time: 5/3/01

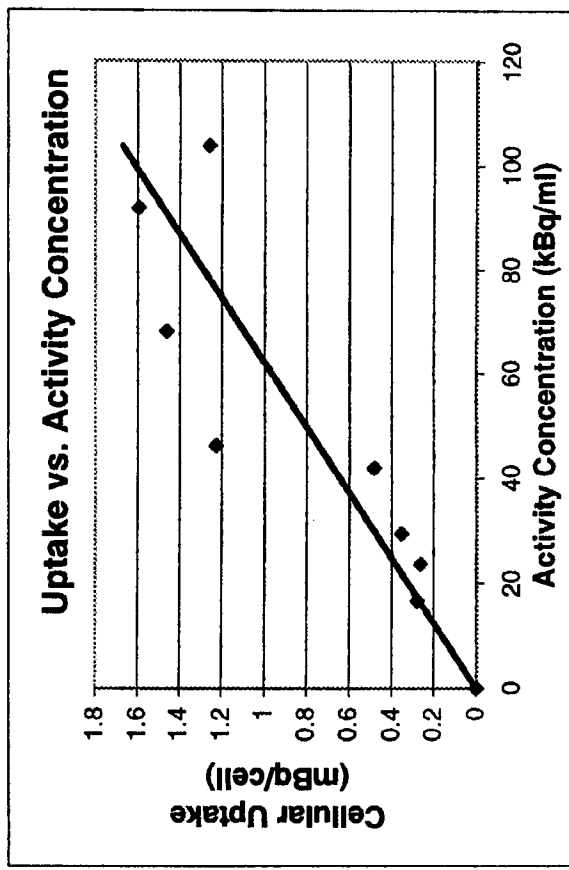
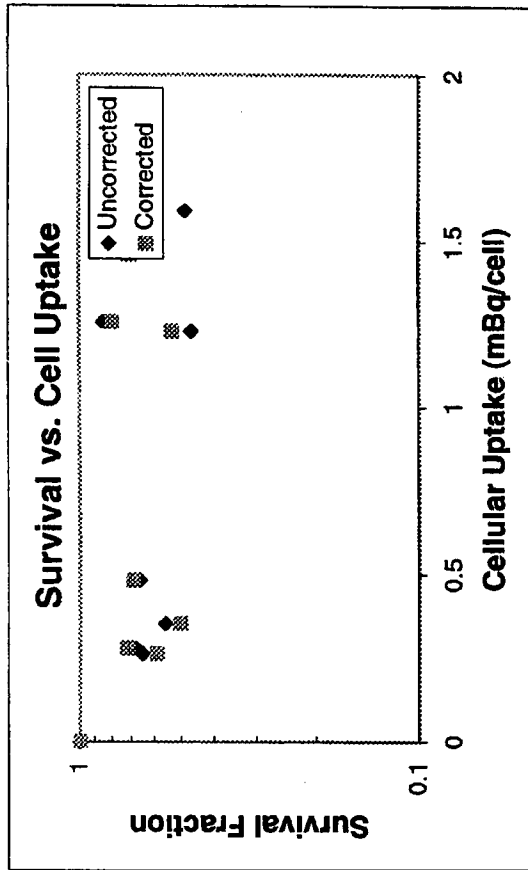
Tube #	Coulter count			Average Cells/ml	Hemocytometer Count in Grid				
	1st	2nd	3rd		1st	2nd	3rd	4th	
1	6479	6522	6564	6522	2605467				
2	7117	6864	6859	6947	2775467				
3	6336	6410	6373	6373	2546000				
4	7404	7377	7432	7404	2958533				
5	7595	7546	7263	7468	2984000				
6	6399	6607	6325	6444	2574267				
7	5950	6039	5761	5917	2363467				
8	6655	6821	6607	6694	2674533				
9	5587	5416	5598	5534	2210267				
10	7052	7258	7144	7151	2857333				

Tube #	Predicted # Cells Seeded	Actual # Cells Seeded	Colony count			Average	PE (%)	SF Uncorrected	SF Corrected
			1st	2nd	3rd				
1	200	261	80	78	95	85	31.531	1.00	1.0000
2	200	278	87	86	83				
3	200	255	59	62	53	58	22.781	0.6837	0.7225
4	200	296	61	52	52	55	18.590	0.6483	0.5896
5	200	298	51	47	44	47	15.862	0.5580	0.5031
6	200	257	64	54	51	56	21.883	0.6640	0.6940
7	200	236	41	33	45	40	16.783	0.4676	0.5323
8	200	267	59	59	61	60	22.309	0.7033	0.7075
9	200	221	42	48	33	41	18.550	0.4833	0.5883
10	200	286	69	76	71	72	25.198	0.8487	0.7992

Summary

Experiment: 5/3/01
 Date/Time:

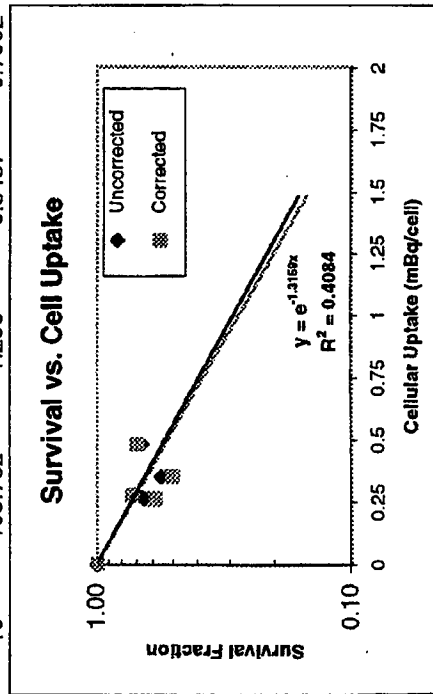
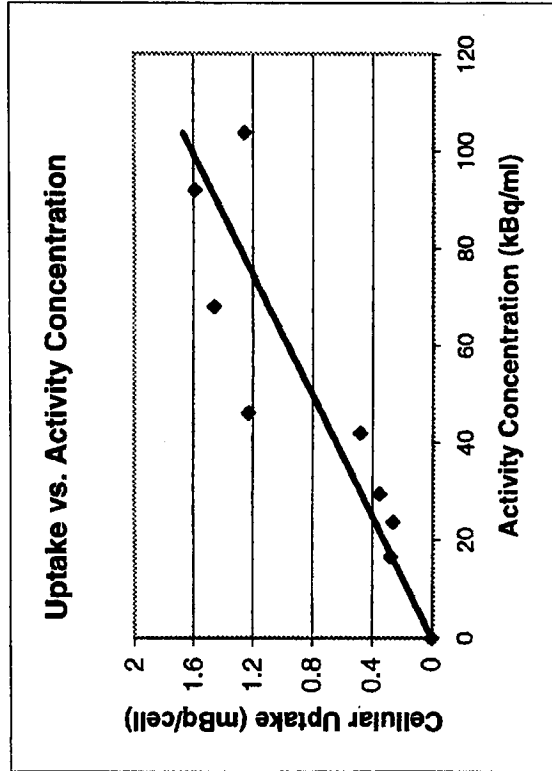
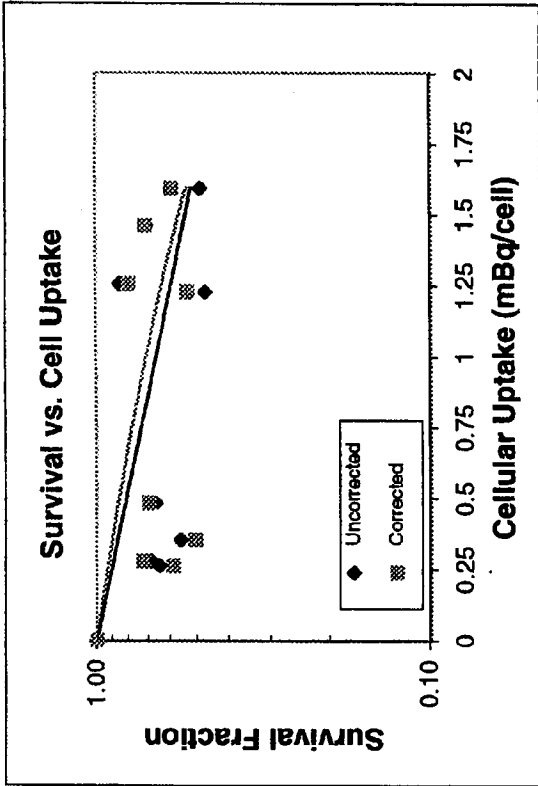
Tube #	Activity Conc. (kBq/ml)	Activity/Cell (mBq/cell)	Survival Uncorrected	Survival Corrected
1	0.000	0.000	1.0000	1.0000
2	0.000	0.000		
3	16.621	0.279	0.6837	0.7225
4	23.704	0.263	0.6483	0.5896
5	29.467	0.354	0.5580	0.5031
6	41.962	0.483	0.6640	0.6940
7	46.161	1.228	0.4676	0.5323
8	68.120	1.462	0.7033	0.7075
9	91.949	1.593	0.4833	0.5883
10	103.782	1.258	0.8487	0.7992



Experiment:
Date/Time: 5/3/01

Tube # Activity Conc. (kBq/ml) Activity/Cell (mBq/cell) Survival Uncorrected Survival Corrected

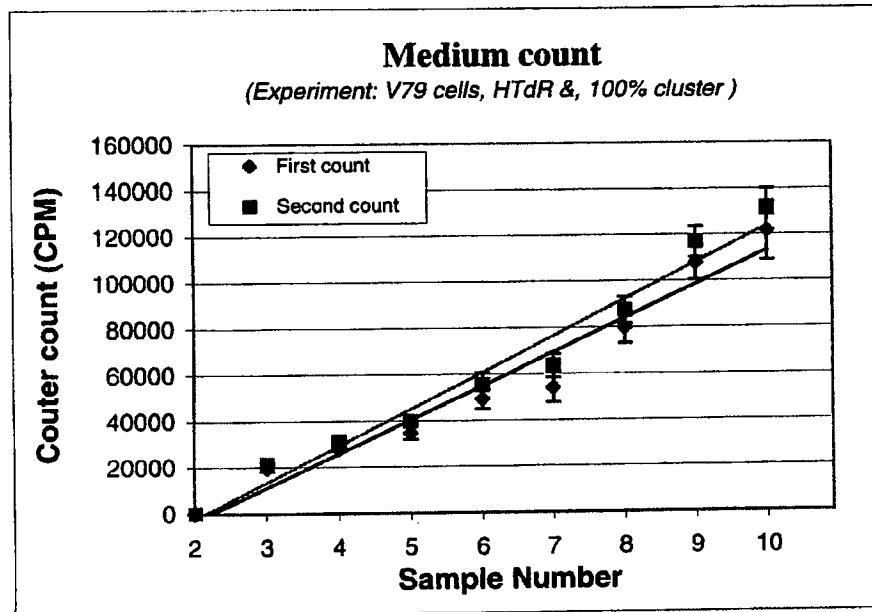
1	0.000	0.000	1.0000	1.0000
2	0.000	0.000	0.6837	0.7225
3	16.621	0.279	0.6483	0.5896
4	23.704	0.263	0.5580	0.5031
5	29.467	0.354	0.6640	0.6940
6	41.962	0.483	0.4676	0.5323
7	46.161	1.228	0.7033	0.7075
8	68.120	1.462	0.4833	0.5883
9	91.949	1.593	0.8487	0.7992
10	103.782	1.258		



Medium Activity

Activity conc. (kBq/ml)	Sample	Coulter count (CPM)					
		1st	2nd	3rd	Mean	Std Dev.	Std Dev. (%)
0	1	5	9	11			
0	2	13	10	9	10	2.7	28
16.62105	3	19516	18031	20814	19454	1392.5	7
23.70429	4	27027	27402	28791	27740	929.3	3
29.46684	5	31642	34666	37136	34481	2751.7	8
41.96244	6	45684	53830	47784	49099	4229.3	9
46.16098	7	53825	47648	60560	54011	6458.0	12
68.11988	8	83953	83053	72093	79700	6602.9	8
91.94912	9	99273	112750	110706	107576	7263.2	7
103.7817	10	111130	117413	135713	121419	12771.6	11

Activity conc. (kBq/ml)	Sample	Coulter count (CPM)					
		1st	2nd	3rd	Mean	Std Dev.	Std Dev. (%)
0.000	1	7	14	10			
0.000	2	9	7	10	10	2.6	27
16.621	3	21080	21150	21824	21351	410.8	2
23.704	4	29814	31682	32317	31271	1301.1	4
29.467	5	37050	39913	41980	39648	2475.7	6
41.962	6	50450	61230	53895	55192	5505.7	10
46.161	7	62655	58785	69060	63500	5189.4	8
68.120	8	89513	91533	80680	87242	5771.9	7
91.949	9	111973	113726	124366	116688	6706.6	6
103.782	10	120866	133246	138546	130886	9073.2	7



Cell suspension activity

Sample	Activity conc. (in medium) (kBq/ml)	Coulter count (CPM)			Mean	Std Dev.	Std Dev. (%)	
		1st	2nd	3rd				
1	0.000	6	9	10				
2	0.000	7	13	10	9	2.5	27	
3	16.621	2917	2641		2779	195.2	7	1393
4	23.704	2969	3048	3103	3040	67.4	2	
5	29.467	4278	3442	4643	4121	615.7	15	
6	41.962	5077	4441	5056	4858	361.3	7	
7	46.161	11143	12341	10489	11324	939.2	8	
8	68.120	14350	15761	15638	15250	781.6	5	
9	91.949	18510	11655	11031	13732	4149.6	30	
10	103.782	15272	12911	13858	14014	1188.2	8	

Sample	Activity conc. (in medium) (kBq/ml)	Second count			Mean	Std Dev.	Std Dev. (%)
		1st	2nd	3rd			
1	0	3	5	11			
2	0	7	11	18	9	5.4	59
3	16.6210519	2476	2140	1352	1989	576.9	29
4	23.7042856	2437	2971	2692	2700	267.1	10
5	29.4668391	3971	2957	4233	3720	673.9	18
6	41.9624395	4449	3906	4528	4294	338.6	8
7	46.1609772	9452	10550	8874	9625	851.3	9
8	68.1198849	12129	13510	13151	12930	716.5	6
9	91.9491151	15584	10376	10118	12026	3084.0	26
10	103.781669	13584	10576	12405	12188	1515.7	12

