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### COLONY FORMING & HPRT ASSAY FOR V79 CELLS

**Experiment Name :** 100 % cluster, 3HTdR);

**Exp.**

**Investigator:** M. Lenarczyk

**Date:** June 21, 2001

#### *Day 1st*

1. Set the rocker-roller at 37°C incubator with 5% CO<sub>2</sub>, set the Coulter Counter, wash cells (from two 150 cm<sup>2</sup> flasks, sub-cultured 15x10(6) cells/T175 flask 24h before; harvested - flask # 1 app 30.6 x10(6) cells, flask # 2 app. 28.5 X 10(6) cells) with PBS, trypsinize cells, each re-suspend in 15 ml MEMB, spin them down (2K rpm, 5", 4oC), re-suspend cells in 15 ml MEMB, pass 5x through 10 cc syringe with 21 gauge needle, count the cells using Couter counter (100 µl cell suspension / 20 ml Isotone
  2. Dilute to ~4,000,000 cells/ml in MEMB [Actual count : 3 952 800 cells/ml)  
coulter count [( 9962+9911+9812) - 13] x 400
  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% CO<sub>2</sub>
- Date/Time:** June 21, 2001/  
**16:15**

5. Prepare MEMB containing radioactivity in hood

64  $\mu$ l  $^3$ HTdR (Stock :  $\mu$ Ci/ $\mu$ l on May 30, 2001) + 3935  $\mu$ l MEMB  
Lot # 3106427

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

**Date/Time: June 21, 2001 / 19:30**

Tube #	$^3$ HTdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ $^3$ HTdR (ml) [16 uCi/ml]
1	0	1.0	1.0	0
2	0	1.0	1.0	0
3	1	1.0	0.875	0.125
4	2.0	1.0	0.750	0.250
5	3.0	1.0	0.685	0.315
6	4.0	1.0	0.500	0.500
7	5.0	1.0	0.375	0.625
8	6.0	1.0	0.150	0.750
9	7.0	1.0	0.125	0.875
10	8.0	1.0	0	1

7. Return test tubes to roller for 12 h

**Date/Time: June 21, 2001 / 19:45**

**Day 2nd**

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 22, 2001 / 9:45 (3.5 hrs)**

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 standard culture MEMA
16. Centrifuge tubes for 10 min at 2000 rpm, 4°C
17. Decant supernatant, click tubes, vortex, and using 200 µl tips transfer the cells in polypropylene micro-centrifuge tubes (Helena tube, 400 µl)
18. Again add 200 µl ice cold standard culture MEMA, pipet 1-2 times @ transfer the remaining cell in the same polypropylene micro-centrifuge tubes (Total volume ~400 ul)
19. Centrifuge tubes for 5 min at 1000 rpm, 4°C
20. Transfer Hellena tubes at 10.5°C for 72 h

**Date/Time: June 22, 2001 / 11:30**

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

transferred on: **Date/Time: June 22, 2001/ 12:00**

counted on: **Date/time:**

**Day 5th**

22. After 72 h, carefully remove the supernatant from the top, vortex pellet and transfer the cells suspension 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: June 25, 2001 /**

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

HL

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2. e

100% MIDER

1 of course.

USER: 6 ID:H3 HOWELL      PRESET TIME: 1.00      MON 25 JUN 2001 17:48  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N      RS232:N  
 H#: 1 AGC:N BCF:N RCM:N  
 CHANNEL 1-LL: 0 UL: 400 ZSIBMA: 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	PDS	CH	CPM	ZSIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	12.00	57.74	1.00	1.48	83.0	
2	**	2	12.00	57.74	1.00	3.05	85.0	
3	**	3	8.00	70.71	1.00	4.62	85.0	
4	**	4	-16.00	50.00	1.00	6.24	83.0	
5	**	5	13.00	55.47	1.00	7.81	85.0	
6	**	6	10.00	63.25	1.00	9.43	85.0	
7	**	7	25302.50	1.99	0.40	10.38	84.0	
8	**	8	26307.50	1.95	0.40	11.34	84.0	
9	**	9	25827.50	1.97	0.40	12.29	85.0	
10	**	10	56520.00	1.88	0.20	13.04	85.0	
11	**	11	57720.00	1.86	0.20	13.84	86.0	
12	**	12	55025.00	1.91	0.20	14.60	84.0	
13	**	13	63620.00	1.77	0.20	15.41	85.0	
14	**	14	71260.00	1.93	0.15	16.11	80.0	
15	**	15	75320.00	1.88	0.15	16.81	80.0	
16	**	16	107693.33	1.57	0.15	17.51	80.0	
17	**	17	116799.99	1.51	0.15	18.21	82.0	
18	**	18	107426.66	1.58	0.15	18.91	81.0	
19	29	1	131840.00	1.42	0.15	19.67	84.0	
20	29	2	122099.99	1.48	0.15	20.38	82.0	
21	29	3	112713.33	1.54	0.15	21.08	81.0	
22	29	4	160500.00	1.29	0.15	21.79	87.0	
23	29	5	154653.33	1.31	0.15	22.50	84.0	
24	29	6	157540.00	1.59	0.10	23.21	84.0	
25	29	7	172913.33	1.24	0.15	23.92	85.0	
26	29	8	188666.66	1.19	0.15	24.63	86.0	
27	29	9	210719.98	1.12	0.15	25.33	85.0	
28	29	10	204693.33	1.14	0.15	26.04	85.0	
29	29	11	194426.66	1.17	0.15	26.75	82.0	

Medium count

100µl

USER: 6 ID:H3 HOWELL      PRESET TIME: 1.00      MON 25 JUN 2001 18:15  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N      RS232:N  
 H#: 1 AQC:N QCF: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	13.00	55.47	1.00	1.42	90.0	
2	**	2	13.00	55.47	1.00	2.98	88.0	
3	**	3	<del>13.00</del>	57.74	1.00	4.55	86.0	
4	**	4	11.00	60.30	1.00	6.18	86.0	
5	**	5	8.00	70.71	1.00	7.74	88.0	
6	**	6	<del>16.00</del>	50.00	1.00	9.37	89.0	
7	**	7	665.00	7.76	1.00	10.94	85.0	
8	**	8	908.00	6.64	1.00	12.51	90.0	
9	**	9	<del>1082.00</del>	6.08	1.00	14.08	90.0	
10	**	10	1847.00	4.65	1.00	15.64	91.0	
11	**	11	1277.00	5.60	1.00	17.27	90.0	
12	**	12	<del>4219.00</del>	3.08	1.00	18.82	92.0	
13	**	13	2163.00	4.30	1.00	20.39	89.0	
14	**	14	3312.00	3.48	1.00	21.97	92.0	
15	**	15	<del>2431.00</del>	4.06	1.00	23.54	90.0	
16	**	16	3679.00	3.30	1.00	25.16	90.0	
17	**	17	2605.00	3.92	1.00	26.72	87.0	
18	**	18	10553.00	1.95	1.00	28.30	96.0	
19	**	1	10244.00	1.98	1.00	29.92	91.0	
20	**	2	9923.00	2.01	1.00	31.49	92.0	
21	**	3	5119.00	2.80	1.00	33.06	89.0	
22	**	4	10375.00	1.96	1.00	34.68	92.0	
23	**	5	12908.75	1.97	0.80	36.05	97.0	
24	**	6	10940.00	1.96	0.95	37.62	94.0	
25	**	7	13732.00	1.97	0.75	38.94	90.0	
26	**	8	8974.00	2.11	1.00	40.57	92.0	
27	**	9	7422.00	2.32	1.00	42.19	91.0	
28	**	10	13837.33	1.96	0.75	43.51	95.0	
29	**	11	<del>4521.00</del>	2.48	1.00	45.13	87.0	
30	**	12	17261.67	1.97	0.60	46.35	94.0	
31	**	13	<u>29731.43</u>	1.96	0.35	47.31	0.0	

Standard  
H3

Cell count (15')

Medicum count

2nd count  
100%, HTR, U79

USER: 6 ID:H3 HOWELL      PRESET TIME: 1.00      TUE 26 JUN 2001 08:56  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N      RS232:N  
 H#: 1 AOC:N GCF: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

30 sec

SAM	POS	CH	CPM	ZSIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	15.00	51.64	1.00	1.47	84.0	
2	**	2	12.00	57.74	1.00	3.10	84.0	
3	**	3	16.00	50.00	1.00	4.68	84.0	
4	**	4	12.00	57.74	1.00	6.29	83.0	
5	**	5	10.00	63.25	1.00	7.85	84.0	
6	**	6	15.00	51.64	1.00	9.47	84.0	
7	**	7	25197.50	1.99	0.40	10.42	83.0	
8	**	8	26107.50	1.96	0.40	11.43	83.0	
9	**	9	25730.00	1.97	0.40	12.38	84.0	
10	**	10	56365.00	1.88	0.20	13.18	84.0	
11	**	11	57645.00	1.86	0.20	13.98	85.0	
12	**	12	54430.00	1.92	0.20	14.78	84.0	
13	**	13	66280.00	1.74	0.20	15.54	85.0	
14	**	14	70886.66	1.74	0.15	16.24	80.0	
15	**	15	75413.33	1.88	0.15	16.94	81.0	
16	**	16	109086.66	1.56	0.15	17.64	80.0	
17	**	17	116219.99	1.51	0.15	18.35	82.0	
18	**	18	107719.99	1.57	0.15	19.06	80.0	
19	29	1	129510.00	1.76	0.10	19.82	85.0	
20	29	2	120706.66	1.49	0.15	20.52	81.0	
21	29	3	114100.00	1.87	0.10	21.23	82.0	
22	29	4	161790.00	1.57	0.10	21.94	86.0	
23	29	5	157180.00	1.60	0.10	22.65	83.0	
24	29	6	157300.00	1.30	0.15	23.36	85.0	
25	29	7	173730.00	1.52	0.10	24.07	84.0	
26	29	8	189450.00	1.45	0.10	24.78	85.0	
27	29	9	212826.66	1.12	0.15	25.48	85.0	
28	29	10	206853.33	1.14	0.15	26.20	84.0	
29	29	11	195930.00	1.43	0.10	26.91	83.0	

(cell count (2nd)) 100%, ATOR, V79

USER: S ID:H3 HOWELL PRESET TIME: 1.00 TUE 26 JUN 2001 09:24  
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N R5232:N  
H#: 1 AQC:N GCF:N RCM:N  
CHANNEL I-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LAR: 0  
DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR: 1.00000  
HALF LIFE (DAYS): N

*100 µl*

SAM	POS	CH	CPM	2SIGZ	TIME	EL TIME	AVG H#	EHR
1	**	1	10.00	63.25	1.00	1.42	90.0	
2	**	2	18.00	47.14	1.00	3.00	87.0*	
3	**	3	13.00	55.47	1.00	4.62	87.0	
4	**	4	11.00	60.30	1.00	6.19	87.0	
5	**	5	11.00	60.30	1.00	7.76	89.0	
6	**	6	15.00	51.64	1.00	9.33	87.0	
7	**	7	831.00	6.94	1.00	10.89	86.0	
8	**	8	1304.00	5.54	1.00	12.45	89.0	
9	**	9	1454.00	5.25	1.00	14.01	90.0	
10	**	10	2546.00	3.96	1.00	15.57	90.0	
11	**	11	2218.00	4.25	1.00	17.19	91.0	
12	**	12	3306.00	3.48	1.00	18.75	91.0	
13	**	13	3097.00	3.59	1.00	20.32	90.0	
14	**	14	3894.00	3.21	1.00	21.94	92.0	
15	**	15	3133.00	3.57	1.00	23.57	89.0	
16	**	16	4604.00	2.95	1.00	25.13	90.0	
17	**	17	3612.00	3.33	1.00	26.70	88.0	
18	**	18	8886.00	2.12	1.00	28.28	95.0	
19	**	1	8236.00	2.20	1.00	29.90	91.0	
20	**	2	8051.00	2.23	1.00	31.47	92.0	
21	**	3	5440.00	2.71	1.00	33.04	88.0	
22	**	4	8920.00	2.12	1.00	34.60	92.0	
23	**	5	11633.33	1.95	0.90	36.07	97.0	
24	**	6	9710.00	2.03	1.00	37.63	93.0	
25	**	7	10669.47	1.99	0.95	39.17	90.0	
26	**	8	8706.00	2.14	1.00	40.72	92.0	
27	**	9	8046.00	2.23	1.00	42.30	91.0	
28	**	10	11911.76	1.99	0.85	43.71	94.0	
29	**	11	7029.00	2.39	1.00	45.27	87.0	
30	**	12	13837.33	1.96	0.75	46.59	94.0	
31	**	13	29548.57	1.97	0.35	47.55	0.0	

Medium count (3rd)

V79, 100%, HTR

PAGE: 1

USER: 6 ID:H3 HOWELL      PRESET TIME: 1.00      WED 27 JUN 2001 13:05  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N      RS232:N  
 H#: 1 ADC:N DCF:N RCM:N  
 CHANNEL I-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

too 30µl

SAM	POS	CH	CPM	ZSIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	9.00	66.67	1.00	1.47	84.0	
2	**	2	7.00	75.59	1.00	3.04	86.0	
3	**	3	7.00	75.59	1.00	4.66	85.0	
4	**	4	10.00	63.25	1.00	6.28	85.0	
5	**	5	10.00	63.25	1.00	7.85	86.0	
6	**	6	8.00	70.71	1.00	9.48	86.0	
7	**	7	25617.50	1.98	0.40	10.48	86.0	
8	**	8	27330.00	1.91	0.40	11.43	85.0	
9	**	9	26747.50	1.93	0.40	12.44	86.0	
10	**	10	57315.00	1.87	0.20	13.24	86.0	
11	**	11	39975.00	1.83	0.20	14.05	86.0	
12	**	12	56055.00	1.89	0.20	14.81	86.0	
13	**	13	65970.00	1.74	0.20	15.56	86.0	
14	**	14	73366.66	1.91	0.15	16.26	81.0	
15	**	15	77960.00	1.85	0.15	16.96	82.0	
16	**	16	110206.66	1.56	0.15	17.66	82.0	
17	**	17	118459.99	1.50	0.15	18.38	83.0	
18	**	18	109739.99	1.56	0.15	19.08	82.0	
19	28	1	132220.00	1.42	0.15	19.83	86.0	
20	28	2	123766.66	1.47	0.15	20.54	83.0	
21	28	3	115486.66	1.52	0.15	21.25	84.0	
22	28	4	160953.33	1.29	0.15	21.96	88.0	
23	28	5	161346.66	1.29	0.15	22.67	85.0	
24	28	6	160040.00	1.58	0.10	23.38	87.0	
25	28	7	178386.66	1.22	0.15	24.08	86.0	
26	28	8	190120.00	1.18	0.15	24.79	86.0	
27	28	9	213459.98	1.12	0.15	25.50	86.0	
28	28	10	205079.98	1.14	0.15	26.22	86.0	
29	28	11	204680.00	1.40	0.10	26.94	84.0	



(cell count (3rd) , v79, 100%, HTR)

USER: 6 ID:H3.HOWELL PRESET TIME: 1.00 WED 27 JUN 2001 13:33  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N  
 H#: 1 AGC:N GCF: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

100 µl

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	4.00	100.0	1.00	1.42	90.0	
2	**	2	7.00	75.59	1.00	3.00	90.0	
3	**	3	6.00	81.65	1.00	4.57	90.0	
4	**	4	11.00	60.30	1.00	6.18	90.0	
5	**	5	10.00	63.25	1.00	7.75	91.0	
6	**	6	5.00	89.44	1.00	9.33	91.0	
7	**	7	1126.00	5.96	1.00	10.89	88.0	
8	**	8	1505.00	5.16	1.00	12.46	92.0	
9	**	9	1569.00	5.05	1.00	14.03	93.0	
10	**	10	2856.00	3.74	1.00	15.66	94.0	
11	**	11	2466.00	4.03	1.00	17.22	92.0	
12	**	12	3151.00	3.56	1.00	18.77	93.0	
13	**	13	3512.00	3.37	1.00	20.35	92.0	
14	**	14	4075.00	3.13	1.00	21.92	93.0	
15	**	15	3555.00	3.35	1.00	23.50	93.0	
16	**	16	5223.00	2.77	1.00	25.07	92.0	
17	**	17	4293.00	3.05	1.00	26.64	89.0	
18	**	18	8576.00	2.16	1.00	28.22	96.0	
19	**	1	7801.00	2.26	1.00	29.83	91.0	
20	**	2	7887.00	2.25	1.00	31.42	93.0	
21	**	3	6578.00	2.47	1.00	32.99	90.0	
22	**	4	8899.00	2.12	1.00	34.56	93.0	
23	**	5	11186.67	1.99	0.90	36.02	95.0	
24	**	6	9503.00	2.05	1.00	37.60	94.0	
25	**	7	9751.00	2.03	1.00	39.17	92.0	
26	**	8	8901.00	2.12	1.00	40.75	92.0	
27	**	9	8637.00	2.15	1.00	42.31	92.0	
28	**	10	11508.89	1.97	0.90	43.78	95.0	
29	**	11	8112.00	2.22	1.00	45.34	89.0	
30	**	12	12872.50	1.97	0.80	46.77	95.0	
31	**	13	29077.14	1.98	0.35	47.73	1.0	

Medium count (4th)

V.79, 100%, H1012

NEW COUNTER!!!

ID# 1133    INCHWELL    COMMENT: *30µl*  
 USER: 3  
 PRESET TIME : 1.00  
 DATA CALC : CPM    HW    YES    SAMPLE REPEATS: 1    PRINTER : STD  
 COUNT BLANK : NO    IC#    NO    REPLICATES : 1    RS232 : OFF  
 TWO PHASE : NO    ACC    NO    CYCLE REPEATS : 1  
 SCINTILLATOR: LIQUID    LUMEX: NO    LOW SAMPLE REJ: 0  
 LOW LEVEL : NO    HALF LIFE CORRECTION DATE: none

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

SAM NO	POS	TIME MIN	H#	CPM	3H %ERROR	LUMEX %	ELAPSED TIME
1	**1	1.00	83.4	11.00	40.30	1.01	1.52
2	**2	1.00	83.8	12.00	57.74	1.44	3.01
3	**3	1.00	81.3	14.00	37.45	0.85	4.62
4	**4	1.00	85.9	4.00	100.00	1.41	6.20
5	**5	1.00	84.1	14.00	53.45	0.58	7.84
6	**6	1.00	84.9	12.00	57.74	0.68	9.44
7	**7	1.00	87.6	26770.03	1.20	0.00	11.07
8	**8	1.00	83.3	27735.04	1.20	0.00	12.68
9	**9	1.00	83.7	27740.04	1.20	0.00	14.30
10	**10	1.00	84.9	60303.10	0.81	0.00	15.82
11	**11	1.00	84.1	62347.11	0.80	0.00	17.48
12	**12	1.00	83.4	58955.12	0.82	0.00	19.12
13	**13	1.00	83.2	69546.15	0.76	0.00	20.77
14	**14	1.00	78.5	75417.18	0.73	0.00	22.41
15	**15	1.00	79.1	80907.20	0.70	0.00	24.08
16	**16	1.00	79.0	115866.3	0.59	0.00	25.74
17	**17	1.00	79.7	123533.4	0.57	0.00	27.32
18	**18	1.00	79.9	114366.3	0.59	0.00	28.98
19	**1	1.00	84.2	138978.5	0.54	0.00	30.66
20	**2	1.00	83.4	130877.4	0.55	0.00	32.32
21	**3	1.00	82.2	120996.4	0.57	0.00	34.01
22	**4	1.00	85.0	170725.6	0.48	0.00	35.69
23	**5	1.00	83.9	169459.7	0.49	0.00	37.40
24	**6	1.00	84.4	168967.7	0.49	0.00	39.08
25	**7	1.00	84.8	187642.8	0.46	0.00	40.80
26	**8	1.00	85.0	199808.9	0.45	0.00	42.51
27	**9	1.00	84.2	222548.0	0.42	0.00	44.25
28	**10	1.00	84.9	214696.0	0.43	0.00	45.96
29	**11	1.00	84.1	214658.1	0.43	0.00	47.70

Cell count (4th)

NEW COUNTER!!!

2 JUL 2001 10:55

ID# 1133    INCHWELL    COMMENT: *100µl*  
 USER: 6  
 PRESET TIME : 1.00  
 DATA CALC : CPM    HW    YES    SAMPLE REPEATS: 1    PRINTER : STD  
 COUNT BLANK : NO    IC#    NO    REPLICATES : 1    RS232 : OFF  
 TWO PHASE : NO    ACC    NO    CYCLE REPEATS : 1

SAM NO	PDS	TIME MIN	H#	CFM	%H ERROR	LUMEX %	ELAPSED TIME
1	11	1.00	90.2	8.00	66.71	0.73	1.01
2	11-1	1.00	88.7 1c	7.00	75.52	0.57	2.12
3	11-2	1.00	89.8	7.00	73.53	0.53	3.27
4	11-4	1.00	88.6	11.00	66.70	0.54	4.33
5	11-5	1.00	90.0 2c	7.00	75.55	0.52	5.51
6	11-6	1.00	89.7	9.00	65.67	0.67	6.44
7	11-7	1.00	89.0	1473.00	5.21	0.01	11.04
8	11-8	1.00	90.1 3c	1873.00	4.62	0.01	12.67
9	11-9	1.00	90.7	1882.00	4.61	0.01	14.27
10	11-10	1.00	90.9	3258.01	2.50	0.01	15.89
11	11-11	1.00	89.8 4c	3012.01	3.54	0.01	17.50
12	11-12	1.00	90.4	3397.01	3.43	0.01	19.13
13	11-13	1.00	89.8	4245.01	3.07	0.01	20.72
14	11-14	1.00	90.3 5c	4484.01	3.59	0.01	22.36
15	11-15	1.00	89.8	4239.01	3.07	0.00	23.97
16	11-16	1.00	89.8	6089.02	2.56	0.00	25.59
17	11-17	1.00	87.0 6c	4937.01	3.05	0.01	27.21
18	11-18	1.00	87.0	8607.03	2.16	0.00	28.85
19	11-1	1.00	91.0	8684.03	2.15	0.00	30.55
20	11-2	1.00	90.9 7c	8491.03	3.17	0.00	32.17
21	11-3	1.00	90.8	7639.03	3.29	0.00	33.67
22	11-4	1.00	91.3	9891.04	2.01	0.00	35.31
23	11-5	1.00	91.0 8c	11904.05	1.83	0.00	36.92
24	11-6	1.00	92.8	10448.04	1.76	0.00	38.56
25	11-7	1.00	90.7	10565.04	1.74	0.00	40.35
26	11-8	1.00	91.3 9c	10388.05	1.76	0.00	41.70
27	11-9	1.00	89.9	9830.04	2.02	0.00	43.30
28	11-10	1.00	91.8	12864.06	1.76	0.00	44.82
29	11-11	1.00	88.5 10	10055.05	1.99	0.00	46.43
30	11-12	1.00	90.3	13865.07	1.70	0.00	48.07
31	11-13	1.00	0.6	61496.32	0.81	0.00	49.71
32	11-14	1.00	-1.1	30845.17	1.14	0.00	51.46

OLD H-2      NEW H-3

TABLE-3

v79, H1dr, 100%

June 25, 2001

Expt. #: ~~14, 2000~~

Date/Time:

2x10<sup>6</sup> cells

Background  
in Coulter

8  
12  
12  
2  
8  
2  
2  
8  
12  
8

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000] - Background	pCi/cell [uCi/ml x 10 <sup>6</sup> Cells/ml]
1	6003 20%		2 52000	0.85
2	5067, 5096, 4980		2 012 933	1.00
3	6240, 6373, 6307		2 526 133	2.77
4	5934, 5676, 5619		2 296 400	0.82
5	5904, 5828, 5876		2 319 200	0.81
6	5902, 5821, 5843		2 339 067	0.87
7	5432, 5617, 5667		2 224 733	0.90
8	5456, 5370, 5454		2 167 467	0.95
9	5410,		2 159 200	0.73
10	5637, 5539, 5477		2 219 867	0.31

Mode - 500ul

Plate for SF

- 1.2
- 2.2
- 3.1
- 1.2
- 1.1
- 2.2 5.7
- 2.2 4.1
- 2.2 2.7

Parameters

Date	6/21/2001
Experiment No.	21-Jun-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/3106-427
Original Calibration Date/Time	5/30/2001 12:00
Present Calibration Date/Time	6/21/2001 19:30
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)	6/21/2001 19:45
Cells Washed (Date/Time)	6/22/2001 11:00
Medium Tubes Counted (Date/Time)	5/25/2001 17:50
Cell Tubes Counted (Date/Time)	5/25/2001 18:15
Vol. Supernatant Counted (μl)	30
Vol. Suspension Counted Cell Activity (μl)	100
Vol. Suspension Coupler (μl)	100
Coupler Manometer Volume (μl)	500
Average Coupler Background Counts	8
Coupler 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) <span style="border: 1px solid black; padding: 2px;">37</span> Time Elapsed Since Original Calibration (d) <span style="border: 1px solid black; padding: 2px;">22</span> Present Activity Concentration (MBq/ml) 36.87
	Time Elapsed Between Add and Wash (hr) 15.25 Time Elapsed Between Add and Count (hr) 94.00 Time Elapsed Between Wash and Count (hr) 79.25
	Background Coupler 1 <span style="border: 1px solid black; padding: 2px;">8</span> Coupler 2 <span style="border: 1px solid black; padding: 2px;">8</span> Coupler 3 <span style="border: 1px solid black; padding: 2px;">8</span>

MediumActivity

Experiment: 37063  
Date: 6/21/2001

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	Ao $\text{kBq/ml}$ at addition
1	12	12	8	12	0	0	0	0	0
2	16	13	10	0	0	0	0	0	0
3	25302	26307	25827	25812	25800	39693	0.5960	0.5963	22.0647
4	56520	57720	55025	56422	56410	86784	1.3031	1.3039	48.2426
5	63620	71260	75320	70067	70055	107777	1.6183	1.6192	59.9120
6	107693	116799	107426	110639	110628	170196	2.5555	2.5570	94.6105
7	131840	122099	112713	122217	122206	188008	2.8229	2.8247	104.5122
8	160500	154653	157540	157564	157553	242388	3.6395	3.6417	134.7415
9	172913	188666	210719	190766	190754	293468	4.4064	4.4091	163.1361
10	204693	194426		199560	199548	306996	4.6096	4.6123	170.6565

(Handwritten note)

CellSuspension

Experiment: 37063  
Date: 06/21/01

Tube #	Suspension count (CPM)			CPM Average	CPM corrected for control	DPM CPM(y e)	A <sub>i</sub> μCi/ml on counting	A <sub>o</sub> μCi/ml after uptake	A <sub>o</sub> kBq/ml after uptake
	1st	2nd	3rd						
1	13	13	12	12	0	0	0.0000	0	0.0000
2	11	8	16	0	0	0	0.0000	0	0.0000
3	665	908	1082	885	873	1343	0.00605	0.00605	0.2239
4	1847	1277	4219	2448	2436	3747	0.01688	0.01689	0.6248
5	2163	3312	2431	2635	2623	4036	0.01818	0.01819	0.6729
6	3679	2605	10553	5612	5600	8616	0.03881	0.03883	1.4367
7	10244	9923	5119	8429	8417	12948	0.05833	0.05836	2.1592
8	10375	12908	10940	11408	11396	17532	0.07897	0.07901	2.9234
9	13732	8974	7422	10043	10031	15432	0.06951	0.06955	2.5732
10	13837	6521	17261	12540	12528	19273	0.08682	0.08686	3.2138

CoulterSurvival

iment: 37063  
e/Time: #####

Tube #	Coulter count			Average Cells/ml	Hemocytometer Count in Grid			
	1st	2nd	3rd		1st	2nd	3rd	4th
1	6003	5803		5903	2358000			
2	5067	5096	4980	5048	2015867			
3	6270	6373	6309	6317	2523733			
4	5934	5676	5619	5743	2294000			
5	5904	5828	5686	5806	2319200			
6	5903	5821	5843	5856	2339067			
7	5438	5627	5667	5577	2227733			
8	5456	5370	5454	5427	2167467			
9	5410			5410	2160800			
10	5637	5539	5497	5558	2219867			

Tube #	Predicted # Cells Seeded	Actual # Cells Seeded	Colony count			Average	PE (%)	SF Uncorrected	SF Corrected
			1st	2nd	3rd				
1	200	236	100	90		85	38.867	1.00	1.0000
2	200	202	78	72					
3	200	252	97	107	93	99	39.228	1.1647	1.0093
4	200	229	58	61	57	59	25.574	0.6902	0.6580
5	200	232	68	51	63	61	26.158	0.7137	0.6730
6	200	234	61	39	49	50	21.234	0.5843	0.5463
7	200	223	57	42	39	46	20.649	0.5412	0.5313
8	200	217	43	47	34	41	19.070	0.4863	0.4906
9	200	216	47	55	49	50	23.294	0.5922	0.5993
10	200	222	61	61	52	58	26.128	0.6824	0.6722



Experiment: 6/21/2001  
 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	1.1647	1.0093
3	22.065	0.089	0.6902	0.6580
4	48.243	0.272	0.7137	0.6730
5	59.912	0.290	0.5843	0.5463
6	94.610	0.614	0.5412	0.5313
7	104.512	0.969	0.4863	0.4906
8	134.741	1.349	0.5922	0.5993
9	163.136	1.191	0.6824	0.6722
10	170.656	1.448		

