14

COLONY FORMING & HPRT ASSAY FOR V79 CELLS

Experiment Name : 100 % cluster, 3HTdR); **Exp.**
Investigator: M. Lenarczyk **Date: May 21, 2001**

1. Set the rocker-roller at 37°C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 150 cm^2 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 Couter counter (100 ul cell suspension / 20 ml Isotone
2. Dilute to ~4,000,000 cells/ml in MEMB [Actual count : 3995 333 cells/ml)

*

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_2 **Date/Time: May 21, 2001/ 18:00**

*- Cell count - Mode - 100 µl $\left[\left(\frac{1996+2007+1990+1997}{4}\right)\right]\times$ = 3 995 333

Final count
= 1996, 2007, 1990
1997

3, 995, 333 cells/ml

MS = 100 µl
2982, 2938, 2992
2970

Cell conc = 5,940,000 cells/ml

12 ml × 4,000,000 cells/ml
= 48,000,000

Vol. = $\frac{48,000,000}{5,940,000}$
= 8 ml + 4/5 ml

5. Prepare MEMB containing radioactivity in hood

60 µl ^{3}HTdR (Stock : 1 µCi/µl on 4/10/01) + 5 ml MEMB

Lot# - NET-027Z Lot#-3106-421-

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

Date/Time: May 21, 2001 / 20:00

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	0.580	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 21, 2001 / 20:15**

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 / 11:00
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 regular
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 2 ml of **standard culture** MEMA~~
19. Decant supernatant, click tubes, vortex, and using 200 µl tips transfer the cells in polypropylene micro-centrifuge tubes (Helena tube, 400 µl)
14. 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)
15. Centrifuge tubes for 5 min at 1000 rpm, 4°C
16. Transfer Hellena tubes at 10.5°C for 72 h

Decant supernatant, click tubes, vortex, transfer the cell suspension in polypropylene micro-centrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips

17. Transfer tubes at 10.5°C for 72 h. 05/22/01 **Date/Time:** 13:00
23. 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 **Date/Time:**
24. 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: 05/25/01 13:30
25. Again add 200 ul 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,

HTdR, 100% cluster, V79

05/22/01 /13:00 —72hr→ 05/25

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 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 **standard culture** MEMA, pass 5x through 3-5 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 dilusion 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 100 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.

Exp: 05/21/01.

V79, 100% cluster, HidR

Medium count (1st)

PAGE: 1

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 THU 24 MAY 2001 14:45
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

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	29- 1	1	14.00	53.45	1.00	1.42	76.0	
2	29- 2	1	5.00	89.44	1.00	3.00	77.0	
3	29- 3	1	11.00	60.30	1.00	4.63	77.0	
4	29- 4	1	10.00	63.25	1.00	6.25	77.0	
5	29- 5	1	10.00	63.25	1.00	7.82	76.0	
6	29- 6	1	12.00	57.74	1.00	9.38	77.0	
7	29- 7	1	22800.00	1.97	0.45	10.40	76.0	
8	29- 8	1	22504.44	1.99	0.45	11.41	76.0	
9	29- 9					11.88		101
10	29-10	1	32180.00	1.88	0.35	12.77	77.0	
11	29-11	1	33826.66	1.99	0.30	13.63	77.0	
12	29-12	1	32825.71	1.87	0.35	14.53	75.0	
13	29-13	1	43968.00	1.91	0.25	15.33	77.0	
14	29-14	1	39446.66	1.84	0.30	16.24	78.0	
15	29-15	1	44848.00	1.89	0.25	17.04	79.0	
16	29-16	1	55820.00	1.89	0.20	17.79	77.0	
17	29-17	1	55850.00	1.89	0.20	18.54	78.0	
18	29-18	1	55470.00	1.90	0.20	19.35	76.0	
19	**- 1	1	70800.00	1.94	0.15	20.10	78.0	
20	**- 2	1	72926.66	1.91	0.15	20.80	77.0	
21	**- 3	1	72206.66	1.92	0.15	21.50	78.0	
22	**- 4	1	96320.00	1.66	0.15	22.26	77.0	
23	**- 5	1	95260.00	1.67	0.15	22.96	78.0	
24	**- 6	1	97546.66	1.65	0.15	23.66	79.0	
25	**- 7	1	124119.99	1.47	0.15	24.36	80.0	
26	**- 8	1	118100.00	1.84	0.10	25.07	79.0	
27	**- 9	1	114119.99	1.53	0.15	25.77	77.0	
28	**-10	1	146066.66	1.35	0.15	26.48	79.0	
29	**-11	1	135526.66	1.40	0.15	27.19	79.0	
30	**-12	1	136560.00	1.71	0.10	27.90	79.0	
31	**-13	1	29508.57	1.97	0.35	28.85	0.0	

Standard DPM-98200

B019632

Exp: 05/21/01

V79, 100% cluster, HTdR

Medium count (2nd).

PAGE: 1

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 THU 24 MAY 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: 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	29- 1	1	13.00	55.47	1.00	1.42	76.0	
2	29- 2	1	8.00	70.71	1.00	3.00	75.0	
3	29- 3	1	14.00	53.45	1.00	4.58	77.0	
4	29- 4	1	10.00	63.25	1.00	6.14	77.0	
5	29- 5	1	12.00	57.74	1.00	7.71	77.0	
6	29- 6	1	10.00	63.25	1.00	9.33	77.0	
7	29- 7	1	22695.55	1.98	0.45	10.33	76.0	
8	29- 8	1	22082.00	1.90	0.50	11.38	76.0	
9	29- 9					11.85		101
10	29-10	1	32151.43	1.89	0.35	12.80	78.0	
11	29-11	1	33220.00	1.88	0.35	13.71	77.0	
12	29-12	1	33496.66	2.00	0.30	14.56	77.0	
13	29-13	1	43672.00	1.91	0.25	15.36	77.0	
14	29-14	1	40036.00	2.00	0.25	16.21	78.0	
15	29-15	1	44144.00	1.90	0.25	17.08	80.0	
16	29-16	1	55540.00	1.90	0.20	17.83	77.0	
17	29-17	1	56410.00	1.88	0.20	18.58	77.0	
18	29-18	1	55240.00	1.90	0.20	19.33	76.0	
19	**- 1	1	71400.00	1.93	0.15	20.08	77.0	
20	**- 2	1	73706.66	1.90	0.15	20.78	78.0	
21	**- 3	1	72853.33	1.91	0.15	21.48	78.0	
22	**- 4	1	95473.33	1.67	0.15	22.18	77.0	
23	**- 5	1	96426.66	1.66	0.15	22.88	78.0	
24	**- 6	1	97420.00	1.65	0.15	23.58	80.0	
25	**- 7	1	123160.00	1.80	0.10	24.28	79.0	
26	**- 8	1	117779.99	1.50	0.15	24.98	78.0	
27	**- 9	1	115286.66	1.52	0.15	25.69	78.0	
28	**-10	1	146950.00	1.65	0.10	26.40	79.0	
29	**-11	1	138186.66	1.39	0.15	27.10	78.0	
30	**-12	1	134060.00	1.41	0.15	27.81	79.0	
31	**-13	1	129797.14	1.96	0.35	28.78	0.0	

Stevolol
DAM 98200

B019633

Plate 200,000 for MN
1,000,000 for 6TGRT

MS = 500 µl; Background = 14

DAY 0 of mutant expression

TABLE-3

Expt. #: V79, 100%, HTGR **Date/Time:** 05/25/01; 3-00 pm

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 400]	pCi/cell [uCi/ml x 10^6 Cells/ml]
1	6134, 6219, 6161	6171	2,468,533	
2	~~6789, 6628, 6961 7301, 7004, 7688~~	6792	2,717,066	
3	6934, 6950, 6751	6878	2,751,333	
4	7188, 7166, 7101	7151	2,860,666	
5	7464, 7568, 7366	7466	2,986,400	
6	~~7036~~, 6720, 6864, 6800	6794	2,717,866	
7	6437, 6516, 6442	6465	2,586,000	
8	6548, 6789, 6661	6666	2,666,400	
9	6044, 6415, 6176	6211	2,484,666	
10	6515, 6607, 6469	6530	2,612,133	

4/6 → 4
4/6 → 6

Mode - 500µl
Bckgr - see above

For survival:

1.2
2.2
3.2
4.2
5.2 5.3
6.2 6.3
7.2 7.3
8.2 8.2
9.2 9.3
10.2 10.3

Cell activity (1st)
V79, 100%, HTGR
May 21, 2001

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 FRI 25 MAY 2001 17:01
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	11.00	60.30	1.00	1.42	88.0	
2	**- 2	1	9.00	66.67	1.00	3.00	86.0	
3	**- 3	1	13.00	55.47	1.00	4.58	87.0	
4	**- 4	1	11.00	60.30	1.00	6.14	89.0	
5	**- 5	1	10.00	63.25	1.00	7.71	89.0	
6	**- 6	1	12.00	57.74	1.00	9.28	90.0	
7	**- 7	1	2450.00	4.04	1.00	10.89	88.0	
8	**- 8	1	2448.00	4.04	1.00	12.46	87.0	
9	**- 9	1	2965.00	3.67	1.00	14.03	89.0	
10	**-10	1	7151.00	2.37	1.00	15.61	88.0	
11	**-11	1	7461.00	2.32	1.00	17.18	90.0	
12	**-12	1	7668.00	2.28	1.00	18.75	90.0	
13	**-13	1	8304.00	2.52	1.00	20.33	91.0	
14	**-14	1	4966.00	2.84	1.00	21.90	88.0	
15	**-15	1	4902.00	2.86	1.00	23.53	89.0	
16	**-16	1	4678.00	2.92	1.00	25.10	90.0	
17	**-17	1	3811.00	3.24	1.00	26.67	89.0	
18	**-18	1	4373.00	3.02	1.00	28.23	89.0	
19	29- 1	1	9558.00	2.05	1.00	29.87	89.0	
20	29- 2	1	8447.00	2.18	1.00	31.43	88.0	
21	29- 3	1	9503.00	2.05	1.00	33.01	90.0	
22	29- 4	1	8970.00	2.11	1.00	34.57	87.0	
23	29- 5	1	9266.00	2.08	1.00	36.14	87.0	
24	29- 6	1	10171.00	1.98	1.00	37.71	89.0	
25	29- 7	1	13630.67	1.98	0.75	39.07	93.0	
26	29- 8	1	12164.71	1.97	0.85	40.49	88.0	
27	29- 9	1	12307.06	1.96	0.85	41.91	88.0	
28	29-10	1	12753.75	1.98	0.80	43.27	87.0	
29	29-11	1	15354.29	1.93	0.70	44.54	88.0	
30	29-12	1	15958.46	1.96	0.65	45.75	89.0	
31	29-13	1	29491.43	1.97	0.35	46.72	0.0	

Cell activity (2nd
V79, HTGR, 100%
May 21, 2001.

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 TUE 29 MAY 2001 10:11
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

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**- 1	1	11.00	60.30	1.00	1.42	88.0	
2	**- 2	1	11.00	60.30	1.00	3.00	87.0	
3	**- 3	1	11.00	60.30	1.00	4.58	88.0	
4	**- 4	1	11.00	60.30	1.00	6.14	89.0	
5	**- 5	1	10.00	63.25	1.00	7.71	89.0	
6	**- 6	1	8.00	70.71	1.00	9.28	90.0	
7	**- 7	1	1909.00	4.58	1.00	10.84	89.0	
8	**- 8	1	1942.00	4.54	1.00	12.40	87.0	
9	**- 9	1	2410.00	4.07	1.00	13.97	89.0	
10	**-10	1	5644.00	2.66	1.00	15.53	87.0	
11	**-11	1	5737.00	2.64	1.00	17.10	89.0	
12	**-12	1	5998.00	2.58	1.00	18.67	90.0	
13	**-13	1	5173.00	2.78	1.00	20.24	90.0	
14	**-14	1	3979.00	3.17	1.00	21.82	89.0	
15	**-15	1	3831.00	3.23	1.00	23.40	90.0	
16	**-16	1	3678.00	3.30	1.00	24.97	90.0	
17	**-17	1	2968.00	3.67	1.00	26.60	90.0	
18	**-18	1	3406.00	3.43	1.00	28.17	90.0	
19	29- 1	1	7419.00	2.32	1.00	29.79	89.0	
20	29- 2	1	6589.00	2.46	1.00	31.36	88.0	
21	29- 3	1	7556.00	2.30	1.00	32.92	90.0	
22	29- 4	1	6643.00	2.45	1.00	34.50	86.0	
23	29- 5	1	7143.00	2.37	1.00	36.07	88.0	
24	29- 6	1	7841.00	2.26	1.00	37.64	88.0	
25	29- 7	1	10642.11	1.99	0.95	39.16	93.0	
26	29- 8	1	9280.00	2.08	1.00	40.73	88.0	
27	29- 9	1	9368.00	2.07	1.00	42.32	89.0	
28	29-10	1	10225.00	1.98	1.00	43.89	86.0	
29	29-11	1	11546.67	1.96	0.90	45.42	89.0	
30	29-12	1	12587.50	1.99	0.80	46.78	88.0	
31	29-13	1	29540.00	1.97	0.35	47.74	0.0	

Standard
DPM-98200

TABLE-4

Expt # V79, 100%, HICR **Date :**

~~38. Count vials for radioactivity~~ **Date/Time :**

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1.2	86	78	72		
2.2	50	76	63		
3.2	71	81	68		
4.2	68	64	66		
5.2	62	55	55		
6.2	81	66	67		
7.2	29(?)	38	46		
8.2	54	53	51		
9.2	38	46	38		
10.2	40	36	36		

2 µg/µl.

3 µg/ml.

CYTOCHALASIN-B

10 × 3 ml

10 sample × [illegible] + 3 ml = 30 ml MEMB

10 sample → 30 ml

35 ml.

3 µg/ml

x – 35 ml

35

105 µg

11

↓

105 µg – Y → 52.5 µl of stock CYT-B (2 µg/µl)

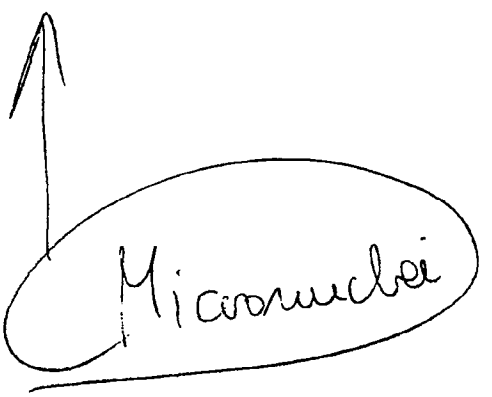
2 µg – µl

Take 52.5 µl of 2 µg/µl Cyt B

Add into the tube with 35 ml MEMB

Mix — (final conc. of Cyt B ~~52.5 µg / 35 ml~~

= 105 µg / 35 ml = 3 µg/ml.



DAY 0 = May 25, 2001.

HPRT mutant expression - DAY 3 May 28, 2001

Exp: V79, 100%, HTdR

Sample:	Coulter Count			# of cells per ml. $\times 10^6$	Total # of cells $\times 10^6$	Volume for 10^6 cells (ml)
1	8643	8671	8699	3.45	20.7	0.29
2	9465	9199	9250	3.7	22.2	0.27
3	7846	7945	7764	3.1	21.9	0.32
4	6119	6208	5965	2.4	14.6	0.41
5	Contamination					
6	9556	9329	9476	3.8	22.6	0.27
7	9301	9238	9357	3.8	22.2	0.27
8	8744	8582	8382	3.4	20.5	0.29
9	8121	8066	8227	3.2	19.4	0.31
10	7639	7846	7535	3.1	18.3	0.33

Background - 36

Mode - 500µl

Procedure:

1. Wash 1x with PBS-A
2. Trypsinize (2ml trypsin/T75, 2-3min RT) & harvest the cells (+8ml MEM
3. Spin the cells down (5 min, 2K rpm, 4°C)
4. Sirinage the cells 3x
5. Count the cells (100µl cell susp. + 20ml Isotone)
6. Plate 10^6 cells / T75 flask + 15ml MEMA

· RPET mutant expression - DAY 6

May 31, ~~2004~~

Exp.: V79, 100%, HTdR,

Sample	Coulter count			# of cells per ml x 10^6	total # of cells in 7ml.	Volume for 10^6 cells.
1	6426	6762 ~~5762~~	6720	2.7	18.5	0.37
2	7890	7715	7858	2.2	15.2	0.46
3	9414	9836	9784	3.9	27.1	0,26
4	6843	6773	6778	2.7	18.9	0.37
5						
6	~~8577~~	7052	6875	2.8	19.5	0.36
7	9266	9352	9420	3.7	26.1	0.27
8	~~6028~~ 8028	~~7562~~ 7731	7731	3.1	21.9	0.32
9	10083	10308	10302	4.1	28.6	0.24
100	9767	9590	9710	3.9	27.1	0,26

Backgr - 17

Mode - 500µl

HPRT mutant expression, DAY 9 , June 3, 2001

Exp: V79, 100%, HTdR,

Sample	Coulter count			# of cells per ml.	Total # of cells in 6 ml.	10^6 cells (vol.)
1	9864	9779	10066	4.0	23.7	0,25
2	11462	11509	11161	4.5	27.3	0,22
3	10164	9996	10152	4.0	24.2	0,25
4	9967	10586	10756	4.2	25.0	0,24
5	———				—	—
6	9956	9819	9979	4.0	23,7	0,25
7	9471	9533	9522	3,8	22.7	0,33
8	10066	10401	10343	4.1	24.6	0.24
9	9493	9510	9830	3.8	23.0	0.26
10	11261	11208	11261	4.5	26.9	0.22

Bckgr - 17

Mode - 500µl

HPET expression DAY 11 June 5, 2001

Exp: V79, 100%, HIdR,

Sample	Cooter count			# of cells per ml x10^6	Total # of cells (in 6ml)	10^6 cells (ml)
1	1668	1672	1642	0.66	3.9	1.52
2	1076	1131	1098	0,43	2,6	2.32
3	4557	4716	4518	1.83	11.00	0.55
4	3971	4078	3996	1,60	9,6	0.63
5						
6	3919	3886	3878	1,55	9.3	0.65
7	1985	1949	1962	0,80	4.7	1.25
8	3083	3019	3061	1,22	7,3	0.82
9	3789	3614	3772	1,48	8,9	0,68
10	4934	4986	4924	1,97	11,8	0,51

Beads - 16

11.0c - 500µl

NOTE : T75 #1, 2 & 7 was capped to tight. !

HPRT - Challenge June, 2001

Exp: V79, 100%, HTdR,

Sample	Coulter count			#cells/ml	Total # (in 12 ml)	0.2×10^6
1	5379,	5556	5437	2178533	13.07	92
2	4488,	4359	4494	1774400	10.6	111
3	4429	4526	4466	1785067	10.7	111,
4	4349	4142	4271	1697200	10.2	118
? 6	5306	5198	5084	2074000	12.4	95
? 7	5048	5141	5032	2025067	12.2	100
? 8	5509	5395	5390	2168133	13.0	91
9	5239	4778	5110	2012533	12,1	100
10	5110	5136	5012	2030000	12,2	100

Beluga- 11

Mode - 500

STOCK 200 000/5ml = 40 000/ml

10x 0,5ml + 4,5ml MEMA = 4000/ml

100x 0.5ml + 4,5 ml MEMA = 400/ml

for PE 0,5 ml / P60' x 3

- 200000/5ml = 40 000/ml

- 0.5ml = 20 000

- 20 000/5ml = 4 000/ml

4000/5ml =

Plating efficiency for HPBI challenge

13 July, 2001.

Exp: 100%, HiDR, V79

1	100	138	140	13 15 .
2	135	128	129	.
3	157	180	160	17 2
4	173	155	180	18 1 1
5				
6	132	150	124	20 22
7	129	128	119	17 21 24
8	130	137	135	24 17 .
9	119	100	110	. 20
10	115	132	127	20 19 19

HPRT — 100% HTdR 479

Plated for [illegible] on June 7

Fixed & stained on June 18

1	0, 0, 0, 0, 0, 0, 0, 1,	C
2	0, 6, 1, 2, 1, 1, 1,	C, C,
3	1, 1, 4, 4, 4, 1, 1, 3, 4,	
4	0, 0, 1, 4, 2, 3, 3, 1, 3,	C,
5		
6	0, 0, 0, 0, 2, 2, 1, 2, 1,	1-0
7	0, 7, 7, 4, 7, 10, 5, 8	C, C
8	0, 0, 0, 0, 0, 0, 1, (1)?, 1,	C
9	0, 0, 2, 1, 2-4, 2, 1, 1, 6, 3,	
10	0, 2, 3, 1, 3, 3, 3, 3	C, C

COLONY FORMING ASSAY – V79 cells / 50 % cluster/ 3HTdR

Experiment Name : ^{3}HTdR toxicity (cluster, 50% labeling); **Exp. June 07, 2001**

Experiment performed by: M. Lenarczyk **Date:** 06/07/01

1. Set the rocker-roller at 37°C incubator with 5% CO_2, set the Coulter Counter, wash cells (from 4x 175 cm^2 flask, sub-cultured 1:2, 24h before) with DPBS, trypsinize cells (2 ml/flask), re-suspend cells in 15 ml MEMB/flask, pool 2 flask in 50mm conical tube, spin the cells down (4 o C, 2000 rpm, 10"), aspirate supernatant, and wash the cells in 15 ml DPBS, aspirate supernatant, pass five times through 10 cc syringe with 21 gauge needle, perform cell count by transfer 100 µl in Coulter cup containing 20 ml IsoLume
2. Dilute to ~2,000,000 cells/ml in MEMB 4528, 4603, 4687 [Actual count :1.840 000 cells/ml)
3. Transfer 1 ml of cell suspension into 20 12 ml tubes (Falcon plastic test tube, 17x100 mm) labeled 1-10 both on cap and wall
4. Keep the tubes in the roller for 3-4 h at 37°C, 5% CO_2 **Date/Time: June., 07, 2001 / 16:00**
5. Prepare MEMB containing radioactivity in hood

   800 µl ^{3}HTdR (Stock :1 µCi/µl on ., , 2001) + 4200 ml MEMB
6. After 3-4 h, remove first set of 10 test tubes from roller and add MEMB with or without radioactivity according to Table below. **Date/Time: June., 07, 2001 / 19:00**

Tube #	^{3}HTdR µCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+^{3}HTdR Working sol. Activity [160µCi/ml] (ml)
1	0	1.0	1.0	0
2	0	1.0	1.0	0
3	10	1.0	875	0.125
4	20	1.0	0.750	0.250
5	30	1.0	0.625	0.375
6	40	1.0	0.500	0.500
7	50	1.0	0.375	0.625
8	60	1.0	0.250	0.750
9	70	1.0	0.125	0.875
10	80	1.0	0	1

7. Return test tubes to roller for 12 h . **Date/Time: June, 07, 2001 / 19:30**

1. 1.8×10^6/ml →
2. Roll Start → 16:00 → → 19:00 (3 hrs).
3. ~~19:30~~ → Add HEMB

↓ 19:30 (July 7, 2001) → 10:45 (July 8, 2001) → (15 hrs)

			MEMB	H-3 + MEMB (160 µCi/ml)
1	0	~~40~~	1	0
2	0	~~40~~	1	0
3	10		0.875	0.125
4	20		0.750	0.250
5	30		0.650	0.375
6	40		0.500	0.500
7	50		0.375	0.625
8	60		0.250	0.750
9	70		0.125	0.875
10	80		0	1.000
				4.500

5 ml MEMB + 800 µCi

4.2 ml + 800 µCi (160 µCi/ml)

4. Take 30 µl for medium activity counting
5. Wash 3x in wash MEMA →
6. Sinuge

6-TO

1. 2.5 mg + 10 ml MEHA + 2.5 mg + 10 ml MEHA → dissolve → 5 mg/20 ml (STOCK SOL).

2. Add STOCK SOL. (sterilize) in 255 ml MEHA → mix → (WORKING SOL)

3. Take 3 ml of working sol & add rest P100' unit 7 ml.
It gives you final concentration of 6.TO

5 mg/275 ml = 5000 µg/275 ml
x — 3 ml
↓
54.55 µg

54.55 µg/3 ml + 7 ml = 54.45 µg/10 ml
↓
5.45 µg/ml

July 7, 2001

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: June, 07, 2001 / 10:45** (*hrs*)
   NOTE: Set of non-labeled cells were not rolled. The roller is broken. !!!!
10. Remove buckets from centrifuge and carefully remove 150 µl of supernatant from labeled tubes and place in pre-labeled gamma-tube.
11. Decant supernatant, click tubes, vortex, re-suspend in 10 ml **wash** MEMA.
    Steps 11-16 are common for labeled as well as non-labeled tubes.
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 5 ml of **regular** MEMA
16. Centrifuge tubes for 10 min at 2000 rpm, 4°C