16

AL-N cell survival & Mutagenesis
(HTdR, 50 % cluster)

Experiment Name : ^{3}HTdR toxicity (cluster, 50% labeling); **Exp.;**
Performed by: M.Lenarczyk **Date:** Feb., 15, 2001

1. Set the rocker-roller at 37°C incubator with 5% CO_2, , wash cells (from three 175 cm^2 flask, sub-cultured 1:2, 24h before) with HBSS, trypsinize cells, each re-suspend in 10ml RPMI, pool, pass five times through 10 cc syringe with 21 gauge needle, count cells
[Actual count : 2 046 533 /ml
2. Prepare cell suspension with final concentration of 2000000/ml
3. Transfer 1 ml of cell suspension into 20 12 ml tubes (Falcon polypropylene TC) labeled 1-10 both on cap and wall.
4. Roll the tubes in standard culture condition **Date/Time:** Feb., 15, 2001 **/ 16:00**
5. Prepare RPMI 1640 containing radioactivity
640 µl HTdR (Stock :1 µCi/µl on Feb., 15, 2001) + 3. 4 ml RPMI 1640
6. Remove test tubes from roller and add RPMI with or without HTdR according to the table:
Date/Time: Feb., 15, 2001 **/ 18:15**

Tube #	^{3}HTdR µCi/ml	Cells in RPMI (ml)	RPMI (ml)	RPMI + ^{3}HTdR [stock -160 µCi/ml] (ml)
1	0	1.0	1.0	0
(2)	0	1.0	1.0	0
(3)	5	1.0	0.940	0.060
4	10	1.0	0.875	0.125
5	20	1.0	0.750	0.250
(6)	30	1.0	0.625	0.375
7	40	1.0	0.500	0.500
8	50	1.0	0.375	0.625
9	60	1.0	0.250	0.750
10	80	1.0	0	1

1, 2, 3 & 10 – OK

4, 5, 6, 7, 8, 9 – ?

see table

S00/22 — No single A+B samples

1 – 2116, 2088, 2134 –

2 – 2006, 1928, 1846

3 – 1511, 1462, 1454

4 – 1085, 1512, 1512

5 – 1720, 1673, 1688

6 – 1850, 1895, 1758

7 – 1433, 1514, 1548

8 – 1457, 1515, 1352

9 ———

10 – 1472, 1489, 1511

(dupa o 3 ml total ?)

2ml-susp 3ml

↓

100µl

(580-22) Mode / Bckgr.
100 - 4 Feb 16, 2007.
50 -

ONLY M-3 Sample

1 - 992, 963, 945 | 1.13 (3 ml)
2 - 1008, 950, 987 (1.15) No siringe
3 - 630, 670, 626 (0.5)
4 - 969, 704, 451 (0.55)
5 -
6 - 600, 592, 610 (0.46
7 - 516, 469, 576 (0.40)
8 - 527, 515, 513 (0.40)
9 - |———|
10 - 638, 556, 655 (0.48)

B019673

7. Return test tubes to roller for 12 h . **Date/Time:** Feb., 15, 2001 / **18:30**
8. Next day, prepare and label 10 gamma-tubes (13 X 100 mm VWR glass test tube)
9. After h incubation period, remove tubes and centrifuge at 2000 rpm at 4°C for 10 min (***precooled centrifuge***). **Date/Time:** Feb., 16, 2001 / 16:30
10. Remove buckets from centrifuge and carefully remove 150 µl of supernatant and place in prelabeled gamma-tube.
11. Decant supernatant, click tubes, vortex, re-suspend in 6 ml HBSS/CS 10%
12. Centrifuge tubes for 10 min at 2000 rpm, 4°C
13. Decant supernatant, click tubes, vortex, re-suspend in 6 ml RPMI/CS 10%.
14. Centrifuge tubes for 10 min at 2000 rpm, 4°C
15. Decant supernatant from 10 unlabeled tubes (set B – without H-3) , click tubes, vortex, re-suspend the pellet in 5 ml RPMI, & transfer cell suspension into set A tubes (e.g. 1 B into 1A into 1B ....10A into 10B)
16. Add 5 ml RPMI and centrifuge tubes for 10 min at 2000 rpm, 4°C
17. Transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 µl) using 200 µl pipet tips
24. Again add 200 µl ice cold RPMI, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 µl)
25. Centrifuge tubes for 5 min at 1000 rpm, 4°C
26. Transfer tubes at 10°C for 72 h. **Date/Time:** Feb.,16, 2001 / 18:30
27. Transfer 30 µl supernatant in three sets of 20 ml scintillation vials containing 6 ml liquid scintillation cocktail (EcoLume) 150 µl supernatant removed earlier (Step 10) and count them for radioactivity **Date/Time:** Feb.,16 , 2001 / 19:00
28. After 72 h, carefully remove the supernatant from the top, re-suspend pellet in 200 ul HBSS & 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 RPMI by using Pasteur pipet
Date/Time: Feb.,19, 2001 / 17:30
29. Again add 200 µl RPMI in microcentrifuge tubes, re-suspend and transfer the cell suspensions in 12 ml tubes
30. Centrifuge the tubes for 10 min at 2000 rpm, 4°C (***precooled centrifuge***)
31. Labeling and preparation of dilution tubes and colony dishes
load P 60's dishes with 4 ml RPMI
load 40 sterile tubes with 4.5 ml RPMI:

32. Decant supernatant, click tubes, vortex, re-suspend in 10 ml HBSS

33. Centrifuge tubes for 10 min at 2000 rpm, 4°C
34. Decant supernatant, click tubes, vortex, re-suspend in 10 ml RPMI
35. Centrifuge tubes for 10 min at 2000 rpm, 4°C
36. Decant supernatant, click tubes, vortex, re-suspend in 2 ml RPMI,
37. Determine cell concentration by Coulter couinter (20ml Isotene II + 100 µl cell suspension)
38. Vortex tube,

    transfere 0.5 ml cell susp. into tube X.5, vortex tube X5, (10x dilution = cells)

    take 0.5 ml of X5 and transfer to tube X.4, vortex tube X4 (100x dilution = cells),

    take 0.5 ml of X4 and transfer to tube X.3, vortex tube X3 ((1000 dilution = cells),

    take 0.5 ml of X3 and transfer to tube X.2, vortex tube X2, (10000 dilution = cells)

39. Transfer 1 ml from dilution tubes into dishes labeled X.2, X.3,(in triplicate). Only X.2 should be seeded for control T-tubes.
40. Transfer 100 µl of cell suspension (in triplicate) to 7 ml scintillation vial containing 6 ml cocktail (EcoLume)
41. Plate rest amount of the cell into 100 mm dish for mutant expression (if you have enogh cells plate 1000 000 cells /plate)
41. Incubate 60 mm dishes for 1 weeks .
42. Count vials for radioactivity **Date/Time:**
43. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
44. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.

Medium count (50%), H3, $A_L N_1$

PAGE:

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 WED 28 FEB 2001 18:26
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
H#: 1 AQC:N QCF:N RCM:Y
RCM-TIME: 0.10 INT:999.95
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:@ 1.00000
HALF LIFE(DAYS):N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	RCM%	ERI
1	**- 1	1	22.00	42.64	1.00	1.42	78.0	0.86	
2	**- 2	1	12.00	57.74	1.00	3.01	76.0	1.15	
3	**- 3	1	57.00	26.49	1.00	4.58	77.0	0.45	
4	**- 4	1	15.00	51.64	1.00	6.14	79.0	0.81	
5	**- 5	1	19.00	45.88	1.00	7.71	76.0	0.87	
6	**- 6	1	67.00	24.43	1.00	9.28	78.0	0.41	
7	**- 7	1	270506.66	0.99	0.15	9.99	81.0	0.00	
8	**- 8	1	296453.31	0.95	0.15	10.72	78.0	0.01	
9	**- 9	1	203113.33	1.15	0.15	11.43	76.0	0.00	
13	**-13	1	397186.66	0.82	0.15	12.20	81.0	0.01	
14	**-14	1	583180.00	0.68	0.15	12.94	77.0	0.01	
15	**-15	1	411586.66	0.80	0.15	13.68	77.0	0.01	
16	**-16	1	600373.31	0.67	0.15	14.43	81.0	0.01	
17	**-17	1	606500.00	1.15	0.05	14.93	77.0	0.01	
18	**-18	1	791106.62	0.58	0.15	15.70	77.0	0.01	
19	**- 1	1	1005246.62	0.52	0.15	16.54	81.0	0.01	
20	**- 2	1	1154726.62	0.48	0.15	17.35	77.0	0.02	
21	**- 3	1	1063660.00	0.87	0.05	17.87	75.0	0.01	
22	**- 4	1	613026.62	0.66	0.15	18.63	81.0	0.01	
23	**- 5	1	611520.00	0.66	0.15	19.38	77.0	0.01	
24	**- 6	1	640846.62	0.65	0.15	20.13	76.0	0.01	
28	**-10	1	1593580.00	0.71	0.05	20.69	81.0	0.02	
29	**-11	1	2277680.00	0.59	0.05	21.26	77.0	0.03	
30	**-12	1	1567240.00	0.71	0.05	21.79	76.0	0.02	
31	**-13	1	24.00	40.82	1.00	23.37	72.0	1.17	

OK DATA IN TABLE

Medium count, ALN, 073, 50%

[illegible] 1st count OK!

SAM	POS	CH	CPM	2SIG%	TIME	EL. TIME	AVG H#	RCM%	ERR
1	**- 1	1	13.00	55.47	1.00	1.46	74.0	22.23	
2	**- 2	1	14.00	53.45	1.00	3.04	72.0	8.02	
3	**- 3	1	56.00	26.73	1.00	4.60	72.0	1.89	
4	**- 4	1	16.00	50.00	1.00	6.18	74.0	2.39	
5	**- 5	1	18.00	47.14	1.00	7.81	73.0	3.39	
6	**- 6	1	134.00	17.28	1.00	9.43	73.0	0.78	
7	**- 7	1	181493.33	1.21	0.15	10.14	76.0	0.01	
8	**- 8	1	193340.00	1.17	0.15	10.86	72.0	0.01	
9	**- 9	1	141590.00	1.68	0.10	11.58	72.0	0.01	
13	**-13	1	284973.31	0.97	0.15	12.33	76.0	0.01	
14	**-14	1	400319.97	0.82	0.15	13.07	74.0	0.02	
15	**-15	1	288360.00	0.96	0.15	13.78	73.0	0.01	
16	**-16	1	413199.97	0.80	0.15	14.52	77.0	0.02	
17	**-17	1	431946.66	0.79	0.15	15.25	75.0	0.01	
18	**-18	1	533253.31	0.71	0.15	16.00	73.0	0.02	
19	**- 1	1	650920.00	0.64	0.15	16.81	75.0	0.03	
20	**- 2	1	749693.31	0.60	0.15	17.58	73.0	0.03	
21	**- 3	1	714766.62	0.61	0.15	18.34	73.0	0.03	
22	**- 4	1	421670.00	0.97	0.10	19.08	77.0	0.02	
23	**- 5	1	421380.00	0.97	0.10	19.82	72.0	0.02	
24	**- 6	1	451693.31	0.77	0.15	20.56	73.0	0.02	
28	**-10	1	1103220.00	0.49	0.15	21.38	77.0	0.04	
29	**-11	1	1537710.00	0.51	0.10	22.23	74.0	0.05	
30	**-12	1	1100826.62	0.49	0.15	23.03	74.0	0.03	
31	**-13	1	37.00	32.88	1.00	24.60	71.0	3.01	
33	**-15	1	29740.00	1.96	0.35	25.57	2.0	0.00	

Medium count (2nd) ALN, H3, 80%

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 SAT 17 FEB 2001 11:53
[illegible] REPEAT: 1 SCR:N RS232: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:Q 1.00000
HALF LIFE(DAYS):N

	SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	RCM%	ERR
	1	**- 1	1	7.00	75.59	1.00	1.42	75.0	5.19	
	2	**- 2	1	12.00	57.74	1.00	2.99	73.0	2.69	
	3	**- 3	1	44.00	30.15	1.00	4.57	73.0	1.00	
	4	**- 4	1	13.00	55.47	1.00	6.13	76.0	2.48	
2	5	**- 5	1	25.00	40.00	1.00	7.70	75.0	1.98	
	6	**- 6	1	84.00	21.82	1.00	9.27	73.0	0.53	
	7	**- 7	1	216960.00	1.36	0.10	9.99	79.0	0.01	
	8	**- 8	1	233246.66	1.07	0.15	10.72	73.0	0.01	
3	9	**- 9	1	168920.00	1.26	0.15	11.43	75.0	0.01	
	13	**-13	1	336266.66	0.89	0.15	12.17	77.0	0.01	
	14	**-14	1	471786.66	0.75	0.15	12.91	74.0	0.01	
5	15	**-15	1	341833.31	0.88	0.15	13.64	75.0	0.01	
	16	**-16	1	498259.97	0.73	0.15	14.38	78.0	0.01	
	17	**-17	1	508973.31	0.72	0.15	15.12	76.0	0.01	
6	18	**-18	1	634333.31	0.65	0.15	15.88	74.0	0.02	
	19	**- 1	1	792746.62	0.58	0.15	16.69	77.0	0.02	
	20	**- 2	1	903666.62	0.54	0.15	17.48	74.0	0.02	
7	21	**- 3	1	834239.94	0.57	0.15	18.26	75.0	0.02	
	22	**- 4	1	501126.66	0.73	0.15	18.99	78.0	0.01	
	23	**- 5	1	502280.00	0.89	0.10	19.74	75.0	0.01	
8	24	**- 6	1	531390.00	0.87	0.10	20.48	74.0	0.01	
	28	**-10	1	1318186.62	0.45	0.15	21.33	78.0	0.03	
	29	**-11	1	1820366.62	0.38	0.15	22.19	75.0	0.04	
10	30	**-12	1	1320786.62	0.45	0.15	23.01	74.0	0.03	
	31	**-13	1	30.00	36.51	1.00	24.58	72.0	1.66	
C	33	**-15	1	29954.29	1.95	0.35	25.55	2.0	0.00	

B019678

Cell count (50%), H3, ALN,

OK

PAGE:

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 WED 28 FEB 2001 18:50
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
H#: 1 AQC:N QCF:N RCM:Y
RCM-TIME: 0.10 INT:999.95
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:0 1.00000
HALF LIFE(DAYS):N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	RCM%	ERI
1	29--1	1	24.00	37.14	1.00	1.42	81.0	0.70	
2	29- 2	1	13.00	55.47	1.00	3.00	79.0	0.72	
3	29- 3	1	18.00	47.14	1.00	4.57	79.0	0.57	
4	29- 4	1	5.00	89.44	1.00	6.14	80.0	1.13	
5	29- 5	1	6.00	81.65	1.00	7.73	85.0	1.39	
6	29- 6	1	7.00	75.59	1.00	9.30	76.0	1.39	
7	29- 7	1	9608.00	2.04	1.00	10.92	80.0	0.01	
8	29- 8	1	8503.00	2.17	1.00	12.48	77.0	0.01	
9	29- 9	1	10525.00	1.95	1.00	14.11	78.0	0.01	
10	29-10	1	47288.00	1.84	0.25	14.92	79.0	0.01	
11	29-11	1	44744.00	1.89	0.25	15.72	78.0	0.01	
12	29-12	1	40684.00	1.98	0.25	16.53	77.0	0.01	
13	29-13	1	19245.46	1.94	0.55	17.70	81.0	0.00	
14	29-14	1	19078.18	1.95	0.55	18.81	78.0	0.01	
15	29-15	1	18075.00	1.92	0.60	19.97	78.0	0.01	
16	29-16	1	45416.00	1.88	0.25	20.78	78.0	0.01	
17	29-17	1	41176.00	1.97	0.25	21.59	78.0	0.01	
18	29-18	1	36860.00	1.90	0.30	22.45	81.0	0.01	
19	**- 1	1	105706.66	1.59	0.15	23.21	79.0	0.01	
20	**- 2	1	99793.33	1.63	0.15	23.92	77.0	0.01	
21	**- 3	1	102253.33	1.61	0.15	24.62	78.0	0.01	
22	**- 4	1	98999.99	1.64	0.15	25.33	77.0	0.01	
23	**- 5	1	74233.33	1.90	0.15	26.04	77.0	0.01	
24	**- 6	1	73920.00	1.90	0.15	26.75	77.0	0.01	
25	**- 7	1	51795.00	1.97	0.20	27.51	81.0	0.01	
26	**- 8	1	64925.00	1.76	0.20	28.26	78.0	0.01	
27	**- 9	1	54525.00	1.92	0.20	29.02	78.0	0.01	
28	**-10	1	126673.33	1.45	0.15	29.72	80.0	0.01	
29	**-11	1	109453.33	1.56	0.15	30.43	83.0	0.01	
30	**-12	1	96220.00	1.66	0.15	31.14	79.0	0.01	

DATA IN TABLE

Cell count (2nd) 50% , H3, ALN

& Medium count (1st) 100%, ALN, H3

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 WED 21 FEB 2001 11:12
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
H#: 1 AGC:N DCF:N RCM:Y
RCM-TIME: 0.10 INT:999.95
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:Q 1.00000
HALF LIFE(DAYS):N

SAM	POS	CH		CPM	2SIG%	TIME	EL TIME	AVG H#	RCM%	ERR
1	**- 1	1		24.00	40.82	1.00	1.42	77.0	1.57	
2	**- 2	1	1	13.00	55.47	1.00	3.01	76.0	0.98	
3	**- 3	1		19.00	45.88	1.00	4.63	75.0	0.93	
4	**- 4	1		10.00	63.25	1.00	6.26	76.0	1.53	
5	**- 5	1	2	9.00	66.67	1.00	7.88	82.0	1.84	
6	**- 6	1		18.00	47.14	1.00	9.45	74.0	1.53	
7	**- 7	1		4995.00	2.83	1.00	11.02	78.0	0.01	
8	**- 8	1	3	5926.00	2.60	1.00	12.58	74.0	0.01	
9	**- 9	1		5773.00	2.63	1.00	14.22	74.0	0.01	
10	**-10	1		17181.67	1.97	0.60	15.38	76.0	0.01	
11	**-11	1	3.2	16480.00	1.93	0.65	16.59	75.0	0.01	
12	**-12	1		22110.00	1.90	0.50	17.65	73.0	0.01	
13	**-13	1		7605.00	2.29	1.00	19.21	79.0	0.01	
14	**-14	1	4	9406.00	2.06	1.00	20.79	75.0	0.01	
15	**-15	1		9898.00	2.01	1.00	22.37	74.0	0.01	
16	**-16	1		19289.09	1.94	0.55	23.47	75.0	0.01	
17	**-17	1	5	22058.00	1.90	0.50	24.54	75.0	0.01	
18	**-18	1		19114.54	1.95	0.55	25.70	78.0	0.01	
19	**- 1	1		40036.00	2.00	0.25	26.56	75.0	0.01	
20	**- 2	1	6	41572.00	1.96	0.25	27.37	75.0	0.01	
21	**- 3	1		37253.33	1.89	0.30	28.28	76.0	0.01	
22	**- 4	1		35430.00	1.94	0.30	29.13	75.0	0.01	
23	**- 5	1	7	41032.00	1.97	0.25	29.94	75.0	0.01	
24	**- 6	1		44584.00	1.89	0.25	30.80	75.0	0.01	
25	**- 7	1		30145.71	1.95	0.35	31.70	80.0	0.01	
26	**- 8	1	8	25290.00	1.99	0.40	32.66	76.0	0.01	
27	**- 9	1		28722.86	1.99	0.35	33.57	76.0	0.01	
28	**-10	1		60475.00	1.82	0.20	34.32	77.0	0.01	
29	**-11	1	10	50000.00	2.00	0.20	35.14	79.0	0.01	
30	**-12	1		58100.00	1.86	0.20	35.95	77.0	0.01	

Cell count (2nd) ALN, 50%, H3

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 PAGE:
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N TUE 20 FEB 2001 10:37
H#: 1 AQC:N QCF:N RCM:Y
RCM-TIME: 0.10 INT:999.95
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#	RCM%	ER
1	29- 1	1		13.00	55.47	1.00	1.42	75.0	5.60	
2	29- 2	1		11.00	60.30	1.00	3.00	75.0	5.08	
3	29- 3	1		13.00	55.47	1.00	4.57	73.0	2.13	
4	29- 4	1	2	8.00	70.71	1.00	6.20	76.0	2.05	
5	29- 5	1		4.00	100.0	1.00	7.83	80.0	1.76	
6	29- 6	1		10.00	63.25	1.00	9.39	73.0	19.15	
7	29- 7	1		3109.00	3.59	1.00	10.97	76.0	0.03	
8	29- 8	1	3	3428.00	3.42	1.00	12.60	73.0	0.03	
9	29- 9	1		4443.00	3.00	1.00	14.17	72.0	0.02	
10	29-10	1		13125.00	1.95	0.80	15.58	74.0	0.02	
11	29-11	1		10884.21	1.97	0.95	17.17	74.0	0.02	
12	29-12	1		11631.11	1.95	0.90	18.69	72.0	0.02	
13	29-13	1		4452.00	3.00	1.00	20.32	77.0	0.03	
14	29-14	1		5216.00	2.77	1.00	21.89	72.0	0.02	
15	29-15	1		5360.00	2.73	1.00	23.51	73.0	0.02	
16	29-16	1		14841.43	1.96	0.70	24.77	73.0	0.02	
17	29-17	1	5	14242.67	1.94	0.75	26.08	73.0	0.02	
18	29-18	1		12533.75	2.00	0.80	27.45	76.0	0.02	
19	**- 1	1		20304.00	1.98	0.50	28.56	74.0	0.01	
20	**- 2	1	6	17815.00	1.93	0.60	29.72	73.0	0.01	
21	**- 3	1		16655.38	1.92	0.65	30.94	72.0	0.02	
22	**- 4	1		24813.33	1.89	0.45	31.95	74.0	0.02	
23	**- 5	1	7	22417.78	1.99	0.45	32.96	74.0	0.02	
24	**- 6	1		22253.33	2.00	0.45	33.97	73.0	0.02	
25	**- 7	1		14285.33	1.93	0.75	35.28	77.0	0.02	
26	**- 8	1	8	15747.69	1.98	0.65	36.49	73.0	0.02	
27	**- 9	1		13336.25	1.94	0.80	37.86	74.0	0.02	
28	**-10	1		40212.00	1.99	0.25	38.66	75.0	0.02	
29	**-11	1	10	31794.29	1.90	0.35	39.57	77.0	0.02	
30	**-12	1		37943.33	1.87	0.30	40.42	76.0	0.02	

Cell count (1st) H3, AL-N, 50%

TABLE-2

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

Tube #	Radioactivity for 100 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.65]	µCi/ml (A_t) on counting [dpm/444000]	µCi/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

TABLE-3

Expt. # : AN, 50%, H-3 **Date/Time :** 29 Feb. 2000 / 17:30

Tube #	Coulter count for 100 µl cell suspension	Avg. count	Cells/ml [Avg. count x 400]	pCi/cell [uCi/ml x10^6 / Cells/ml]	Recovery (%)
1	3473, 3378, 3365	3391	1356533	2713067	68
2	3769, 3862, 3883	3824	1529600	3059200	76
3	2594, 2682, 2596	2610	1044000	2088000	52
4	2616, 2535, 2493	2534	1013600	2027200	51
5	3196, 3153, 3156	3154	1261733	2523467	63
6	2979, 3024, 3078	3013	1205200	2410400	60
7	2578, 2630, 2637	2601	1040400	2080800	52
8	2809, 2778, 2828	2791	1116400	2232800	56
~~X~~ 3.2	2502, 2654, 2549	2554	1021733	2043467	51
10	3005, 2944, 2760	2889	1155600	2.311200	58

Bckg. - 14
Mode - 500 µl

ALN-H3 50% H3 MARCH
021401-19-30 [illegible] 3.2.2

TABLE-4

Expt # **Date :**

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	Ab SF	
1	49	44	26	40] 39	30%] 27 = 1	135
2	32	38	40	37	24%	152
3	22	(11)	21	22	21 = 0,78	104
4	24	23	24	24 (17)	24 = 0,89	101
5	(14)	25	25	25 (18)	20 = 0,74	126
6	27	24	—	26	21 = 0,78	121
7	15	15	—	15	14 = 0,52	104
8	30	28	21	26	23 = 0,85	112
32. — 9	36	37	—	37	36 = 1.33	[illegible]
10	20	15	18	14	12 = 0,44	116

No colonies – I plated [illegible] cells / dish
[illegible] X6 – dilution.

X6 [illegible] – 0.5
X3
X4

Mutant expression
Start: Feb. 19 -0day

	($\times 10^6$/plate)
1	~1.4
2	1.5
3	1.0
4	1.0
5	1.3
6	1.2
7	1.0
8	1.1
9	1.0
10	1.1

Day 0.

For Mn — ~250 µl of each sample + 1 ml RPMI w/ Cytoch. B (~2 µg/ml)
on Feb. 19, 2001.

TABLE-3 Cell counts for labeled samples after overnight roll with H-3 (cells were NOT syringe !!!)

Expt. # : AL N, 50%, H-3, **Date/Time :** Feb, 16, 2001

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	~~pCi/cell [uCi/ml x 10^6 Cells/ml]~~	Total # of cells (in 3ml)
1	992, 963, 945				
2	1008, 950, 987				
3	630, 670, 626				
4	969, 704, 451				
5					
6	600, 592, 610				
7	516, 469, 576				
8	527, 515, 513				
~~3~~ 2					
10	638, 556, 655				

Mode - 500gel

Bckgr - 22 counts

TABLE-4 Cell counts after transferring non-labeled cells to labeled ones (cells were NOT syringe !!!) (exp with 50%, H-3)

Expt # : 50%, A, N, H3 **Date :** Feb 16, 2001.

Tube.dilution	~~Colony~~ 1 Count	~~Colony~~ 2 Count	~~Colony~~ 3 Count	Avg ~~Colony~~ count/	~~# of SF~~ cell per/ml
1	2116	2088	2134		
2	2006	1928	1846		
3	1511	1462	1454		
4	1085	1512	1512		
5	1720	1673	1688		
6	1850	1845	1758		
7	1433	1514	1548		
8	1457	15 15	1357		
~~9~~					
10	1472	1489	1511		

Parameters

Date	Feb., 15, 2001		
Experiment No.	Feb., 15, 2001		
Investigator	M.Lenarczyk		
Cell Line	AL-N		
Modifier	none		
Radionuclide	H-3		
Half-life (days)	4500.45	I-125=59.408, H-3=4500.45, Po-210=138.376, I-131=8.02	
Radiation Yield	1	I-125=1.47, H-3=1.0, Po-210=1.0, I-131=8.02	
Radiochemical	3HTdR		
Manufacturer/Lot	NCN/3106-398		
Original Calibration Date/Time	02/15/01/ 12:00	Original Activity Concentration (MBq/ml)	37
Present Calibration Date/Time	Feb., 15, 2001	Time Elapsed Since Original Calibration (d)	0
Fraction of Cells Labeled	0.5	Present Activity Concentration (MBq/ml)	37.00
Liquid Scintillation Cocktail	EcoLume		
Volume of LSC Cocktail (ml)	6		
Volume/Type Counting Vial	7/Plastic vial with cup		
Model of Counter	Beckman LS5000TD		
Counting Efficiency	0.65		
Activity Added (Date/Time)	Feb., 15, 2001/ 18:30		
Cells Washed (Date/Time)	Feb., 16, 2001/ 16:30	Time Elapsed Between Add and Wash (hr)	22.50
Medium Tubes Counted (Date/Time)	Feb., 28, 2001/ 18:26	Time Elapsed Between Add and Count (hr)	312.00
Cell Tubes Counted (Date/Time)	Feb., 28, 2001/ 18:50	Time Elapsed Between Wash and Count (hr)	312.00
Vol. Supernatant Counted (µl)	30		
Vol. Suspension Counted Cell Activity (µl)	100		
Vol. Suspension Coulter (µl)	100		
Coulter Manometer Volume (µl)	500		
Average Coulter Background Counts	14		
Coulter Calibration Parameter	400		
Hemocytometer Counting (Yes or No)?			

Background		
Coulter 1	Coulter 2	Coulter 3
14	14	14

MediumActivity

Experiment: Feb., 15, 2001
Date: Feb., 15, 2001

Tube #	Medium count (CPM) 1st	2nd	3rd	CPM Average	CPM corrected for control	DPM CPM/(y e)	At µCi/ml on counting	Ao µCi/ml at addition [At/e-0.693t/T]	Ao kBq/ml at addition
1	*22*	*12*	*57*	*32*	*0*	*0*	*0*	*0*	*0*
②	*15*	*19*	*67*		*0*	*0*	*0*	*0*	*0*
③	270507	296453	203113	256691	256659	394860	5.9288	5.9407	219.8062
4				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
5	397187	583180	411587	463985	463953	713773	10.7173	10.7388	397.3353
⑥	600373	606500	791106	665993	665961	1024555	15.3837	15.4145	570.3380
7	1005247	1154727	1063660	1074545	1074513	1653096	24.8213	24.8710	920.2272
8	613026	611520	640846	621797	621765	956562	14.3628	14.3916	532.4882
9				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
10	1593580	2277680	1567240	1812833	1812801	2788925	41.8758	41.9597	1552.5075

CellSuspension

Experiment: Feb., 15, 2001
Date: Feb., 15, 2001

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	*29*	*13*	*18*	*13*	*0*	*0*	*0.00000*	*0*	*0.0000*
②	*5*	*6*	*7*		*0*	*0*	*0.00000*	*0*	*0.0000*
③	9608	8503	10525	9545	9532	14665	0.06606	0.06619	2.4491
4	47288	44744	40684	44239	44226	68039	0.30648	0.30710	11.3626
5	19245	19078	18075	18799	18786	28902	0.13019	0.13045	4.8267
⑥	45416	41176	36860	41151	41138	63289	0.28508	0.28566	10.5693
7	105707	99793	102253	102584	102571	157802	0.71082	0.71224	26.3530
8	99000	74233	73920	82384	82371	126725	0.57083	0.57198	21.1632
9	51795	64925	54525	57082	57069	87798	0.39549	0.39628	14.6623
10	126673	109453	96220	110782	110769	170414	0.76763	0.76917	28.4592

CoulterSurvival

riment: Feb., 15, 2001
e/Time: Feb., 15, 2001

Tube #	Coulter count 1st	2nd	3rd	Average	Cells/ml	Hemocytometer Count in Grid 1st	2nd	3rd	4th
1	*3473*	*3378*	*3365*	*3405*	*1356533*				
②	*3769*	*3862*	*3883*	*3838*	*1529600*				
③	2594	2682	2596	2624	1044000				
4	2616	2535	2493	2548	1013600				
5	3196	3153	3156	3168	1261733				
⑥	2979	3024	3078	3027	1205200				
7	2578	2630	2637	2615	1040400				
8	2809	2778	2828	2805	1116400				
9	2502	2654	2549	2568	1021733				
10	3005	2944	2760	2903	1155600				

Tube #	Predicted # Cells Seeded	Actual # Cells Seeded	Colony count 1st	2nd	3rd	Average	PE (%)	SF Uncorrected	SF Corrected
1	200	*136*	*49*	*44*	*26*	*38*	*26.448*	*1.00*	*1.0000*
②	200	*153*	*32*	*38*	*40*				
③	200	104	22	11	21	18	17.241	0.4716	0.6519
4	200	101	24	23	24	24	23.349	0.6201	0.8828
5	200	126	14	25	25	21	16.908	0.5590	0.6393
⑥	200	121	27	24		26	21.158	0.6681	0.8000
7	200	104	15	15		15	14.418	0.3930	0.5451
8	200	112	30	28	21	26	23.588	0.6900	0.8918
9	200	102	36	37		37	35.724	0.9563	1.3507
10	200	116	20	15	18	18	15.288	0.4629	0.5780

B019691

Experiment:
Date/Time: Feb., 15, 2001

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	219.806	4.692	0.4716	0.6519
4	#DIV/0!	22.420	0.6201	0.8828
5	397.335	7.651	0.5590	0.6393
6	570.338	17.539	0.6681	0.8000
7	920.227	50.659	0.3930	0.5451
8	532.488	37.913	0.6900	0.8918
9	#DIV/0!	28.701	0.9563	1.3507
10	1552.508	49.254	0.4629	0.5780

mBq/cell = ? Gy