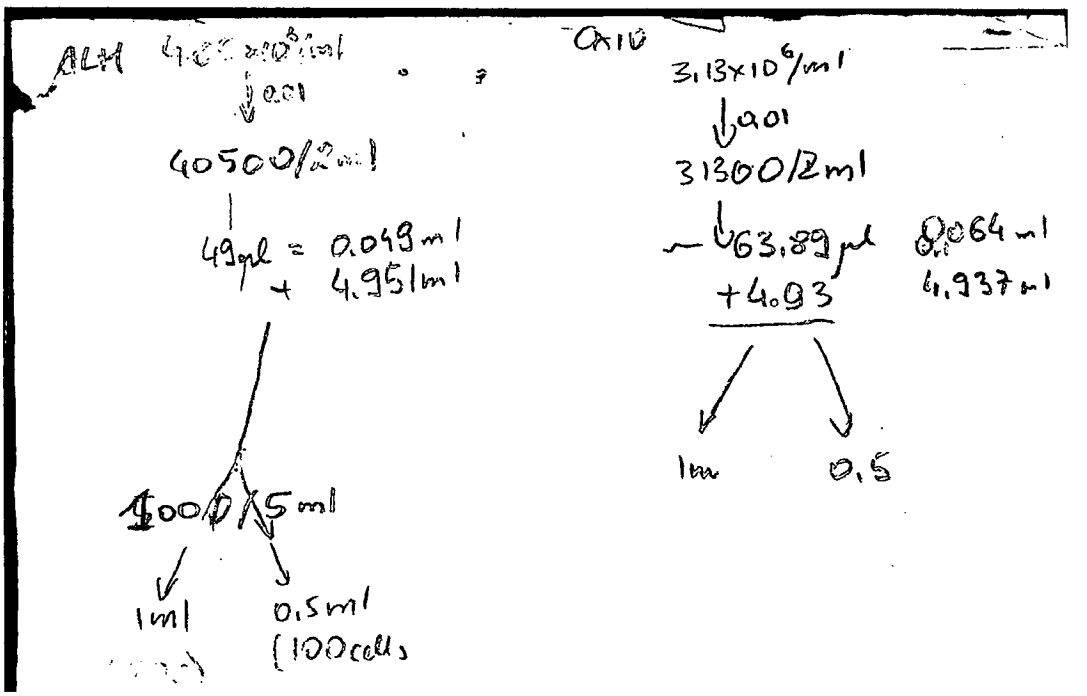

ALH
40500/2ml
49µl = 0.049ml
+ 4.951ml
1ml
0.5ml
(100cells
3.13x10⁶/ml
31300/2ml
63.89µl
+4.93
0.064ml
4.937ml
1ml
0.5

Conical tube, F12 medium.

7 1 AL

Toxicity of AL-N cells for 100% cluster conformation at different temperature
COLONY FORMING ASSAY

Experiment Name : 100% cluster, no labeling); **Exp.**
Investigator: M. Lenarczyk **Date:** Jan., 05, 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 flask, sub-cultured 1:2, 24h before) with HBSS, trypsinize, resuspend in 20 ml F12FCS8, pool, pass 5x through 10 cc syringe with 21 gauge needle, count the cells by transfer 100 µl in Coulter cup containing 20 ml Isotone II

2. Dilute to ~2,000,000 cells/ml in F12FCS8 [Actual count : 2.0[illegible] cells/ml) (100%) 4.14×10^6
3. Transfer 2 ml of cell suspension into 12 ml tubes (Falcon polypropylene test tube, 17x100 mm)
4. Roller the tubes for overnight at 37°C, 5% CO_2 **Date/Time:Jan., 05, 2001 / 18:30**
5 After overnight incubation period, remove tubes from incubator/roller. **Date/Time: Jan., 06, 2000 / 10:30**
6. Pass 5x through 10 cc syringe with 21 gauge needle cell susupension from one tube only, then count the cells by transfer 100 µl in Coulter cup containing 20 ml Isotone II (6) 5063, 5317, 5301 / 3 - 17 * 400 = 2.08 /ml × 2 = 4.17×10^6 (± 100%) After rolling
7. Transfer 2 ml cell suspension from each Falcon tube(s) into conical 15 polypropylene (Stardsted) tube
8. Wash 2 x Falcon tube with 5 ml wash F12NCS8 and transfer into Conical tube
9. Centrifuge at 2000 rpm at 4°C for 10 min (***precooled centrifuge***).
9. Remove buckets from centrifuge.
11. Decant supernatant, click tubes, vortex, re-suspend in 5 ml wash F12, wash Falcon tube with 5 ml F12 and tranfere into conical tube
12. Repeat step 11 three times .
13. Centrifuge tubes for 10 min at 2000 rpm, 4°C
14.Decant supernatant, click tubes, vortex, re-suspend in 5 ml of F12FCS8
15. Centrifuge tubes for 10 min at 2000 rpm, 4°C
16. Decant supernatant, click tubes, vortex,
16a. Resuspend cells in 2 ml F12, sirnge them 5X and re-count cells using Coulter counter (100 ml cells suspension / 20 ml Isotone II). Do thet for one tube only. Then centrifuge those tube, after washing. decant suspernatant, click, vortex @

sample A (4549 + 4701 + 4460)/3 - 17 * 400 : 1.[illegible] = 10^6/[illegible] (87%)
× 2 ml
3.64×10^6 --> in cluster

87% -- recovery after [illegible]

TABLE-3

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

Recovery from elutriator

Temp	Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000] x 6	pCi/cell [uCi/ml x 10^6 Cells/ml]
14°C	1 A	3958, 3820, 3830	3869	1,545333	75 %
8.5	2 B	4853, 4705, 4686	4748	1.896800	92 %
11	3 C	5244, 5281, 5162	5229	2089200	101 %
11	4 D	5671, 5807, 5802	5760	2301600	111 %
10.5	5 E	5270, 5462, 5447	5393	2154800	104 %
10.5	6 F	5807, 5771, 5929	5836	2331867	112 %
	7				
	8				
	9				
	10				

Bckg - 6
Mode - 500 µl

A_L-N cells

Dec. 04-05/2001.

Sample	Temp° for cluster	
A (-drop)	14°C	✓
B	8.5 ~~14.5~~	✓
C	11	✓
D	11	✓
E	10.5	✓
F	10.5	✓

TABLE-3

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

Tube #	Coulter count for 100 ul cell suspension $\times 10^6$/ml 0.5ml	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [uCi/ml x 10^6 / Cells/ml]	Abs PE
1	15 $\times 10^6$ 0.75/5 0.15/ml →	0.075	0.0075	1500	
2	1.9 0.95/5 0.19		0.0095	1900/247	0.13 0.10
3	2.1 1.1/5 0.22		0.0110	2200/288	0.13 0.10
4	2.3 1.25/5 0.25		0.0125	2500/267	0.11 0.09
5	2.2 1.1/5 0.22		0.0110	2200/292	0.13 0.10
6	2.3 1.15/5 0.23		0.0115	2300/252	0.11 0.09
7				200/247	1.24 -1
8					
9					
10					

TABLE-4

Expt # **Date :**

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

	Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
A – 14°C						
B – 8,5°C	200	255	237		492/2 8,C 246	
C – 11°C		324	274	267	288	
D – 11°C		268	282	251	801/3 4.6 267	
E – 10,5°C		288	320	267	875/3 4,3 292	
F – 10,5°C		286	237		523/2 4,7 262	
Control 37°C	200	231	262	250	742/3 247	1

Note 1000x dilution for plated cells only !!! 4 700, 00

CONTROL 37°C AFTER WASH (1/6)

ALL – 100 – 137, 131, 137
200 – 231, 262, 250

Toxicity of AL-N cells for 100% cluster conformation at different temperature
COLONY FORMING ASSAY

RPMI

Experiment Name : 100% cluster, no labeling); **Exp.**
Investigator: M. Lenarczyk **Date:** Jan., 09, 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 flask, sub-cultured 1:2, 24h before) with HBSS, trypsinize, resuspend in 20 ml RPMI1640, pool, pass 5x through 10 cc syringe with 21 gauge needle, count the cells by transfer 100 µl in Coulter cup containing 20 ml Isotone II

2. Dilute to ~2,000,000 cells/ml in RPMI1640 [Actual count : 1 889 867 cells/ml) (2.11ml = 4 000 000 cells)
3. Transfer 2 ml of cell suspension into 12 ml tubes (Falcon polypropylene test tube, 17x100 mm)
4. Roller the tubes for overnight at 37°C, 5% CO_2 **Date/Time:Jan., 09, 2001 / 18:00**
5 After overnight incubation period, remove tubes from incubator/roller.

Date/Time: Jan., 10, 2000 / 11:30

6. Pass 5x through 10 cc syringe with 21 gauge needle cell susupension from one tube only, then count the cells by transfer 100 µl in Coulter cup containing 20 ml Isotone II
7. Take 100 µl cells for to count them (100 ml / 20 ml Isotone II)
8. Take 100 µl cells suspension from only one tube and plate them for survival (CONTROL AFTER ROLLING)

3255 cells/ml → 0.108 ml + 5ml F12 → 1 ml / P60's x 3 = 200 cell/dish
↘ 0.5 ml / P60's x 3 = 100 cell/dish

9. Add 8 ml RPMI1640CS8 to each tube
10. Centrifuge at 2000 rpm at 4°C for 10 min (***precooled centrifuge***).
11. Remove buckets from centrifuge.
12. Decant supernatant, click tubes, vortex, re-suspend in 10 ml wash RPMI1640CS8,
13. Repeat step 12 two times .
14. Re-suspend cells in 2 ml RPMI1640CS8, syringe them 5X and re-count cells using Coulter counter (100 ml cells suspension / 20 ml Isotone II). Do this for one tube only. Then centrifuge tube, decant supernatant, click, vortex @

17. Using 200 µl tips transfer cells suspension into polypropylene Helena micro- tubes with attached caps (Helena Plastics, 400 µl)
18. Again add 200 µl ice cold RPMI1640, re-suspend and transfer the cell suspensions in the same polypropylene Helena micro-centrifuge tubes (Total volume ~400 µl)
19. Centrifuge tubes for 5 min at 1000 rpm, 4°C
20. Transfer tubes at 10°C for 72 h **Date/Time: Jan., 10, 2000 / 13:00**
21. After 72 h, carefully remove the supernatant from the top, re-suspend pellet in 200 µl wash F12 and transfer the content to ten 12 ml tubes (Falcon polypropylene test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash F12 by using Pasteur pipet

Date/Time: Jan., 13, 2000 / 11:45

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 P 60's with 4 ml RPMI1640
    - load tubes with 4.5 ml RPMI1640 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 RPMI1640
29. Centrifuge tubes for 10 min at 2000 rpm, 4°C
30. Repeat step 29
31. Decant supernatant, click tubes, vortex, re-suspend in 2 ml wash RPMI1640CS8, pass five times through 3 cc syringe with 21 gauge needle
32. Count cells using Coulter counter 100 µl cells/ 20 ml Isotone II
33. Vortex tube, transfer 0.5 ml into 12ml 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.
34. 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.
35. Incubate P 60's for 7-9 days
36. After 1 week, wash colonies 3x with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
37. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.

AFTER ROLLING.

TABLE-3-1

Expt. #: AL N, different temp conditions **Date/Time:** JAN. 10, 2000

100% Cluster,

CLUSTER TEMP (°C)

Tube #	Coulter count for 100 ul cell suspension	Avg. count Bckg-18	Cells/ml [Avg. count x 4000]	% Recovery after rolling pCi/cell [uCi/ml x 10^6 Cells/ml]	CLUSTER TEMP (°C)
1	4784, 4924, 4924	4877	1943600	102 %	8/20°
2	4660, 4691, 4534	4628	1844133	97	8/20°
3	4222, 4365, 4164	4250	1692933	89	10.5
4	4812, 4949, 4845	4868	194267	102	10.5
5	5105, 5043, 4862	5003	199410	105	12
6	3755, 3782, 3766	3767	1499867	79	12
7	4908, 5022, 4747	4892	1949733	102	14
8	4334, 4888, 4929	4717	1879600	99	14
9					
10					

After 3x wash with RPMI 1640

	Bckgr.
5146	43
4981	46
5224	69
5117 - 53	

↓ x400 ↓

2.03×10^6 / ml

Bckg - 18
Mode - 500 µl

0 - temp for 1 & 2 was unstable discarded these samples !!

Sample A - 2 was used for re-counting, so final # of cells ≃ 3.6×10^6/cluster

TABLE-4

Expt # **Date :** ALN – 100% cluster RPMI

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

	Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF	
1	–						
2	–					Abs PE	SF
3	200	720	700	801	740	3.2 0.46	0.53
4	200	655	528	608	597	2.4 0.50	
5	200	425	443	514	461	2.61 0.35	0.36
6	200	420	502	427	450	3.00 0.30	
7	200	309	310	309	309	2.6 0.24	0.24
8	200	336	307	291	311	3.1 0.20	
Control		970	857	861	896	3.60 0.90	1

10,5 12° 14°

Plated x 2 dilution based on cell suspension on Table 3.2

↓

1000 x dilution only !!!

CONTR. AFTER ROLL

100 – 563, 546, 552

200 – 970, 857, 861

CONTR AFTER WASH – 37°C

100 –

200 –

Recovery from cluster (after 72 hrs)

TABLE-3 2

RPMI

Expt. # : AL-N, 100% cluster, different temp. **Date/Time :** JAN/13 / 12.30

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] 0/6 - recovery
1	Because of no stable			
2	temp. clusters for sample 1 & 2 were discarded			
3 AN	3995, 4004, 4023	4007	1600400	94/80
4 AN	3050, 3128, 3087	3088	1232933	64/62
5 AN	3183, 3310, 3296	3263	1302800	65/65
6 AN	3886, 3794, 3817	3832	153053	102/77
7 AN	3362, 3383, 3317	3354	1339333	67/65
8 AN	4065, 3998, 3934	4000	1597600	85/80
9				
10				

Temp (°C)
10,5
12
12
14
14

Bckgr. - 5
Mode - 500 pl

vs after rolling / vs before rolling

4

Toxicity of AL-H @ CX 10 cells for 100% cluster conformation at different temperature
COLONY FORMING ASSAY

Experiment Name : 100% cluster, no labeling); **Exp.**
Investigator: M. Lenarczyk **Date:** Jan., 10, 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 flask, sub-cultured 1:2, 24h before) with HBSS, trypsinize, resuspend in 20 ml RPMI1640CS8, pool, pass 5x through 10 cc syringe with 21 gauge needle, count the cells by transfer 100 µl in Coulter cup containing 20 ml Isotone II

CX10 same as ALH
↓
ALH- 0.01+5ml
↓
0.25 or 4.75 FI?
↓
1ml or 0.5ml
(200) (100)
dish dish
only?

2. Dilute to ~2,000,000 cells/ml in F12FCS8 [Actual count AL-H 19777867 @ CX10 2037067 cells/ml)

6.Plate AL-H @ CX10 cells for survival (200 cell/P 60's x3) = CONTROLS 37oC 3.

3. Transfer 2 ml of cell suspension into 12 ml tubes (Falcon polypropylene test tube, 17x100 mm)

4. Roller the tubes for overnight at 37°C, 5% CO_2 **Date/Time: Jan., 10, 2001 / 15:00**

5 After overnight incubation period, remove tubes from incubator/roller. **Date/Time: Jan., 11, 2000 /** 13:00

6. Pass 5x through 10 cc syringe with 21 gauge needle cell susupension from one tube only, then count the cells by transfer 100 µl in Coulter cup containing 20 ml Isotone II

6.a Take 100 µl of cell suspension from one tube only (for each cell line) and plate ALH @ CX10 cells for survival (200 cell/P 60's x3) = CONTROLS AFTER ROLLING

6R — 100µl for survival (for one tube of each line)

v7.Centrifuge at 2000 rpm at 4°C for 10 min (***precooled centrifuge***).

v9. Remove buckets from centrifuge.

v11. Decant supernatant, click tubes, vortex, re-suspend in 10 ml wash RPMI1640CS8,

12. Repeat step 11 two times .

AVOID this step.

16a. Re-suspend cells in 2 ml F12, syringe them 5X and re-count cells using Coulter counter (100 ml cells suspension / 20 ml Isotone II). Do that for one tube only. Then centrifuge tube, decant supernatant, click, vortex @

17. Using 200 µl tips transfer cells suspension into polypropylene Helena micro- tubes with attached caps (Helena Plastics, 400 µl)

18. Again add 200 µl ice cold F12FCS8, re-suspend and transfer the cell suspensions in the same polypropylene Helena micro-centrifuge tubes (Total volume ~400 µl)

19. Centrifuge tubes for 5 min at 1000 rpm, 4°C
20. Transfer tubes at 10°C for 72 h **Date/Time: Jan., 11, 2000 / 17:**
21. After 72 h, carefully remove the supernatant from the top, re-suspend pellet in 200 µl wash F12 and transfer the content to ten 12 ml tubes (Falcon polypropylene test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash F12 by using Pasteur pipet

Date/Time: Jan., 14, 2000 /

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 P 60's with 4 ml F12NCS8
    - load tubes with 4.5 ml F12NCS8 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 RPMI1640CS8
29. Centrifuge tubes for 10 min at 2000 rpm, 4°C
30. Repeat step 29
31. Decant supernatant, click tubes, vortex, re-suspend in 2 ml wash RPMI1640CS8, pass five times through 3 cc syringe with 21 gauge needle
33. Count cells using Coulter counter 100 µl cells/ 20 ml Isotone II
34. Vortex tube, transfer 0.5 ml into 12ml 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.
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. Incubate P 60's for 7-9 days
37. After 1 week, wash colonies 3x with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
38. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.

Note: Only 3 dilution for cell survival.. !!
X5 – stock (2 ml cell suspension) – No dilution
X4 — 0.5 stock + 4.5 medium – 10x dilution
X3 – 0.5 ml of X4 + 4.5 medium – 100x dilution
X2 – 0.5 ml of X3 + 4.5 medium – 1000x dilution

100% cluster vs temp.
RPMI 1640, A_2H_1 + CX10 Cells were 3

22 hrs. at rolling

TABLE-3

Expt. # : ALH & CX10 cell survival at different temp in cluster condition

Date/Time : JAN, 11, 2001 /

A_2H $10^6 \times 1.98$ for rolling 4x10⁶ cell in ml.
CX10 2.04

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]	Temp
AFTER WASHING					
1 A_2H	10262, 9899, 10314	10158	4054933	1.00	(8)
2 A_2H	9266, 9939, 9670	9625	3841600	1.05	(8)
3 A_2H	8794, 9261, 9369	9141	3648133	1.11	10.5
4 A_2H	9329, 9561,	9445	3769600	1.05	10.5
5 A_2H	9928, 10037, 9876	9947	3970400	1.00	12
6 A_2H	9188, 9392, 9408	9329	3723333	1.08	12
7 A_2H	10896, 10896, 11031	10941	4368000	0.91	14
8 A_2H	9539, 10072, 9842	9818	3918667	1.03	14
Bdlg-1					
11771, 11527 11895					
9	8177, 7726, 7699	7867	3138533	1.30	(8)
10	8243, 8165, 8326	8245	3289467	1.25	(8)
CX10					
3 (3.5ml)	7709, 7660, 7688	7686	3065867	1.29	10.5
4	6282, 6171, 6261	6238	2486800		10.5
5	7671, 7568, 7682	7640	3047733		12
6	7655, 7529, 7529	7571	3020000		12
7	7726, 7693, 7573	7664	3057200		14
8	6256, 6235, 6336	6276	2501867		14

Bdlg-21, Mode - 500 µl

O — discarded ina... table temp

TABLE-4

Expt # **Date :**

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

Tube.dilution		Colony 1	Colony 2	Colony 3	Avg Colony	SF
3	0,5	539	526	439	501	
4	0.5	443	466	425	445	
5	0,5	283	282	264	276	
6	0,5	262	286	274	274	
7	0,5	186	200	180	189	
8	0,5	205	213	208	209	
3	0,5	436	404	395	412	
4	1,0	788	~~732~~	796	772	
5	0,5	340	381	378	366	
6	0,5	345	362	357	355	
7	0,5	251	270	250		
8	1,0	456	495	400		

A_LH

CX10

↓ vol plate of X 2 dilution

X2 dilution = 1000 × dilution of stock suspension only !!!

CONTROLS AFTER ROLL

A_LH – 100 – 428
200 – 736, 678, 912

CX10 – 100 – 756, 688
200 – 740

WORK FOR 37°C

ALH 100 – 627, 646
200 – 1000, 950, 920

CX10 100 – 547
200 – 1014, 1050

NOTE: ①Medium was at ~ 20°C, old RPMI reason to loose cells ??
② Aspirated vs pouring off.

TABLE-3

Expt. # : ALM & CX10 (RPMI) 100% different temp. **Date/Time :** JAN, 14, 2001

	Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 400]	pCi/cell [uCi/ml x10^6 Cells/ml]	
	1					
	2					
ALM	3	6891, 7301, 8315	7503	2997600		3.65
	4	5850, 5863, 5659	5791	2312667		3.77
	5	5128, 5348, 5165	5213	2081867		3.97
	6	6302, 6435, 6501	6404	2561467		3.72
	7	6376, 6559, 6596	6501	2600533		4.34
	8	5499, 5541, 5270	5428	2171067		3.92
CX10	93	8393, 8677, 8465	8512	3401067		(3.07)
	104	7858, 7901, 8210	7980	3195067		(2.45)
	5	4802, 4588, 4550,	4638	1855066		(3.05
	6	4918, 5099, 4981	4990	1996133		(3.02)
	7	4789, 4814, 4841	4804	1921600		(3.05)
	8	4341, 4425, 4321	4353	1741333		(2.51

Bdg- 9
Made 500µl

round bottom tube, F12 medium

AL cell survival for cluster condition
(Colony forming assay

Experiment Name : (cluster, no labeling); **Exp.**
Investigator: M.Lenarczyk AL H AND DN6 line **Date:**
Jan/03/2001

1. Set the rocker-roller at 37°C incubator with 5% CO_2, set the Coulter Counter, wash cells (from 150 cm^2 flask, sub-cultured 1:2, 24h before) with PBS with PBS-A (no Ca^{++}, no Mg^{++}) , acutase / trypsinize cells, each re-suspend in F12FCS10 , pool,, count the cells by transfer 100 µl in Coulter cup containing 20 ml isotone (Coulter balanced electrolyte solution) NOTE: a) second 150 flask were used for CX10 cell line, cells were trypsinase (2ml/flask), re-suspend in 15 ml F12FCS10, and spin down, resuspend in 20 ml F12FCS10 pooled with acutase sample, and then passed 5x through 3 cc syringe with 21 gauge needle, b) AL-H were also spin down , resuspend in F12 FCS10 and then passed 5x through 3 cc syringe with 21 gauge needle before they were counted using Coulter counter (100 µl/ 20 ml Isotone II)

2. Dilute to ~4,000,000 cells/ml in F12FCS10 [Actual count : AL H - 2973867 → 1.34 ml + 6.70 F12; DN6 - 3431200 → 4.17 ml + 6.80 F12. cells/ml)
3. Transfer cell suspension into 12 ml tubes (Falcon polypropylene test tube, 17x100 mm) labeled 1-10 both on cap and wall
4. Keep the tubes in the roller for overnight (3-4 hrs + at 37°C, 5% CO_2 **Date/Time:**
   Jan., 03, 2001 / 18:45
5. After ~..... h incubation period, remove tubes
   a) passed the cell suspension 5x through 3 cc syringe with 21 gauge needle (~~for each~~ one tube) only for each cell line
   b) using Coulter counter re-count cells from one sample/test tube (100 µl/20ml Isotene II)
   c) vortex, all centrifuge cells/tubes at 2000 rpm at 4°C for 10 min (***precooled centrifuge***). **Date/Time:**
   Jan., 04, 2001 / ~~10:45~~ 13:15

ad b) DN6 - (6187 + 6351 + 6282)/3 - 17 × 400 = 2.5 × 10^6/ml
AL H - (6816 + 7139 + 7415)/3 - 17 × 400 = 2.84 × 10^6/ml

✓ 11. Decant supernatant, click tubes, vortex, re-suspend in 10 ml wash F12**CS10**
✓ 12. Centrifuge tubes for 10 min at 2000 rpm, 4°C
✓ 13. Decant supernatant, click tubes, vortex, re-suspend in 10 ml wash F12**CS10**
✓ 14. Centrifuge tubes for 10 min at 2000 rpm, 4°C
✓ 15. Decant supernatant, click tubes, vortex, re-suspend in 10 ml wash F12**CS10**
✓ 16. Centrifuge tubes for 10 min at 2000 rpm, 4°C
✓ 17. Decant supernatant, click tubes, vortex, re-suspend in 7 ml of culture F12FCS10
✓ 18. Centrifuge tubes for 10 min at 2000 rpm, 4°C
19. Decant supernatant, click tubes, vortex @ transfer the cell suspension in polypropylene micro

!!! Cells are growing

11
@6
Loose drop from sample A AL H

18A. Recount same sample as in 5c for both lines after sitting the cells 5x

18.B From sampl A → 0.1 ml for SVA. DN6 - 4178 + 4353 + 4401)/3 - 28 × 400 = 1,71 × 10^6/ml | +0.25 = (78%)
AL-H - (4864 + 4975 + 4949)/3 - 28 × 400 = 1.96 × 10^6/ml | + 0.28 (78%)

centrifuge tubes with attached caps (Helena Plastics, 400 µl) using 200 µl pipet tips

20. Again add 200 µl ice cold ~~MEMA~~ F12FCS8, re-suspend @ 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: Jan, 04, 2001 /** 16:30
23. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 µl wash F12NCS8 and transfer the content to 12 ml tubes (Falcon polypropylene test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash F12NCS10 by using Pasteur pipet **Date/Time: Jan., 07, 2001 /**
25. Again add 200 µl wash F12NCS8 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 , P 60's dishes with 4 ml F12FCS10
    - load sterile tubes with 4.5 ml wash F12N CS10 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 F12NCS8
29. Centrifuge tubes for 10 min at 2000 rpm, 4°C
30. Decant supernatant, click tubes, vortex, re suspend in 10 ml wash F12NCS8
31. Centrifuge tubes for 10 min at 2000 rpm, 4°C
32. Decant supernatant, click tubes, vortex, re suspend in 2 ml wash F12FCS8, pass 5X through 3 cc syringe with 21 gauge needle

later 33. Determine cell concentration by transfer 100 µl to Coulter cup

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 200 µl of cell suspension (in triplicate) to 20 ml scintillation vial containing 6 ml cocktail (Aquasol)~~ Ecolume (no-labeling !!!)
37. Incubate P 60's dishes for app. 1 week
38. After 1 week, wash colonies 3 x with normal (1X) saline, and 2 times with methanol. Stain them 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. Jan, 4, 2001

Temperature condition

Sample	A L-H	DN G
A	14° C V	14°C ✓
B	8.5 V	8.5°C V
C	11.° V	11° C ✓
D	11 V	11°C V
E	10.5 V	10.5 V
F	10.5 V	10.5 V

12.5 - lab

10.5 - brown

14.0 - new

14.0 -

10.5

11.

12.0

14.0

10.5
11
12.5
14.0

TABLE-3

Expt. # : A, H & DN6 survival at different temp condition in cluster. **Date/Time :**

	Tube #	Coulter count for 100 µl cell suspesion.	Avg. count	Cells/ml [Avg. count x 400]	% Recovery pCi/cell/~~cluster~~ [uCi/ml x 10^6 Cells/ml]
$A_L H$	1 A	3308, 3202, 3169	3226	1286933	64 %
	2 B	5224, 5084, 5156	5155	2058267	103 %
	3 C	4585, 4913, 4794	4764	1902000	95 %
	4 D	5187, ~~5893~~, 4913, 4893	4998	1995467	99.8
	5 E	4709, 4805, 4758	4757	1899333	95 %
	6 F	5089, 5379, 5198	5222	2085200	105 %
DN6	7 A	2888, 2932, 2920	2913	1161733	58%
	8 B	3470, 3324, 3510	3435	1370267	69 %
	9 C	3702, 3672, 3608	3661	1460667	73 %
	10 D	3293, 3378, 3306	3326	1326667	66 %
	E	4094, 3993, 4008	4032	1609067	81 %
	F	3905, 3803, 3840	3849	1536133	77 %

Background - 9

Node - 500µl

TABLE-4

Expt # : **Date :**

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
A	808				
B	704				
C					
D					
E	—				
F	607	600			

A, H

!!! Volume of plate / x2 dilution = 1000 x dilution of cell suspension

CONTROL – 37°C

ALH 100 – 802, 768, 711
200 – 1131, 1170, 1180

DNG 100 – 617, 529
200 – 1060, 1019, 1023

DNG

A –
B – 8°C – 334
C – 11°C – 732
D –
E –] 10.5 – 784
F –] 10.5 – 674, 440

DN6 – $0.01 \times 1.71 \times 10^6 = 17100$ cells in 0.02 ml F12

1. 17100 x $0.01 < 17.1 < 10^6$ → 17100 + 3.4 ml F12

↓

0.5 ml + 4.5 ml F12 (5000/5ml)

↓

a) 1 ml → P 60's x 3 = ~~200~~ 500 / P 60's
b) 0,5 ml → P60's x 3 = ~~400~~ 250 / P/60's

] 37°

A4 $0.01 \times 1.96 \times 10^6 = 19600$ cells in 0.02 ml F12

$0.01 \times 1.96 \times 10^6 = 19600$ + 3.9 ml F12 → Dea

↓

0,5 ml + 4,5 ml F12 (5000/ml)

↓

a) 1 ml → P60's => 200 / P 60's
b) 0,5 ml → P60's => 100 / P 60's

] 37°

3,42ml

17.100 -

17100 / 0,02 → 5000 / ml.

5000 / 5ml → 0,5 + 4,5

1000 / 5ml

~~1000~~ /

~~5000~~ /

17100 - 3,42

5000 / 1

↓

0,5ml + 4,5ml.

↓ 5000/5ml 1000/ml

↓

1ml × 3 = 200/P

0,5ml × 3 = 100/P

4

DN6

4 × 200 cells in 1 ml × 4

800 cells - 4 ml (

1 ml = 200   3 ml

0,5 ml = 100   1,5 ml

4.5

3 × 200 = 600

3 × 100 = 300

100

x - 1200

1 - $1,7 \cdot 10^6$

1000 - 5ml

1 ml = 200 × 3 = 3ml

0,5 ml - 100 × 3 = 1,5 ml

1,9660 - 4,5 → 3,91

3,9

A, H

5000/ml

↓

0,5 ml + 4,5 ml

↓

~~5000/5ml 1000/ml~~

3 × 200 = 600

1200 cell - ?

1

0,7ml.

17100 / 0,02

1000 / x = 0,0012 ml

↓

17100 / 4,5 ml

~~1ml~~

0,5ml

DN6 -

$1,7 \times 10^6$/ml

$1,7 \times 10^6$ -

~~0,5~~ 10x ← 1000 / 1ml

10000/1ml

17100/x

1,71 - 0,02 =

1,69

$10^6 \times 1,7$ - 1000

$10^3 \times 1,20$ = x = 0,7

0,7µl

10µl

17100/1,71

10000 1 ml

↓

0,5 = 5000

+4,5 (10x)

B019577

Belg - 6410, 6388, 6314 - 2,545,867/ml x 15 → 38188 000
CX10 - ~~5661, 5229, 5707~~ - ~~2223?~~ ~~/ml x 15 = 33,348,000~~ cells
ALN - 12360, 12500, - 5042,267/ml x 15 = 75,634,000 cells

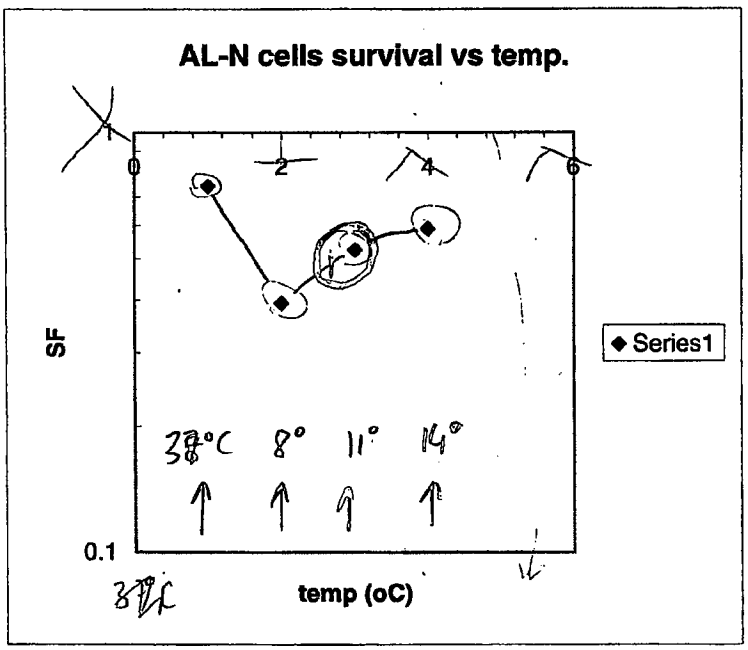
PAGE 1

Exp. Dec, 20, 2000 A_L cells, cluster & temp → SURVIVAL

4x10^6

	Temp	A_L N	CX10			
1.1	37°C	100 / P60's	1.1x3 100 / P60's	- Control I		3
1	37°C	200 cells / P60's	1.2x2 200 / P60's	- Control II		3
(2)	~~15°C~~ 14°C	4x10^6 /cluster	(2) 4x10^6 /cluster	2 CL		
(3)	~~15°C~~	4x10^6 /cluster	(3) 4x10^6 /cluster	2 CL		
(4)	~~15°C~~	4x10^6 /cluster	(4) 4x10^6 /cluster	2 CL		
5	~~15°C~~	200 / P60's	200 / P60's		3x3 B	6
6	~~15°C~~	200 / P60's	200 / P60's			6
7	~~15°C~~ 14°C	200 / P60's	200 / P60's			6
(8)	8°C ~~12 11.2~~	4x10^6 /cluster	(8) 4x10^6 /cluster	2 CL		
(9)	~~12 11.2~~	4x10^6 /cluster	(9) 4x10^6 /cluster	2 CL		
(10)	~~12 11.2~~	4x10^6 /cluster	(10) 4x10^6 /cluster	2 CL		
11	~~12~~ 11.2	200 / P60's	200 / P60's			6
12	~~12~~ 11.2	200 / P60's	200 / P60's			6
13	~~12 11.2~~	200 / P60's	200 / P60's			6
(14)	~~10.5~~ 11.2	4x10^6 /cluster	(14) 4x10^6 /cluster	2 CL		
(15)	10.2 ~~10.5~~ 11.2	4x10^6 /cluster	(15) 4x10^6 /cluster	2 CL		
(16)	10.2 ~~10.5~~ 11.2	4x10^6 /cluster	4x10^6 /cluster	2 CL		
17	~~10.5~~ 11.2	200 / P60's	200 / P60's			6
18	~~10.5~~ 11.2	200 / P60's	200 / P60's			6
19	~~10.5~~ 11.2	200 / P60's	200 / P60's			6
			qtx	18 Hellena's		60
		9x4x10^6 = 36x10^6	9x4x10^6 = 36x10^6		x 4ml	
					P2 240ml	

	37oc	8	11	14
AL-H	118	94	110	96
	184	63	100	140
	144			
	148.6667	78.5	105	118
AbsPE	0.743333	0.3925	0.525	0.59
SF	1	0.528027	0.706278	0.793722



17. Using 200 µl tips transfer cells suspension into polypropylene Helena micro- tubes with attached caps (Helena Plastics, 400 µl)
18. Again add 200 µl ice cold F12FCS8, re-suspend and transfer the cell suspensions in the same polypropylene Helena micro-centrifuge tubes (Total volume ~400 µl)
19. Centrifuge tubes for 5 min at 1000 rpm, 4°C 4569
20. Transfer tubes at 10°C for 72 h **Date/Time: Jan., 06, 2000 /**

✓ 21. After 72 h, carefully remove the supernatant from the top, re-suspend pellet in 200 µl wash F12 and transfer the content to ten 12 ml tubes (Falcon polypropylene test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash F12 by using Pasteur pipet

Date/Time: Jan., 9, 2000 / 15:00

✓ 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 P 60's with 4 ml F12NCS8
    - load tubes with 4.5 ml F12NCS8 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 F12NCS8

✓ 29. Centrifuge tubes for 10 min at 2000 rpm, 4°C

✓ 30. Repeat step 29

✓ 31. Decant supernatant, click tubes, vortex, re-suspend in 2 ml wash F12NCS8, pass five times through 3 cc syringe with 21 gauge needle

33. Count cells using Coulter counter 100 µl cells/ 20 ml Isotone II
34. Vortex tube, transfer 0.5 ml into 12ml 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. Count vials for radioactivity **Date/Time :**
38. Incubate P 60's for 7-9 days
39. After 1 week, wash colonies 3x 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

~~23. Transfer~~ -

Date/Time – Dec 23, 200 / 12:00

24
↓

X-5 - ~ 4x10^6
X-4 - 4x10^5/5
X-3 4x10^4/5
X-2 4x10^3/5 → 4000/5 →

24 - After 72hr → transfer cluster into 12 ml Falcon propylene tube containing 10 ml MEMA wash !!!
↓

26. As same as regular procedure [illegible]
27. Ⓐ 10", 2000 rpm, 4°C → F12 medium
28. ~~Ⓐ 15", 2000 rpm, 4°C~~ Decant supern, click tube, vortex, re-suspen 10ml F12
~~28~~.
28 Ⓐ 10' 2000, rpm, 4°C
29 same as 28
30. Ⓐ 10", 2000, rpm, 4°C
31. same as 28
32. Decant sup, click tube, vortex, resuspen in 2 ml F12 pass 5x, 3cc syringe 21 gauge needle
33. Transfer 100 µl cell susp into coulter vials with 20 ml Ecolume to determine cell concentration
34. Transfer ~~1 ml~~ 0,5 ml cell suspen + 4,5 ml F-12 (X5)
35. vortex X5, '
36 Transfer 0,5 → 4,5 ml F12 (X4)
37. vortex
38. Transfer 0,5ml → 4,5ml F12 (X3), vortex
39. Transfer 0,5ml → 4,5ml F12 (X2) vortex
40 Plate 1 ml of X2 into [illegible] PGO's with 4 ml F12

B019581

± 10%

3.6 – 4.4

TABLE-3

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

10^6 cells in volume (ml)

Temp °C	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]
14°	1 2 ALN	5892, 5839, 5732	5841	2338400	0,490
	2 3 ALN	6119, 6113, 6118	6117	2440667	0.420
	3 4	5792, 5755, 5850	5799	2313600	0,490
7.5-8°	4 8	6634, 6682, 6469	6595	2632000	0,390
7.5-8°	5 9	6891, 7004, 6795		2752667	0, 360
7.5-8°	6 10	6623, 6693, 6666	6660	2658267	0. 370
11	7 14	6378, 6447, 6511	6445	2572133	0, 390
11	8 15	6783, 6870, 6735	6796	2712400	0,370
11	9 16	6768, 6511, 6618	6632	2646933	0,390
14	10 2 ox	5671, 5713, 5698	5694	2271600	0,440
14	3 ox	5022, 5068, 5146	5079	2025467	0,495
7.8°C	8 ox	4965, 4955, 5063	4994	1991733	0.500
7.5-8°C	9 ox	6490, 5844, 6018	6117	2440933	0,420

Backgr - 15 at the end 133

Mode - 500µl

5×10^6/ml

↓

4×10^6 / 2ml

1.1 ALN 100 37°C – 531, 35, 47,
1.2 ALN 200 37°C – 59×2, 92×2, 72×2
(118) (184) (144)

2.2 ALN 14°C – 277, 274, 277,

3,2 ALN 14°C – 327, 118×2 (236)

4,2 ALN 14°C – 394, 348, 319

15,2 ALN 8°C – 324×2, 306×2, 256×2
(648) (612) (512)

16.2 ALN 8°C – 286×2, 266×2, 259×2
(552) (532) (518)

14,2 ALN 8°C – 242×2, 274×2, 282×2
(484) (548) (564)

8.2 ALN 11°C – 345×2, 288×2, 275×2
(690) (656) (550)

9.2 ALN 11°C – 276×2, 271×2, 297×2
(552) (542) (694)

10.2 ALN 11°C – 329×2, 284×2, 308×2
(658) (568) (616)

5526 / 9 = 514

2 ALN 200 14° – 96,

3 ALN 200 14° – 70×2 (140)

14. ALN 200 11° – 55×2, CONT, (110)

15. ALN 200 11°

15 ALN 200 11°C – 50×2 (100)

16 ALN — 11°C – ?

8 ALN 200 7.5C – 57×2 (44)

9 ALN 200 7.5°C – 52×2 or 63

1,1 CX 100 37° – 14, 13, 10

1,2 CX 200 37° – 26, 37, 33

3.2 CX 14° – 93, 78,

2.2 CX10 14° – 46, 48, 45

4

5

6

7

8.2 CX10 8°C – 98, 84, CONT

9,2 CX10 8°C – 101, 49×2, 87 (98)

2 CX 200 14°C – ?

3 CX 14°C –

4 CX 200 14°C – ?

6.3 CX 200 14°C, – 21?

8 CX 200 7.5°C – 10

9 CX 200 7.5°C – 11×2 (22)

10 CX 200 7.5°C – 9?

13,7

ALN

ALN 100 (37°C) - 53, 85, 47 - (45)/100
200 (37°C) 118, 185, 144 - (149)/200

5.2, 6.2, 7.2] ALN (18°) - 648, 552, ⌊__⌋

8, 9] ALN 200 (7.5°C) - 94, 63, ⌊__⌋, ⌊__⌋] 0.33

2.2, 3.2, 4.2] ALN (14°C) - 277, 274, 277
- 327, 236, ⌊__⌋
- 394, 348, 319

2., 3.] ALN 200 (14°) - 96, 140, ⌊__⌋

8.2, 9.2] ALN (11°C) - 690,
- ⌊__⌋

14, 15, 16] ALN 200 (11°) - 110, CONT,
- ⌊__⌋, 100,
- ⌊?⌋

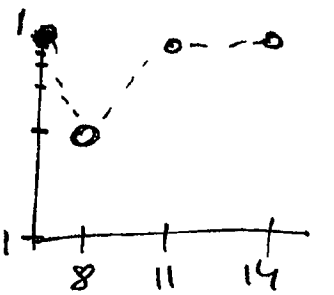
PE / SF

ALN ④ 37°C - 45/100 + 45] 66% - 1.00
149/200 - 74%

② 8°C 77/200 - 33% (7.5% - 0.50
552/⌊__⌋ -

③ 11°C 105/200 - 0.53 - 0.80
694/⌊__⌋ -

⑤ 15°C 118/200 - 0.59 - 0.89
307/⌊__⌋ -



PE SF

CX10 - 100 (37°C) 12/100 - 12
① 200 (37°C)

② 200 (7.5°C) 14/200 - 0.07

③ (11°C)

⑤ (15°C

Experiment:
Date/Time: 12/14/00

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	1.993	0.093	1.3174	0.5613
4	3.725	0.169	1.2399	0.7177
5	7.777	0.340	0.8876	0.4776
6	11.401	0.549	0.9017	0.3631
7	15.083	0.973	0.7397	0.3263
8	19.003	1.742	0.4600	0.2204
9	21.601	1.306	0.3713	0.1418
10	27.115	2.481	0.3713	0.1765