WB Cells
Stacey

WB Protocol

Date: 6/11/00 Ex.#

Exp. Name Name:

1. Set Rocker-Roller in 37°C incubator with 5% CO_2

-turn on coulter counter or have a hemacytometer ready

-turn centrifuge on so it can start to cool

2. Wash 1-150 cm^2 flasks with 5ml of PBS twice and then trypsinize the cells using 3 ml of trypsin and place flasks in incubator for 3-4 min. until cells lift when flask in "smacked"

3. Neutralize the trypsin by adding 7 ml of D-Medium and resuspend the cells by pooling into a 50 ml tube (total vol.=10 ml=3 ml trypsin+7 ml D-Medium)

4. Centrifuge the tube at 2000 rpm, 4°C, 10 min.

5. Decant supernatant, gently break pellet by running tube alon a test tube rack 3 times

-resuspend pellet in 10 ml of D-Medium and vortex

6. gently syringe cells 5 times sing a 5-cc syringe with a 21 gauge needle

7. Do cell count using coulter counter

-100ul of cells in 20 ml of Isotone

-do 3 times: 1. 4418 2. 4541 3. 4428

(if setting is at 50 ul on coulter counter multiply ave. by 4000 or if setting is at 500 ul on coulter counter then multiply ave. by 400)

CELL COUNT: 4462 x 400 = 1,784,800

8. Dilute cells to 2 X 10^6 cells/ml in D-Medium

FINAL VOL: 10.0

ACTUAL CELL COUNT: 1381

9. Transfer 2 ml (4 X 10^6 cells/ml) of cell suspension into six 14 ml Falon polypropylene tubes and label tube and cap

10. Place tubes in Rocker-Roller for 12 hours at 37°C, 5% CO_2

DATE/TIME:

6/11/00

3:40pm

tubes 1, 3, 4 - looks good no clumps

B013072

WBS

Live Ct 4462

Cell Conc = 4462 × 400
= 1,784,800 cell/mL

Need 15mL of 2,000,000 cells/mL
= 30,000,000 cells

Vol. Required = $\frac{30,000,000}{1,784,800}$ = 16.8mL

We need 9 mL of 2,000,000 cells/mL
= 18,000,000 cells

Vol. required = $\frac{18,000,000}{1,784,800}$ = 10.08

1 D-M 1-Mems

UBC

Cove #. 2385

cell conc = 2385 × 400 = 954,000

Need = 6 tubes, 2 mls each = 12 mls
2×10^6 cell/mL = 24 mil cells

Need to make more 15 mls, 2×10^6 cell/mL
= 30 mil cells

Vol. Req. $\frac{30,000,000}{954,000} = 31.4$ mls.

D-mer

9 mls

9 mls of cells
2×10^6 cell/ml

Vol Req. $\frac{18,000,000}{954,000} =$

tube 1 & 2 6/2/00

Day 2: condition 1 (wash and plate)

1. Wash cells with 5 ml of D-Medium and centrifuge at 2000 rpm, 10 min. 4°C

2. Decant and break pellet as previously described, vortex and resuspend cells in 2 ml of D-Medium using a 2 ml pipet

3. Syringe cells 5 times using a 3 cc syringe with a 21 gauge needle

-DO CELL COUNT: tube 1 2019 2559 2506 tube 2 2627 2698

4. Do 4, 10-fold dilutions

-set up 8 dilution tubes: (each condition must be done in duplicate)

-place 4.5 ml of D-Medium into the 2 sets of 4 tubes and label the tubes 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} and also label the dilution tube with the corresponding tube number which contains the cells

5. Perform the dilutions by placing 0.5ml of cells into the dilution tubes 10^{-1}.

-vortex the tube.

-then take 0.5ml of the cells from that dilution tube and place it into the next tube, 10^{-2}

-vortex the tube

-then take 0.5ml of the cells from that dilution tubes and place into the next tube, 10^{-3}

-vortex the tube

-finally do the same for the last dilution tube

6. Seed in triplicate, 1ml of the diluted cells into a 60 mm culture dish, label dish

-Use dilutions-10^{-3} and 10^{-4} *repeat for next 14ml tube

7. Incubate the dishes for 7 days in a 37°C, 5% CO_2 incubator

DATE: 6/2/00 9:00

7 days later:

8. Remove culture dishes from incubator

9. Remove media and label underside of culture dishes selected for count (50-250 colonies)

10. Wash 3 times with normal saline and then 2 times with methanol

11. Stain colonies with 0.1% crystal violet and count colonies under fluroscent light

Day 2: condition 2 (clustors) tube 3

1. Remove tubes from the rocker-roller

2. Wash with 5 ml of D-Medium and centrifuge at 2000 rpm, 10 min., 4°C

3. Decant supernatant and break pellet, as described before, vortex and place cell suspension (200ul) into a Helina tube using a 200ul pipet tip

4. Wash 14 ml test tube with 200 ul of D-Medium and place the wash into the Helina tube using a 200ul pipet tip to get a final vol. of 400ul in the Helina tube

5. Centrifuge the Helina tubes at 1000 rpm, 4°C, 5 min.

6. Place the tubes in Rainin pipet rack and then place into refrigerator at 10.5°C for 72 hours

DATE: 6/2/00 10:00

Day 5: 72 hours later:Date:

1. After 72 hour incubation, remove the Helina tubes from the refrigerator

-set up 2, 14ml tubes with 10 ml of D-Medium in them and label 1 and 2

-remove supernatant from the Helina tube and place into corresponding 14 ml tube

-then break pellet in Helina tube and place the cells into the 14 ml tube

-remove some medium from the top of the 14 ml tube and place it into the Helina tube and wash the Helina tube to collect any remaining cells and place back into the 14 ml tube

2. centrifuge the tubes in a precooled centrifuge for 10 min, 2000 rpm, 4°C

3. While waiting for centrifuge, Label 2 sets of 4 glass dilution tubes with the following dilutions --10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} and labe the dilution tube with the corresponding 14 ml tube #

-place 4.5 ml of D-Medium into each of the dilution tubes

4. When done centrifuging, decant the supernatant and break the pellet as previously described and then vortex.

-resuspend the cells in 2 ml of D-Medium with a 2 ml pipet

-then syringe the cells 5 times using a 3cc syringe with a 21 gauge needle

DO CELL COUNT:

5. Do four-10 fold dilutions

Perform the dilutions by placing 0.5ml of cells into the dilution tube 10^{-1}.

-vortex the tube.

-then take 0.5ml of the cells from that dilution tube and place it into the next tube, 10^{-2}

-vortex the tube

-then take 0.5ml of the cells from that dilution tubes and place into the next tube, 10^{-3}

-vortex the tube

-finally do the same for the last dilution tube

6. Seed in triplicate, 1ml of the diluted cells into a 60 mm culture dish, label dish

-Use dilutions-10^{-3} and 10^{-4}

-Repeat for next 14 ml tube

7. Incubate the dishes for 7 days in a 37°C, 5% CO_2 incubator

8. Following staining and counting procedure as described above.

tube 4

Day 2, condition 3: incubate 72 hours 10.5°C in rocker-roller

1. Remove cells from rocker-roller(37°C)
2. Wash cells with 5 ml of D-Medium and centrifuge tubes at 2000 rpm, 10 min. 4°C 1407
3. Decant the supernatant and break pellet as previously described, vortex collect : 1328
-resuspend the cells in 2 ml of D-Medium and syringe 3 times using a 3cc syringe with a 21 gauge needle 1323
4. Place tubes in a rocker-roller which is in a 10.5°C refrigerator for 72 hours.

DATE: 10:00
6/24/00

Day 5: 72 hours later:

1. After the 72 hours, remove the tubes from the rocker-roller

2. add 5 ml of D-Medium and centrifuge tubes at 2000 rpm,10 min, 4°C

3. Decant the supernatant, break pellet, vortex, and resuspend the cells in 2 ml of D-Medium

4. syringe the cells with a 3cc syringe, 21 gauge needle, 5 times

5. Do four, 10-fold dilutions

-label 2 sets of 4 glass dilution tubes as follows:

10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} and labe the dilution tube with the corresponding 14 ml tube #

-place 4.5 ml of D-Medium into each of the dilution tubes

*Perform the dilutions by placing 0.5ml of cells into the dilution tube 10^{-1}.

-vortex the tube.

-then take 0.5ml of the cells from that dilution tube and place it into the next tube, 10^{-2}

-vortex the tube

-then take 0.5ml of the cells from that dilution tubes and place into the next tube, 10^{-3}

-vortex the tube

-finally do the same for the last dilution tube

6. Seed in triplicate, 1ml of the diluted cells into a 60 mm culture dish, label dish

-Use dilutions-10^{-3} and 10^{-4}

-Repeat for next 14 ml tube

7. Incubate the dishes for 7 days in a 37°C, 5% CO_2 incubator

8. Following staining and counting procedure as described above.

Mediums:

1. MEMA: MEM, 10% heat inactivated FCS, L-Glutamine, pcn/strep

2. MEMB: MEM(Ca free), 10% heat inactivated FCS, L-Glutamine, pcn/strep
   -prevents cells from sticking together
   -for suspension

3. Lindane: a chemical that inhibits formation of gap junctions

4. Triated thymidine: Has short range of 3H β-particles
   -allows only self-irradiation of labeled cells and no cross-irradiation of unlabeled cells

5. DMSO Dimethyl sulfoxide: "eats-up" free radicals, scavanger

6. Wash MEMA: MEM, 10% calf serum, L-Glutamine, pcn/strep

7. D-medium=5%FBS, 50ug/ml gentamicin

New Cell Lines:

Cell	Gap Junction	HGPRT
WB-r	+	-
WB-s	+	+
WB-aB1	-	-

Split cells:

1. chech flask for confluency, 70-80%
2. obtain a waste container
3. remove medium from flask and dispense into waste container
4. wash flask with 10 ml of PBS and remove PBS into waste
5. wash flask with 2 ml of trypsin (use 3ml of trypsin for WB cell line) and put into incubator for 3-6 minutes depending on cell line
6. take out of incubator and "smack" flask to losen cells and then check under scope to make sure majority of cells are off bottom
7. obtain new culture flasks and label with date, cell type, and initials
8. take 25 ml pipet and fill new flasks with 20 ml of new media
9. add 10 ml of media to flask with cell to neutralize the trypsin and note total vol.=
10. put correct amount of cells into new flasks and leave a small amount in the old flask and add 20 ml of media.
11. incubate

Making Media: MEM_

1. obtain a pouch of media which is good to make 1 L
2. fill round bottom flask with about 300ml of sterile H2O
3. add pkt of MEM powder
4. wash pkt out with some sterile water to remove all powder
5. then shake flask
6. move to hood and get 25ml pipet and add 30 ml of sodium bicarb (30ml/L)
7. then take flask back to sterile water and fill to 1 liter mark
8. move back to hood and do filtration
9. put filter on new bottle, pour MEM through the filter and vaccume will pull the media through. *never let filter get dry* trun off vaccume then switch to next bottle
10. add L-glut. PCN/strep, and 10% FCS day of use
11. Label bottle MEM_, date, and whatever has been added

L-Glut., pcn/strep, FCS10%:

1. use 17X100 mm snap cap tubes
2. put 10 ml of pcn/strep into 1 tube and 10ml of L-Glut. into another tube
1tube of L-Glut. and 1tube of pcn/strep will go into 1 liter of media

FCS10%= 100ml in 1liter

Freezing cells:

1. prepare freezing mixture
2. remove media from flasks to be frozen
3. wash cells with PBS-5ml and remove PBS
4. wash cells with trypsin-2ml and place in incubator for 3-6 min. depending on cell type
5. "smack" side of flask to losen cells
6. put 5 ml of media into each flask to neutralize trypsin and the repool cells into a 50 ml tube and centrifuge at 2000 rpm, 7 min
7. decant the supernatant, break-up pellet and resuspend in correct amount of freezing medium, depends on how many tubes freezing
8. place in cryofreezer apparatus.

for WB cells freezing medium: 50ml
-DMSO 10%=5 ml
-FBS 15%= (10% already in d-media) 5ml
-d-medium=40ml

D-MEDIUM

IN 300 ML STERILE, DISTILLED WATER

1. D-powder=8.97 g (GIBCO Formula ~~Cat~~ # 78-5470 EF)
2. Na pyruvate(piruv)=10 ml of 0.1M
3. NaCl=.835 g
4. Glucose= 1g

MIX, place magnetic bar in beaker and place on stirring machiene
-prepare pH meter by placing in buffer solution that has a pH close to what is eventually desired and turn machiene from standby to pH.
-then take pH of the buffer
-once reading is made turn back to standby and rinse probe with dH2O
-test another pH buffer just for accuracy
-then test sample
-leave probe in beaker for next step

5. add NaOH 1M(~ 0.9ml) to get to pH 6.5, while stirring
6. add 1 g of $NaHCO_3$ (sod. Bicarb) ***add slowly or will get ppt**leave stirring rod in place

-get a final pH of ~7.17, leave probe in beaker
-add water up to 1 L

7. then filtrate solution
8. then add FBS to a concentration of 5% so, 50ml FBS in 1000ml media or 25 ml FBS in 500ml media
9. gentamicin 25ug/ml add: 2.5 ml to 1000ml of media

Freezing mixture for V79: 10%DMSO, 15%FBS

-for 100 ml

1. filter 10 ml of DMSO
-use 10 cc syringe and fill barrel with DMSO
-then remove needle and replace with sterile filter
-push down plunger on syringe and dispense DMSO into a sterile test tube
2. if using MEMA which already has 10% FBS then just add 5 ml to bring the % up to 15
-if using new MEMA use 15 ml of FBS
*IN ADDITION, if using New MEMA also need to add pcn/strep, and l-glut.

HARVARD UNIVERSITY
SCHOOL OF PUBLIC HEALTH

LABORATORY OF RADIOBIOLOGY
(617) 432-1184

665 HUNTINGTON AVENUE
Boston, Massachusetts 02115

FACSIMILE TRANSMISSION

FAX NUMBER: (617) 432-0107

ATTENTION: Edouard Azzam

DEPARTMENT: Radiology – Radiation Research

FAX NO: 973 - 972 6474

Telephone: 973 - 972 5323

Total #of sheets: 01 (including cover sheet)

FROM: Sonia de Toledo

DEPARTMENT: C.C.B.

TELEPHONE: 617 - 432 1182

Faxed by: Sonia

COMMENTS:

Habib - Good Morning, the car is in for repair today. I may call you later about the car.

D-Mediums 1 L
*D-powder 8.97 g
Na piruv. 10 mL of 0.1M
NaCl 0.835 g
glucose 1 g

add NaOH 1N (≈ 0.9 mL) to pH 6.5
1 g $NaHCO_3$
final pH ≈ 7.17

Sonia

D- [illegible]

- 2.5 mL sent
- 5% FBS = 50 mL in 1000 mL

(.5 ml / 1000

2000:1

10 µg.

10 mg / ml.

.5

5 mg/ml.

.025 µg 50

5mg 5 / 2000

5 mg in 1000 ml

5000 µg / 1000 ml

5 µg/ml

LIFE TECHNOLOGIES.

Order ID C- 10070

Custom Order Quote

End User Name: ALUPAM BISHAYEE

Phone: (973) 972-6961 *FAX*: (973) 972-6474

Ship To Address: UNIV OF MEDICINE & DENTISTRY @ NJ

Shipping Temp: Ambient

Carrier: AB

Freight Charges: Prepay&Add

Customer Formula #/LTI Catalog Number Modification: *Special Packaging of*

Product Info

Minimum Essential Medium Cat #

with/without	Component Description	Concentration
w	1.5X Amino Acids	
w	1.5X Vitamins	
w	1X L-Glutamine	
w	2X Non-Essential Amino Acids	
w	Earle's Salts	
wout	D-Glucose	
wout	Phenol Red	
wout	Sodium Bicarbonate ($NaHCO_3$)	

SKU	Suffix	Qty	Unit Fill	Pkg size	UOM	Container	QCCODE	QCLeadT	UnitPrice	Storage Temp	SubGrp Descripti
78-5470	EF	10	10	1	L		15AR	0	$104.60	2 to 8C	

1bot. 0.5mL/1L gentamycin

Bridepart

X-46966

4-6 weeks

1000L 20 bottle

500 ml

154.50

3090.00

LIFE TECHNOLOGIES

Order ID C- 10072

Custom Order Quote

End User Name ALUPAM BISHAYEE

Phone (973) 972-6961 *FAX* (973) 972-6474

Ship To Address UNIV OF MEDICINE & DENTISTRY @ NJ

Shipping Temp Ambient

Carrier AB

Freight Charges Prepay&Add

Customer Formula #/LTI Catalog Number Modification: 78-5470 *Special Packaging of*

Product Info

Minimum Essential Medium

with/without	Component Description	Concentration
w	1.5X Amino Acids	
w	1.5X Vitamins	
w	1X L-Glutamine	
w	2X Non-Essential Amino Acids	
w	Earle's Salts	
w	Sodium Bicarbonate (NaHCO3)	
wout	D-Glucose	
wout	Phenol Red	

SKU	Suffix	Qty	Unit Fill	Pkg size	UOM	Container	QCCODE	QCLeadT	UnitPrice	Storage Temp	SubGrp Descriptic
LIQUID	DJ	20	0.5	1	L	P1173		0	$39.55	2 to 8C	
LIQUID	DJ	8	0.5	1	L	P1173		0	$61.75	2 to 8C	

2 weeks

Monday, May 22, 2000

B013088

Experiment Name: V79 mixing **Exp.#:** 5/26/00, 1
Experiment Performed By: A.B. **Date:**

1. Set rocker-roller in 37°C incubator with 5% CO_2
-set coulter counter
-set centrifuge to cool

2. Wash 2- 150 cm^2 flasks with 5 ml of PBS and then Trypsinize the cells using 2 ml of trypsin
-put flasks into incubator for 3 minutes

3. Neutralize the trypsin and resuspend the cells in 8 ml of MEMB

4. Repool the cells in a 50 ml tube and then centrifuge at 1800 rpm, 4°C, 10 min.

5. Decant supernatant, gently break pellet, and resuspend in 10-20 ml of MEMB

6. Gently syringe cells 5 times with a 3cc or 5cc syringe, 21 gauge needle

7. Perform cell count using coulter counter **Cell count**=

8. Dilute cells to ~ 1 X 10^6 cells/ml in MEMB FINAL Vol.=

   - do cell count again to double check-

9. Transfer 2 ml of cell suspension into twenty 14 ml tubes (Falcon plastic test tube-polypropylene) Label each 1-10-each tube will have 2,000,000cells

10. Roll tubes in rocker-roller for 3-4 hours at 37°C, 5% CO_2 **Date/Time**

11. After incubation irradiate 10 of the tubes and keep the other ten in the rocker-roller

*keep the tubes to be irradiated cool

Tube #	Total Dose (Rads)	Dose Rate (Rad/Min)	Time (min)	Attenuation
1	0	0	0	0
2	0	0	0	0
3	500	739.0	.67	X-0
4	700	739.0	.95	X-0
5	1000	739.0	1.35	X-0
6	3000	2981.0	1.01	X-0
7	5000	2981.0	1.71	X-0
8	7000	2981.0	2.35	X-0
9	10,000	2981.0	3.35	X-0
10	30,000	2981.0	10.06	X-0

While irradiating keep tubes over ice, irradiate 1 tube at a time, c̄ "Slot" cut in rack (Acrylic) which will hold tube

12. When done irradiating wash tubes in 8 ml of cold MEMA wash and centrifuge at 2000 rpm, 4°C, 10 min.

13. Then decant supernatant and break up pellet as described before → remove ~ 140 from each pellet & place in Ht tube each.

14. Add the unirradiated cells and media to the irradiated cells, vortex and wash with 5 ml of MEMA wash (6 mls total) corresponding tube IR → 1 2 3 / UL → 1 2 3 (~ 200 ml approx.)

15. Centrifuge the cells for 10 min at 2000 rpm 4°C

16. Decant supernatant and break-up pellet and vortex and transfer the cells to a ~~skinny~~, Helina tube (450 ml) using 200 ul pipet tip

17. Wash test tube with 200 ul of MEMA and transfer to the skinny tube

18. Centrifuge tubes at 1000 rpm, 4°C for 5 min. - 1st place in testtubes

19. Place tubes in a rack and put into refrigerator at 10.5°C for 72 hours
Date/Time: 4/26/00 5:45

20. After 72 hours, remove tubes 1-10, carefully remove the supernatant using a long-necked pasteur pipet and resuspend pellet in 10 ml of MEMA in a 14 ml tube. Wash the skinny tubes with the MEMA to remove all cells.

21. Centrifuge tubes in a precooled centrifuge for 10 min, 2000 rpm, 4°C

22. Label and prepare tubes for dilution and colony dishes
-load 60 mm petri dish with 4 ml MEMA
-load 14 tubes with 4.5 ml MEMA and label them 1-4, 2-4, 3-4, 4-3, 4-3, 5-3, 5-4, 6-3, 6-4, 7-3, 7-4, 8-3, 8-4, 9-3, 9-4, 10-3, 10-4-

23. Decant supernatant, break-up pellet, and resuspend in 10 ml MEMA wash and centrifuge again

24. Decant supernatant, break-up pellet, vortex, and resuspend in 2 ml MEMA and then syringe the cells 5 times using a 3 cc syringe, 21 gauge needle. **Do Cell Count:**

25. Peform four 10-fold dilutions (0.5 ml cells into 4.5 ml MEMA) to get 10^{-1}-10^{-4}

26. Seed 1ml in triplicate into the 60 mm culture dish

27. Then incubate for 7 days in the CO_2 incubator at 37°C

28. Remove dishes from incubator. Remove meduim

29. Wash each dish 3 time with normal saline and 2 times with methanol

30. Stain with 0.1% crystal violet and count colonies

1-4
2-4
1·2
2·2
3·2, 3·3
4·2, 4·3, 4·4
5·2, 5·3, 5·4
6·2, 6·3, 6·4
7·2, 7·3, 7·4
8·2, 8·3, 8·4
9·3, 9-4, 10·3
10·3, 10·4, 10·5

10^{-1} 10^{-2} 10^{-3} 10^{-4}
1-3 1-4
4-2 4-3 4-4

Cell ct 100ml 7 June

Cell 50ml
avg x 4000 cells
Background
00065

(1) 680, ~~736~~, 708, 665

(2) 690, 672, 693

(3) 663, 715, 666, ~~743~~

(4) 670, ~~743~~, 701, 679

(5) 789, 765, 761, 804 → 2nd sample 811 847

(6) Flush → 904, 843, 851

(7) ~~854~~ 671, 663, 719

(8) ~~844~~, 774, 736, 758

(9) ~~863~~, ~~837~~, 732, 748, 711

(10) ~~947~~, ~~851~~, 804, ~~848~~, 759, ~~813~~ 758

804, 759, 758

B013092

V79 COLONY FORMING ASSAY

Experiment Name : ^{137}Cs toxicity (acute, cluster, suspension); **Exp. # : ;**

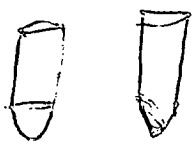
Experiment performed by: **Date:**

Day 1

1. Set the rocker-roller at 37^{0}C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 150 cm^2 flask, subcultured 1:2, 24h before) with 5 mlsPBS, 2mls trypsinize cells, resuspend in 8 ml MEMB for each flask, pool, vortex, pass five times through 3 cc syringe with 21 gauge needle, perform cell count by transfering 100 ul in Coulter cup containing 20 ml isotone (Coulter balanced electrolyte solution) *Cell Count=
2. Dilute to ~4,000,000 cells/ml in MEMB (final volume 11 ml) [Actual count : cells/ml)
3. Transfer 0.5 ml of cell suspension into twenty 14 ml tubes (Falcon plastic test tube, 17x100 mm) labeled 1-10 both on cap and wall
4. Roll the tubes for 3-4 h at 37°C, 5% CO_2 **Date/Time:**
5. After ~3-4 h incubation period, remove tubes, add 8 ml wash MEMA, vortex and centrifuge at 2000 rpm at 4°C for 10 min (***precooled centrifuge***). **Date/Time:**
6. Decant supernatant, click tubes, vortex, resuspend in 3 ml wash MEMA cold
7. Centrifuge tubes for 10 min at 2000 rpm, 4°C
8. Decant supernatant, click tubes, resuspend in 200 ul ice cold MEMA,
   -Irradiate 10 of the tubes with the proper dosages
   -vortex remaining ten tubes of "stock cells" to prevent clumping
9. After all of the tubes have been irradiated, wash the tubes twice with 2 ml MEMA wash
   **after first wash add the 200 ul of unirradiated cells to each of the ten 17 X 100mm tubes.
10. After last decant, vortex and one tube at a time using a 1000 ul sterile micropipet tip
    -transfer the cells into a "skinny" (Helina) tube
    -wash the skinny tube with 1000ul of MEMA
    *Centrifuge the skinny tubes at 1000 rpm, 4°C, 5min.
11. Transfer tubes at 10°C for 72 h. **Date/Time:**

Day 3

12. After 72 h, for tubes 1-5, carefully remove the supernatant using long-neck Pasteur pipet, resuspend the pellet in 400 ml



Coulter Counter

- push power buttons on both machines at same time. - Hit correct CT
- ISOtone II - PC Back-ground check
  - maintains osmolarity

- turn to reset.
- ① 500ul = 5 sec count
- 500ul = 12 sec count
- then turn to count
- after flush system
① open fill knob   clockwise
② open reset
③ close rest
④ close fill

C. cells

100 uL of cells in 20mls ISOtone II

- leave on same setting for flush & count
- mix cells

5.672x10^6 cells/mL = 5.672x10^6 cells/mL / 1x10^6 Con

2x10^6 cells/2mL need
45mls - need } need 45mls of 1x10^6 per mL
45x10^6 tot. cells needed.

B013094

13. The tubes were irradiated using Mark I irradiator (^{137}Cs gamma-ray), two tube (one tube for pellet and one for the suspension) at a time for a single dose-point, while placing onto a Rainin pipet tip box containing ice as per the Table below

Tube #	Total Dose (Rad)	Dose rate (Rad/min)	Time (min)	Attenuat.
1	0	0	0	0
2	0	0	0	0
3	300	97.3	3.08	X-10
4	600	739.8	0.81	X-0
5	1200	739.8	1.62	X-0
6	0	0	0	0
7	0	0	0	0
8	300	97.3	3.08	X-10
9	600	739.8	0.81	X-0
10	1200	739.8	1.62	X-0

14. After irradiation, carefully remove the supernatant from the top for tubes 6-10, resuspend pellet in 200 ul wash MEMA and transfer the content from all tubes to ten 14 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
15. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 14 ml tubes
16. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***
17. Labeling and preparation of dilution tubes and colony dishes
    - load 60 mm petri dishes with 4 ml MEMA
    - load T-tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2, X.3, X.4, X.5 etc.
18. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
19. Centrifuge tubes for 10 min at 2000 rpm, 4°C
20. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times

5/26

Initial cell count ⟶ 1449, 1374, 1431

Avg cell count = 1418

Cell conc. = 1418 × 4000

= 5,672,000 cells/mL

we need 45 mL of 1000,000 cells/mL

= 45,000,000 cells

Vol of reagents = $\frac{45,000,000}{5,672,000}$

= 7.9 mL

did 45 mLs b/c need 20 tubes of 2 mL + 4 mLs extra.

Take 8 mL of stock
Add 37 mL of diluent

Avg = 268

275
241
288

Cell conc = 1,072,000 cells/mL

Freezing tubes ≈ 1.5 mls cell & media per tube

~~[illegible]~~

Freezing mixture . prepare 50 ml

add ~~DMSO~~ — 10% — 5 ml

FBS — 15% — ~~7.5 ml (8.0 ml)~~ + 5.0 ml

d-medium — 40 ml

mena 5/26

~~[illegible]~~mls FBS 10%

45

5ml L glu

5ml strep/pen

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