RRRC Culture & Cryopreservation of Rat Embryonic Stem Cells

Cover Page

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1. Materials

1.1 Equipment:

Inverted microscope

Benchtop Centrifuge

Water bath (37℃)

Incubator (37℃, 5% CO_2, high humidity)

Sterile biosafety cabinet

Pipet-Aid

1.2 Supplies:

Milli-Q water

Beakers: 250 ml, 500 ml

Hemocytometer

Minifuge

70% alcohol in wash bottle

Kimwipe

Item	**Company**	**Cat #**
Serological pipettes		
5 ml	Fisherbrand	13-678-11-D
10 ml	Fisherbrand	13-678-11-E
25 ml	Fisherbrand	13-678-11
9-inch glass pasteur pipets	Fisherbrand	13-678-20-D
Pipette tips, 20 µl, 200 µl, 1000 µl		
Plastic Conical tubes		
15 ml	Fisher (Corning)	430055
50 ml	Fisher (Corning)	430290
Eppendorf tubes (Natural)		
0.5 ml	Fisherbrand	05-408-120
1.5 ml	Fisherbrand	05-408-129

Eppendorf tubes (Amber)		
0.5 ml	Fisherbrand	05-408-125
Tissue culture plates		
60 mm	Fisher (BD Falcon)	353004
4-well	Fisher (Nunc)	144444
6-well	Fisher (BD Falcon)	353046
12-well	Fisher (BD Falcon)	353043
24-well	Fisher (Corning)	3526
Filter bottle units (SFCA membrane, pore size: 0.2 μm)		
250 ml	Fisher (Thermo Scientific)	09-740-39A
500 ml	Fisher (Thermo Scientific)	09-740-28C
Steriflip-GP 50 ml filter units (pore size: 0.2 μm)	Fisher (Millipore)	SCGP00525
Syringe (optional, refer to pg 6)		
5 ml	Fisherbrand (BD Falcon)	301603
10 ml	Fisherbrand (BD Falcon)	309604
Millex-GP 0.22 μm filter (optional, refer to pg 6)	Fisherbrand (Millipore)	SLGP033RS
NALGENE® Cryo 1℃ Freezing Container	Fisher (Thermo Scientific)	5100-0001
CryoTube™ vials	Fisher (Thermo Scientific)	375353

1.3 Reagents

Reagents	**Company**	**Cat #**
0.1% gelatin solution (optional, refer to pg 10)	Millipore	SF008
HCL	Sigma	H9892
High-grade ethanol (200 proof, absolute, anhydrous)	Pharmco-AAPER	111000200CSGL
Embryo transfer water (optional, refer to pg 6)	Sigma	W1503
GMEM	Sigma	G5154
DMEM/F12	Sigma	D6421
Neurobasal™ medium	Invitrogen	21103-049
Fetal bovine serum (FBS)	HyClone	SH30070.03
Penicillin/Streptomycin	Invitrogen	15140-122

B-27 supplement	Invitrogen	17504-044
GlutaMAX™-I	Invitrogen (Gibco)	35050-061
β-mercaptoethanol	Millipore	ES-007-E
Insulin	Sigma	I1882
apo-Transferrin	Sigma	T1147
Progesterone	Sigma	P8783
Putrescine	Sigma	P5780
Sodium selenite	Sigma	S5261
BSA 7.5% solution	Invitrogen	15260-037
Accutase solution	Sigma	A6964
0.05% Trypsin/EDTA (optional, refer to pg 12)	Invitrogen	25300-054
Dimethyl sulfoxide (DMSO)	Sigma	D2438

1.4 Inhibitors

Inhibitors	**Company**	**Cat #**
CHIR99021	Stemgent	04-0004
	or	
	BioVision	1677-5
PD0325901	Stemgent	04-0006
	or	
	Selleck	S1036
PD184352 (optional, refer to pg 9)	Selleck	S1020
SU5402 (optional, refer to pg 9)	Calbiochem	572630

1.5 Feeder Cells

Feeder cells	**Company**	**Cat #**
CF-1	Millipore	PMEF-CF

Materials section complete

2. Stock solution preparation

(Nichols and Ying, 2006)

Refer to Rat ES cell media worksheet II

Notes:

1. Prepare and aliquot all stock reagents at least one day prior to preparing media.
2. Here, you have the option to filter sterilize all stock solutions individually, or you can filter the final N2B27+2i medium following preparation.
3. To filter smaller volumes of reagents, you can use a 5 or 10 ml syringe along with a screw-top 0.22 μm filter, or a 50 ml steriflip filtration device; as opposed to the larger and more expensive filter bottle units.
4. The RRRC uses embryo transfer water for all stock solutions requiring sterile water.

apo-Transferrin 100 mg/ml stock solution: Dissolve 500 mg in 5 ml sterile water overnight at 4℃. Prepare 1 ml aliquots and store at -20℃ for up to 1 year.

BSA 7.5% solution: Prepare 1 ml Aliquots in 1.5 ml Eppendorf tubes and store at -20℃ for up to 1 year.

Insulin 25 mg/ml stock solution: Dissolve 100 mg insulin in 4 ml sterile 0.01 M HCl overnight at 4℃. Prepare 100 µl aliquots in 0.5 ml amber Eppendorf tubes. Store at -20 ℃ for up to 1 year.

Progesterone 0.6 mg/ml stock solution: Dissolve 6 mg in 10 ml high-grade ethanol. Prepare aliquots (volume is of your own preference) and store at -20℃ for up to 1 year.

Putrescine 160 mg/ml stock solution: Dissolve 1.6 g in 10 ml sterile water. Prepare 0.5 ml or 1 ml aliquots and store at -20℃ up to 1 year.

Sodium selenite 3 mM stock solution: (Important! Prepare this solution in a fume hood). Based on the balance in your lab, you may need to prepare a second dilution to make a 3 mM stock. To do this, first dissolve 25.9 mg in 5 ml sterile water to make a 30 mM stock and then add 0.5 ml of this stock into 4.5 ml sterile water to obtain a 3 mM stock solution. Prepare 0.5 ml aliquots and store at -20℃ for up to 1 year.

3. Inhibitor Preparation

Refer to Rat ES cell media worksheet IV

Calculations:

2 inhibitors	MW	Working conc	Stock (1000X)
CHIR99021	465.35	3 µM (1,396.05 µg/L)	1,396 µg/ml (3 mM, 2 mg/1.43 ml DMSO)
PD0325901	482.20	0.5 µM (241.1 µg/L)	241.1 µg/ml (0.5 mM, 2 mg/8.2 ml DMSO)

1. Prepare inhibitors as follows:

Catalog #	Inhibitor	Amount	DMSO	Aliquot
Stemgent 04-0004	CHIR99021	2 mg	1.43 ml	200 µl
Stemgent 04-0006	PD0325901	2 mg	8.2 ml	200 µl

2. Prepare aliquots in 0.5 ml amber Eppendorf tubes, label and store at -20℃ up to 1 year.

Inhibitor preparation section complete

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4. Medium Preparation

Note:

1. Prepare and aliquot all stock reagents at least one day prior to preparing media.
2. If at any time during the culture of rat ES cells the sterility of any reagent or medium becomes compromised, perform a second round of filtration to re-sterilize the solution(s).

4.1 Mouse Embryonic Fibroblast (MEF) Medium:

Refer to Rat ES cell media worksheet I

GMEM + 10% FBS + 1% GlutaMAX™-I (2 mM) + 1% penicillin/streptomycin: For 250 ml MEF medium, add 25 ml FBS, 2.5 ml GlutaMAX™-I solution and 2.5 ml penicillin/streptomycin solution to 220 ml GMEM and filter. Store at 4 ℃ and use within one month.

4.2 N2B27+2i Medium (Nichols and Ying, 2006)

Refer to rat ES cell media worksheets III & V

1. Prepare 10 ml N2 stock solution (100X) (insulin-free):

Refer to rat ES cell media worksheet III

Note: N2 stock solution is also commercially available and contains insulin in the formulation. However, N2 stock made in-house is prepared insulin-free. The insulin is added near the end of N2B27+2i medium preparation to ensure that precipitation of this reagent does not occur. Insulin will precipitate at pH 7.2-7.4 at high concentration.

Briefly spin tubes in microfuge before use to bring all contents down to bottom of tube.

Mix: 1 ml apo-Transferrin
0.67 ml BSA
33 µl progesterone — Aliquot at 1 ml
100 µl putrescine
10 µl sodium selenite — Store aliquots at −20 ℃ up to 1 year
8.187 ml DMEM/F12

2. Prepare 200 ml rat ES cell media with 2 inhibitors (N2B27+2i)

Refer to rat ES cell media worksheet V

Briefly spin tubes in microfuge before use to bring all contents down to bottom of tube.

Beaker 1- **DMEM/F12-N2 medium**	Beaker 2- **Neurobasal-B27 medium**
100 ml DMEM/F12	100 ml Neurobasal medium
1 ml N2 stock	2 ml B27
	1 ml GlutaMAX™-I
N2B27+2i medium	
Mix contents of both beakers by pouring one into the other	
Immediately rinse the empty beaker 10X with Milli-Q water to avoid residue from forming on the glass	
Stir media with 5 ml serological pipet tip	
With constant stirring, add 100 µl insulin **drop-by-drop**, stir well to avoid precipitation***** of the insulin	
CHIR99201 200 µl	
PD0325901 200 µl	
β-mercaptoethanol 2 ml	
Mix well	
Pour into 250 ml filter bottle unit	
Immediately rinse the empty beaker 10X with Milli-Q water to avoid residue from forming on the glass	
Aliquot media into tubes @ 40 ml each	
Store at 4℃; use within one month	

*Insulin is added drop-wise, along with constant stirring to prevent insulin precipitation into the medium (as suggested by Dr. Ying; personal communication).

Note: N2B27 medium can be prepared using two or three inhibitors (2i or 3i); the Rat Resource & Research Center (RRRC) currently uses 2i medium. Refer to the list of references located at the end of this document for complete information regarding these media types.

4.3 Cryopreservation Medium

Refer to Rat ES cell media worksheet VI

Make fresh immediately prior to freeze the cells.

Mix: 90% MEF medium

10% DMSO Filter sterilize and store on ice

5. Methods

5.1 Rat ES Cell Culture

Notes:

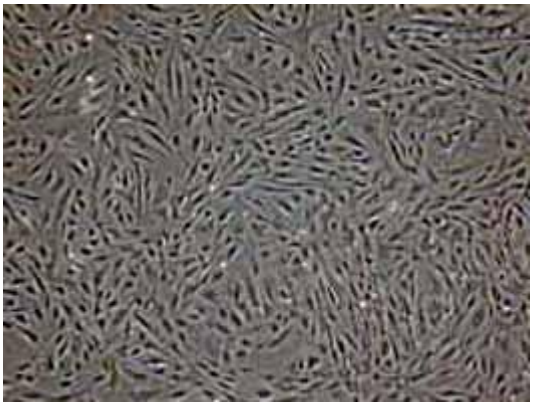
1. Always warm culture media prior to getting started each day. Ethanol-sterilize tubes and place in incubator at 37℃ for 10 min.
2. It is suggested across various cell culture protocols to gelatinize culture vessels prior to plating feeder cells; this is an optional step.
3. Always have feeder plates prepared at least one day prior to plating ES cells.

5.1.1 Plate feeder cells and rat ES cells

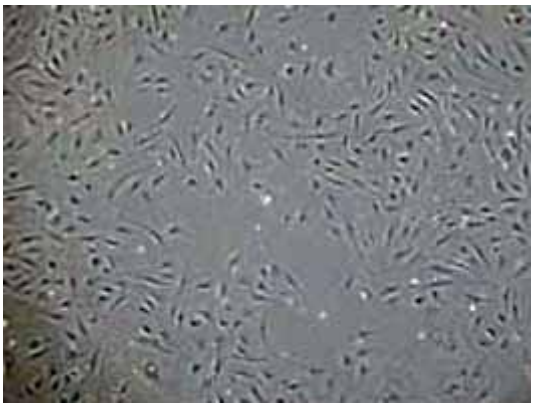
1. Plating MEF feeder cells: One day prior to ES cell culture, thaw one vial of CF-1 feeder cells in 37 ℃ water bath by agitating the vial in the water; after thawing, sterilize vial with 70% ethanol. Using a 5 ml serological pipette, transfer the feeder cells aseptically (in a hood) into a 50 ml conical tube (first take 3 ml MEF medium and then the medium in the vial and expel into 50 ml tube). Centrifuge the cell suspension at 200 g for 3 min. Aspirate the supernatant and resuspend cells in the appropriate volume of MEF media. Plate the cells at the density suggested by the manufacture (usually one vial of feeder cells can plate five to eight 60 mm plates). Transfer the plates to an incubator at 37 ℃ with 5% CO_2 and maximal humidity (90%-100%). Change MEF medium the following day, and every 48 h thereafter.

2. The rat ES cells were cryopreserved at approximately 1×10^6 cells/vial and can be plated onto one 60 mm tissue culture plate per vial of cells.

3. On the day of ES cell culture, before thawing the ES cells, take a look at the feeder cell plate to check if the feeders grew well enough for plating ES cells. Refer to the pictures on the next page for Millipore's recommendations on feeder cell density.



MEFs at correct density



Density is too low

Density is too high

4. Since the ES cells were cryopreserved in 1 ml NALGENE cryovials, there is a possibility that liquid nitrogen (LN_2) can get into the vials when they are stored, and this is a potential explosive hazard when thawing in a 37 ℃ water bath. To prevent this from happening, place the vial on dry ice in a small foam box with a cover, before thawing the ES cells. Let the vial sit in the foam box for a few minutes to allow the LN_2 to evaporate (alternatively, the vial can also be put into a -80 ℃ freezer for a few minutes if the freezer is not too far away from the liquid nitrogen dewar). The vial can be subsequently thawed in 37 ℃ water bath in a manner similar to feeder cell thawing; sterilize with ethanol.

5. Using a 5 ml serological pipette, pick up 3 ml N2B27+2i medium and then the medium in the vial, and transfer all to a 15 ml conical tube. Centrifuge the cell suspension at 150 g for 4 min. Aspirate the supernatant, and resuspend the cells in 4 ml N2B27+2i. Aspirate the medium from a 60 mm feeder cell plate and transfer the ES cell suspension to the plate. Replace the medium daily with fresh N2B27+2i. (**Note:** Components in the N2B27+2i ES cell media are light sensitive; it is suggested the media be kept away from light if possible).

6. Care is taken when changing media on ES cell plates due to poor attachment of rat ES cells to the feeder cells (due to low levels of integrin expression) (Buehr et al. 2008; Li et al. 2008). Aspirate the medium slowly when changing the media to prevent the unwanted removal of ES cell colonies. Alternatively, change half volume (~2 ml) of the medium or simply add half volume (2 ml) of fresh medium to each culture dish. Rat ES cell colonies usually attach to the feeders well when they are small.

5.1.2 Passage rat ES cells

1. Rat ES cells are routinely passaged every 48-72 hr. Sterile-filtered accutase solution or 0.05% trypsin solution is used for disassociating the cells (Buehr et al. 2008; Li et al. 2008). Since N2B27+2i is a serum-free medium, a PBS wash step prior to enzymatic treatment is not necessary.

2. To passage, detach ES cell colonies from feeder layer by gently pipetting up and down the ES cell culture medium several times. Collect the ES cell colonies into a 15 ml centrifuge tube and centrifuge at 150 g for 4 min. after aspirating the supernatant, resuspend the ES colonies in the 15 ml centrifuge tube with appropriate amount of accutase solution or 0.05% trypsin/EDTA and incubate 5-8 min at 37^{o} C. Following the incubation, gently pipette the solution 3-5 times to break up any remaining cell clumps (minimize air bubbles). After the ES colonies become a single cell suspension, centrifuge at 150 g for 4 min. Aspirate the supernatant and re-suspend the cells in appropriate volume of N2B27+2i. Aspirate the MEF medium from the appropriate number of feeder cell plates and transfer the cell suspension to each plate. For culture using 60 mm plates, rat ES cells are routinely passaged at a ratio of 1:5 or 1:6.

5.1.3 Cryopreserve rat ES cells

1. To freeze rat ES cells, begin by passaging the cells as described in section 5.1.2.

2. On the day of freezing cells, obtain single cell suspension as described in section 5.1.2. Count cells using a hemocytometer.

3. Pellet cells by centrifuging tube at 150 g for 4 min.

4. In the hood, aspirate the medium and store cell pellets on ice. Resuspend the cell pellet in the appropriate volume of freshly prepared freezing medium based on the total cell number. The cells are usually frozen at 1×10^6 cells/ml. Aliquot 1 ml of cells per cryotube and place these vials into the NALGENE CRYO 1 ℃ freezing container.

5. Place the freezing container(s) into a -80℃ freezer overnight and transfer vials to liquid nitrogen the next day, for long-term storage.

References

Buehr M, Meek S, Blair K, Yang J, Ure J, Silva J, McLay R, Hall J, Ying QL, Smith A. 2008. Capture of authentic embryonic stem cells from rat blastocysts. Cell 135(7):1287-1298.

Li P, Tong C, Mehrian-Shai R, Jia L, Wu N, Yan Y, Maxson RE, Schulze EN, Song H, Hsieh CL, Pera MF, Ying QL. 2008. Germline competent embryonic stem cells derived from rat blastocysts. Cell 135(7):1299-1310.

Nichols J, Ying QL. 2006. Derivation and propagation of embryonic stem cells in serum- and feeder-free culture. Methods Mol Biol 329:91-98.

Tong C, Li P, Wu N, Yan Y, Ying QL. 2010. Production of p53 gene knockout rats by homologous recombination in embryonic stem cells. Nature 467(7312):211-213

Disclaimer

This protocol is intended for use as an internal SOP at the RRRC. Each laboratory should amend this protocol to be consistent with the specific aspects and procedures of their individual laboratory.

Worksheet I: Preparation of MEF medium

Refer to Rat ES cell SOP, page 8

1. Prepare MEF medium for feeder cells as follows:

Catalog #		Reagent	250 ml	500 ml	Actual Volume	LOT #
Sigma	G5154	GMEM	220	440		
Hyclone	SH30070.03	FBS	25	50		
Invitrogen	15140-122	Penicillin/streptomycin	2.5	5		
Invitrogen	35050-061	GlutaMAX™-I	2.5	5		

2. Filter solution through a 250 ml or 500 ml filter bottle unit; label, store at 4℃ and use within one month.

Filter sterilized: YES / NO

References

Buehr M, Meek S, Blair K, Yang J, Ure J, Silva J, McLay R, Hall J, Ying QL, Smith A. 2008. Capture of authentic embryonic stem cells from rat blastocysts. Cell 135(7):1287-1298.

Li P, Tong C, Mehrian-Shai R. Jia L, Wu N, Yan Y, Maxson RE, Schulze EN, Song H, Hsieh CL, Pera MF, Ying QL. 2008. Germline competent embryonic stem cells derived from rat blastocysts. Cell 135(7):1299-1310.

Nichols J, Ying QL. 2006. Derivation and propagation of embryonic stem cells in serum- and feeder-free culture. Methods Mol Biol 329:91-98.

Today's date:_____

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Worksheet II: Preparation of Stock Solutions

Refer to Rat ES cell SOP, page 6

1. Prepare each stock reagent, aliquot into the appropriate tube, label and store as follows:

Catalog #	Reagent		Dissolve			Aliquot volume	Tube size/color	Storage conditions		Actual		LOT #	Filter sterilized
			amt	solution	notes			Temp	Length	Amt	Vol		
Sigma T1147	apo-Transferrin		500 mg	5 ml embryo transfer water	Overnight at 4℃	1 ml	1.5 ml natural	-20℃	1 yr				
Invitrogen 15260-037	BSA		N/A	N/A	-	1 ml	1.5 ml natural	-20℃	1 yr				
Sigma I1882	Insulin		100 mg	4 ml sterile 0.01M HCl	Overnight at 4℃	100 µl	0.5 ml amber	-20℃	1 yr				
Sigma P8783	Progesterone		6 mg	10 ml high-grade ethanol	-	50 µl	0.5 ml amber	-20℃	1 yr				
Sigma P5780	Putrescine		1.6 g	10 ml embryo transfer water	-	100 µl	0.5 ml amber	-20℃	1 yr				
Sigma S5261	Sodium Selenite	1st dilution	25.9 mg	5 ml embryo transfer water	= 30 mM stock	N/A	N/A	N/A	N/A				
	Prepare in fume hood	2nd dilution	0.5 ml of 30 mM stock	4.5 ml embryo transfer water	= 3 mM stock	50 µl	0.5 ml amber	-20℃	1 yr				

References

Buehr M, Meek S, Blair K, Yang J, Ure J, Silva J, McLay R, Hall J, Ying QL, Smith A. 2008. Capture of authentic embryonic stem cells from rat blastocysts. Cell 135(7):1287-1298.

Li P, Tong C, Mehrian-Shai R. Jia L, Wu N, Yan Y, Maxson RE, Schulze EN, Song H, Hsieh CL, Pera MF, Ying QL. 2008. Germline competent embryonic stem cells derived from rat blastocysts. Cell 135(7):1299-1310.

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12/12/2011 **Your initials:**_____

Worksheet III: Preparation of N2 Stock Solution

Refer to Rat ES cell SOP, page 8

1. Prepare N2 stock solution by adding the following components in a 15 ml or 50 ml conical tube: Briefly spin reagent tubes in microfuge before use, to bring all contents down to bottom of tube.

N2 Stock	10 ml	20 ml	Actual volume	LOT #
apo-Transferrin	1 ml	2 ml		
BSA	0.67 ml	1.34 ml		
Progesterone	33 µl	66 µl		
Putrescine	100 µl	200 µl		
Sodium selenite	10 µl	20 µl		
DMEM/F12	8.187 ml	16.374 ml		

Filter sterilized: YES / NO

2. Aliquot N2 stock solution at 1.0 ml (1.5 ml natural Eppendorf tubes); store at -20℃ up to 1 yr.

References

Buehr M, Meek S, Blair K, Yang J, Ure J, Silva J, McLay R, Hall J, Ying QL, Smith A. 2008. Capture of authentic embryonic stem cells from rat blastocysts. Cell 135(7):1287-1298.

Li P, Tong C, Mehrian-Shai R. Jia L, Wu N, Yan Y, Maxson RE, Schulze EN, Song H, Hsieh CL, Pera MF, Ying QL. 2008. Germline competent embryonic stem cells derived from rat blastocysts. Cell 135(7):1299-1310.

Nichols J, Ying QL. 2006. Derivation and propagation of embryonic stem cells in serum- and feeder-free culture. Methods Mol Biol 329:91-98.

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Worksheet IV: Preparation of CHIR99201 and PD0325901 inhibitors

Refer to Rat ES cell SOP, page 7

1. Prepare inhibitors as follows:

Catalog #	Inhibitor	Amount	DMSO	Aliquot	Actual		LOT #	
					Amt (mg)	DMSO (µl)	inhibitor	DMSO
BioVision 1677-5	CHIR99021	2 mg	1.43 ml	200 µl				
Stemgent 04-0006	PD0325901	2 mg	8.2 ml	200 µl				

Filter sterilized: YES / NO

2. Prepare aliquots in 0.5 ml amber Eppendorf tubes, label and store at -20℃ up to 1 yr.

References

Buehr M, Meek S, Blair K, Yang J, Ure J, Silva J, McLay R, Hall J, Ying QL, Smith A. 2008. Capture of authentic embryonic stem cells from rat blastocysts. Cell 135(7):1287-1298.

Li P, Tong C, Mehrian-Shai R. Jia L, Wu N, Yan Y, Maxson RE, Schulze EN, Song H, Hsieh CL, Pera MF, Ying QL. 2008. Germline competent embryonic stem cells derived from rat blastocysts. Cell 135(7):1299-1310.

Nichols J, Ying QL. 2006. Derivation and propagation of embryonic stem cells in serum- and feeder-free culture. Methods Mol Biol 329:91-98.

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12/12/2011

Worksheet V: Preparation of N2B27+2i medium

Refer to Rat ES cell SOP, pages 8 & 9

1. Prepare N2B27+2i medium as follows:

	200 ml	**400 ml**	**Amt**	**LOT #**
Beaker 1				
DMEM/F12	100 ml	200 ml		
N2 stock	1 ml	2 ml		
Beaker 2				
Neurobasal™	100 ml	200 ml		
B27	2 ml	4 ml		
GlutaMAX™-I	1 ml	2 ml		
Mix contents of both beakers by pouring one into the other				
Immediately rinse the empty beaker 10X with Milli-Q water to avoid residue from forming on the glass				
Stir media with 5 ml serological pipet tip				
With constant stirring, add insulin **drop-by-drop**, stir well to avoid precipitation of the insulin				
Insulin	100 µl	200 µl		
CHIR99201	200 µl	400 µl		
PD0325901	200 µl	400 µl		
β-mercaptoethanol	2 ml	4 ml		
Mix well				
Filter solution through a 250 ml filter bottle unit				
Immediately rinse the empty beaker 10X with Milli-Q water to avoid residue from forming on the glass				
Aliquot media into tubes @ 40 ml, label				
Store at 4℃; use within one month				

Filter sterilized: YES / NO

References

Buehr M, Meek S, Blair K, Yang J, Ure J, Silva J, McLay R, Hall J, Ying QL, Smith A. 2008. Capture of authentic embryonic stem cells from rat blastocysts. Cell 135(7):1287-1298.

Li P, Tong C, Mehrian-Shai R. Jia L, Wu N, Yan Y, Maxson RE, Schulze EN, Song H, Hsieh CL, Pera MF, Ying QL. 2008. Germline competent embryonic stem cells derived from rat blastocysts. Cell 135(7):1299-1310.

Nichols J, Ying QL. 2006. Derivation and propagation of embryonic stem cells in serum- and feeder-free culture. Methods Mol Biol 329:91-98.

Today's date:_____

Your initials:_____

12/12/2010

Worksheet VI: Preparation of Cryopreservation Medium

Refer to Rat ES cell SOP, page 9

1. Prepare cryopreservation medium by mixing the following components:

Component	Actual volume	Preparation date	LOT #
MEF medium (90%)			N/A
DMSO (10%)		N/A	

2. Filter sterilize and store on ice

Filter sterilized: YES / NO

References

Buehr M, Meek S, Blair K, Yang J, Ure J, Silva J, McLay R, Hall J, Ying QL, Smith A. 2008. Capture of authentic embryonic stem cells from rat blastocysts. Cell 135(7):1287-1298.

Li P, Tong C, Mehrian-Shai R. Jia L, Wu N, Yan Y, Maxson RE, Schulze EN, Song H, Hsieh CL, Pera MF, Ying QL. 2008. Germline competent embryonic stem cells derived from rat blastocysts. Cell 135(7):1299-1310.

Nichols J, Ying QL. 2006. Derivation and propagation of embryonic stem cells in serum- and feeder-free culture. Methods Mol Biol 329:91-98.

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