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Before You Begin

- Immediately transfer the frozen vials to liquid nitrogen storage.
- Read this entire User's Guide before handling or using iPS Cells.
- iPS cells are for life science research use only.
- Only technically qualified individuals experienced in handling DMSO and human biological materials should access, use, or handle iPS cells.

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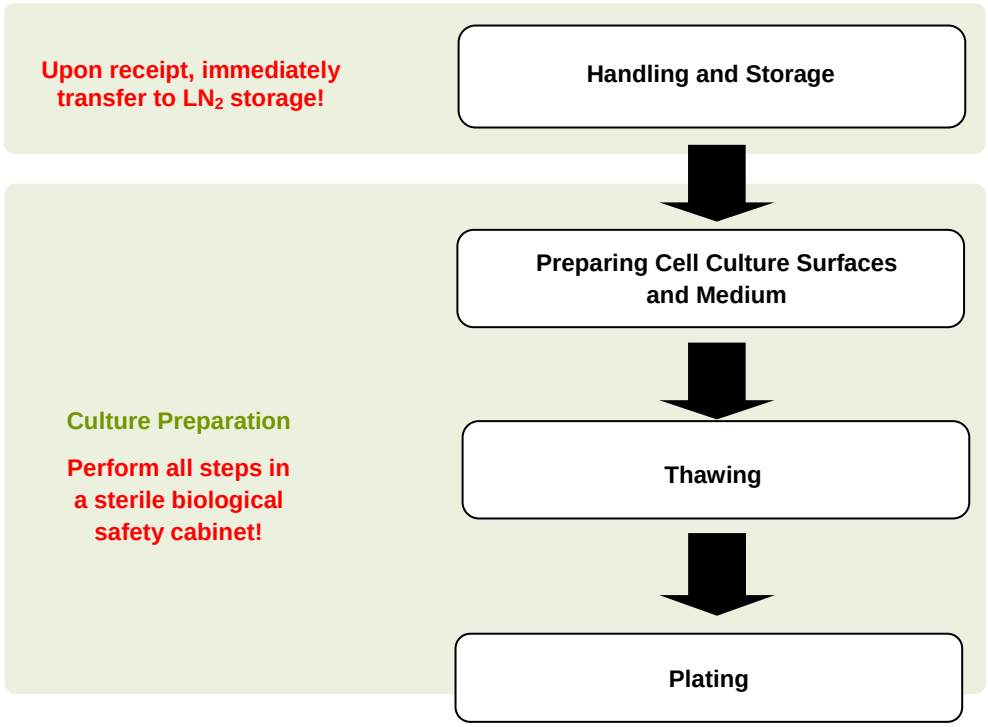
Required Equipment and Consumables

Item	Vendor	Catalog Number
Equipment		
37°C Water Bath	Multiple Vendors	
Biological Safety Cabinet with UV Lamp	Multiple Vendors	
Cell Culture Incubator	Multiple Vendors	
Liquid Nitrogen Storage Unit	Multiple Vendors	
Pipettors	Multiple Vendors	
Tabletop Centrifuge	Multiple Vendors	
Consumables		
15 ml Centrifuge Tubes	Multiple Vendors	
6-well Cell Culture Plates	Nunc	140675
Dulbecco's Modified Eagle Medium:Nutrient Mixture F-12 (DMEM/F-12)	Life Technologies	11330-032
Dulbecco's Phosphate Buffered Saline without Ca^{2+} and Mg^{2+} (D-PBS)	Life Technologies	14190-144
Essential 8 Medium*	Life Technologies	A14666SA
Growth Factor Reduced Corning Matrigel Matrix (Matrigel)*	Corning	354230
Rho Kinase, H1152	EMD Scientific	555550
mTeSR1*	StemCell Technologies	05857
Pipettes	Multiple Vendors	
Sterile Tissue Culture Grade ddH_20	Multiple Vendors	
Vitronectin*	Life Technologies	A14701SA

* iPS cells can be maintained in two different cell culture conditions: (1) Essential 8 Medium and vitronectin or (2) mTeSR1 and Matrigel. Order the necessary components accordingly.

Technical Support and Training

Workflow Diagram



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Chapter 2. Handling and Storage

iPS cells are provided as cryopreserved single-cell suspensions in 1.5 ml cryovials. Upon receipt, directly transfer the cryobox containing iPS cells to the vapor phase of a liquid nitrogen cell storage dewar. We strongly recommend transferring the entire cryobox into the storage rack to avoid transferring individual vials.

> *It is critical to maintain cryopreserved iPS cells at a stable temperature. Minimize exposure of cryopreserved iPS cells to ambient temperature when transferring vials to liquid nitrogen storage.*

Chapter 3. Culturing iPS Cells in mTeSR1

Notes

Cellular Dynamics International (CDI) recommends plating iPS cells into cell culture plates that are pre-coated with Matrigel and used in conjunction with mTeSR1.

The following sections detail preparing Matrigel and mTeSR1 and thawing iPS cells using these reagents.

> *It is critical to perform all subsequent steps in a sterile biological safety cabinet.*

Preparing Matrigel Aliquots

1. Equilibrate a sterile box of 200 µl pipette tips and a sterile container of 1.5 ml tubes at -20°C overnight.
2. Thaw a bottle of Matrigel on ice at 4°C overnight.
3. Calculate the volume of Matrigel required to aliquot 1 mg/tube, which is the amount required to coat a 6-well cell culture plate.

   Note: *Each lot of Matrigel has a different concentration. Perform the calculation each time a new lot of Matrigel is used.*

   $$Volume\ per\ Tube\ = \frac{1\ mg/tube}{Concentration\ of\ Matrigel}$$

   For example, the calculation for a lot of Matrigel with a concentration of 13.841 mg/ml is as follows:

   $$Volume\ per\ Tube\ = \frac{1\ mg/tube}{13.841\ mg/ml} = 0.072\ ml$$

4. Place the 4°C Matrigel, -20°C sterile pipette tips, and -20°C sterile 1.5 ml tubes on ice just before use. Spray the ice container thoroughly with 70% ethanol and place in a biological safety cabinet.
5. Aliquot the calculated volume of Matrigel into 1.5 ml tubes, switching tips when the Matrigel begins to clog the tip and/or the measurement becomes inaccurate. Place the tubes on ice as they are filled.

   > *It is essential to perform this step on ice. Matrigel will solidify at room temperature.*

6. Store aliquots at -20°C for up to 1 year.

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Preparing the 6-well Cell Culture Plate

1. Add 6 ml of ice-cold DMEM/F-12 to a 15 ml centrifuge tube.
2. Remove an aliquot of Matrigel from -20°C.
3. Immediately add 1 ml of the DMEM/F-12 to the aliquot and mix using a 1 ml pipettor, gently pipetting up and down. Transfer the Matrigel solution to the 15 ml centrifuge tube containing ice-cold DMEM/F-12 and mix to achieve a final concentration of 0.167 mg/ml (1 mg Matrigel/6 ml DMEM/F-12).
4. Add 1 ml/well of Matrigel solution to a 6-well cell culture plate. Scale volumes appropriately for other vessel formats to add Matrigel solution at 0.017 mg/cm^2.
5. Incubate at room temperature for at least 1 hour before plating iPS cells.

   Note: *If necessary, wrap the plate in aluminum foil and store at 4°C for up to 1 week. Incubate plates for 1 hour at room temperature before use.*

Preparing mTeSR1

Prepare the mTeSR1 according to the manufacturer's instructions.

Note: *If necessary, store mTeSR1 at 4°C for up to 2 weeks.*

Preparing mTeSR1 + H1152

1. Prepare a 100 µM stock solution of H1152 in sterile tissue culture grade ddH_20 according to the manufacturer's instructions.
2. Prepare the mTeSR1 + 1 µM H1152 in a 50 ml centrifuge tube by diluting 0.1 ml of 100µM H1152 in 10 ml of mTeSR1.
3. Filter the mTeSR1 + 1 µM H1152 using a 0.2 µm filter.

   Note: *If necessary, store the 50 ml centrifuge tube containing mTeSR1 + 1 µM H1152 at 4°C, protected from light, for up to 1 week.*

Thawing iPS Cells into mTeSR1

Maintain iPS cells in liquid nitrogen until immediately before thawing to ensure maximal performance of the cells. Completing the following steps in a time-efficient manner facilitates optimal iPS cells viability and performance.

Note: Cellular Dynamics International (CDI) *does not recommend thawing more than 1 vial at one time.*

1. Equilibrate mTeSR1 and mTeSR1 + H1152 to room temperature, spray with 70% ethanol, and place in a biological safety cabinet.
2. Remove the iPS cells cryovial from the liquid nitrogen storage tank.

   Note: *If necessary, place cryovials on dry ice for up to 10 minutes before thawing.*

3. Immerse the cryovial in a 37°C water bath (avoid submerging the cap) and gently swirl until only a small piece of frozen material remains (approximately 3 - 5 minutes).

4. Immediately remove the cryovial from the water bath, spray with 70% ethanol, and place in a biological safety cabinet.
5. Gently transfer the iPS cells cryovial contents drop-wise to a sterile 15 ml centrifuge tube using a 1 ml pipettor.
6. Rinse the empty iPS cells cryovial with 1 ml of room temperature mTeSR1 to recover any residual cells from the vial. Transfer the 1 ml of mTeSR1 rinse from the cryovial drop-wise to the 15 ml centrifuge tube containing iPS cells suspension.
7. Slowly add 8 ml of room temperature mTeSR1 to the 15 ml centrifuge tube containing iPS cells suspension.
8. Centrifuge the cell suspension at 200 x g for 4 minutes.
9. Aspirate the supernatant to just above the cell pellet.
10. Gently resuspend the cell pellet in 2.5 ml of mTeSR1 + H1152 using a 5 ml serological pipette.

Plating iPS Cells into a Pre-coated 6-well Cell Culture Plate

1. Aspirate the Matrigel solution from 1 well of a pre-coated 6-well cell culture plate.
2. Immediately add the 2.5 ml of iPS cells suspension to the well.
3. Evenly distribute the cells by shaking the plate back and forth then side to side.
4. Culture iPS cells in a cell culture incubator at 37°C, 5% CO_2 for 24 hours.
5. 24 hours post-plating iPS cells, aspirate the spent mTeSR1 + H1152 and replace with 2.5 ml of mTeSR1.
6. Maintain iPS cells according to the mTeSR1 manufacturer's instructions.

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Chapter 4. Culturing iPS Cells in Essential 8 Medium (Xeno-free Conditions)

Cellular Dynamics International (CDI) recommends plating iPS cells into cell culture plates that are pre-coated with vitronectin and used in conjunction with Essential 8 Medium.

The following sections detail preparing vitronectin and Essential 8 Medium, and thawing iPS cells using these reagents.

Preparing the 6-well Cell Culture Plate

1. Dilute the vitronectin in D-PBS to 5 µg/ml according to the manufacturer's instructions.
2. Add 1 ml/well of vitronectin solution to a 6-well cell culture plate. Scale volumes appropriately for other vessel formats to add vitronectin solution at 0.5 µg/cm^2.
3. Incubate at room temperature for at least 1 hour before plating iPS cells.

   Note: *If necessary, wrap plates in laboratory film and store at 4°C for up to 1 week. Incubate plates for 1 hour at room temperature before use.*

Preparing Essential 8 Medium

Prepare the Complete Essential 8 Medium according to the manufacturer's instructions.

Note: *If necessary, store Complete Essential 8 Medium at 4°C for up to 2 weeks.*

Preparing Complete Essential 8 + H1152 Medium

1. Prepare a 100 µM stock solution of H1152 in sterile tissue culture grade ddH_20 according to the manufacturer's instructions.
2. Prepare the Complete Essential 8 + 1 µM H1152 medium in a 50 ml centrifuge tube by diluting 0.1 ml of 100 µM H1152 in 10 ml of Complete Essential 8 Medium.
3. Filter the Complete Essential 8 + 1 µM H1152 medium using a 0.2 µm filter.

   Note: *If necessary, store the 50 ml centrifuge tube containing Complete Essential 8 + 1 µM H1152 medium at 4°C, protected from light, for up to 1 week.*

Thawing iPS Cells into Complete Essential 8 Medium

Maintain iPS cells in liquid nitrogen until immediately before thawing to ensure maximal performance of the cells. Completing the following steps in a time-efficient manner facilitates optimal iPS cells viability and performance.

Note: Cellular Dynamics International (CDI) *does not recommend thawing more than 1 vial at one time.*

1. Equilibrate Complete Essential 8 Medium and Complete Essential 8 + H1152 medium to room temperature, spray with 70% ethanol, and place in a biological safety cabinet.
2. Remove the iPS cells cryovial from the liquid nitrogen storage tank.

   Note: *If necessary, cryovials can be placed on dry ice for up to 10 minutes before thawing.*
3. Immerse the cryovial in a 37°C water bath (avoid submerging the cap) and gently swirl until only a small piece of frozen material remains (approximately 3 - 5 minutes).
4. Immediately remove the cryovial from the water bath, spray with 70% ethanol, and place in a biological safety cabinet.
5. Gently transfer the iPS cells cryovial contents drop-wise to a sterile 15 ml centrifuge tube.
6. Rinse the empty iPS cells cryovial with 1 ml of room temperature Complete Essential 8 Medium to recover any residual cells. Transfer the 1 ml of Complete Essential 8 Medium rinse from the cryovial drop-wise to the 15 ml centrifuge tube containing iPS cells suspension.
7. Slowly add 8 ml of room temperature Complete Essential 8 Medium to the 15 ml centrifuge tube containing iPS cells suspension.
8. Centrifuge the cell suspension at 200 x g for 4 minutes.
9. Aspirate the supernatant to just above the cell pellet.
10. Gently resuspend the cell pellet in 2.5 ml of Complete Essential 8 + H1152 medium using a 5 ml serological pipette.

Plating iPS Cells into a Pre-coated 6-well Cell Culture Plate

1. Aspirate the vitronectin solution from 1 well of a pre-coated 6-well cell culture plate.
2. Immediately add the 2.5 ml of iPS cells suspension to the well.
3. Evenly distribute the cells by shaking the plate back and forth then side to side.

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4. Culture iPS cells in a cell culture incubator at 37°C, 5% CO_2 for 24 hours.
5. 24 hours post-plating iPS cells, aspirate the spent Complete Essential 8 Medium + H1152 and replace with 2.5 ml of Complete Essential 8 Medium.
6. Maintain iPS cells according to the Complete Essential 8 Medium manufacturer's instructions.