Human Induced Pluripotent Stem Cell (iPSC) Handling Protocols (Matrigel and mTeSR Media)

General Guidelines for Handling Human iPSC cells

- iPSC are cryopreserved in plastic cryovials and shipped on dry ice. If storing the iPSC before thawing, store in liquid nitrogen vapor. Storage directly in liquid nitrogen may result in cracking of a-rings.
- It is highly recommended that a small number of vials are cryopreserved as a master stock before beginning any experimentation.

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Media and Reagents

Matrigel

mTeSR1

ROCK Inhibitor (Y-27632)

Dispase or Accutase

Cryopreservation Medium: 50% mTeSRI, 40% Knock-out Serum Replacement, 10% DMSO, 10 LM Y-27632 (ROCK Inhibitor)

Coating Plates With Matrigel

1. Thaw aliquot of Matrigel and add to cold medium and mix well. (this should be performed according to manufacturer's instructions)
2. Immediately use Matrigel solution to coat 6-well plates.
3. Gently swirl plate to spread the Matrigel solution evenly across the plate.
4. Use plates immediately or seal with parafilm and store at 2-8°C for up to 7 days. To use plates after storage at 2-8°C, remove parafilm and place in 37°C incubator for 30 minutes.
5. Aspirate Matrigel and add warm mTeSRl.

Thawing Human iPS cells

1. Remove iPSC from liquid nitrogen vapor or dry ice and thaw quickly in 37°C water bath.
2. Transfer cell suspension to sterile 15 ml tube containing warm mTeSR1 supplemented with 10 M Y-27632 (ROCK Inhibitor) and mix cells.
3. Centrifuge conical tube containing cells at 228 ± 24 g for 2 min at room temperature.
4. Aspirate medium and resuspend cells into 2 ml of warm mTeSRlsupplemented with 10 MY-27632.
5. Plate cells in 1 well of 6-well plate coated with Matrigel.
6. Maintain cells by daily medium exchange. **WARNING**: Failure to replace medium daily can result in spontaneous differentiation.
7. Colonies should be observed within 2-5 days (May vary depends on lines).

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Passaging of Human iPSC

Passage iPSCs when colonies approach borders of an adjacent colony. Ideally, iPSCs should be passaged before individual colonies begin differentiating in the center of colony (approximately 700 microns in diameter). To avoid spontaneous differentiation, do not allow colonies to overgrow.

Split ratios range from 1:3 to 1:6 and are indicated on the Certificate of Analysis for each iPSC line. We recommend that you record the passage information for each cell line to determine the exact growth kinetics of a particular line in your laboratory.

1. Remove spent medium from culture and rinse with PBS.
2. Add I ml/well dispase or accutase and place in incubator for 5 minutes or until edges of colonies begin to roll up.
3. Aspirate enzyme solution.
4. Add 1 ml of warm mTeSRl to cells.
5. Using cell scraper, gently dislodge cells from plate.
6. Transfer the detached cell aggregates to a 15 ml conical tube containing mTeSRI. Rinse each well with an additional I ml of mTeSRl to collect any remaining aggregates. Add the rinse to conical tube containing cells.
   NOTE: Do not dissociate iPSCs into a single cell suspension. Passage iPSCs as clusters containing 10-20 cells. The most effective way to maintain cell clusters is to minimize pipetting ofthe cells.
7. Centrifuge conical tube containing cells at 228 ±24 g for 2 min at room temperature.
8. Remove the supernatant from conical tubes and resuspend cells in appropriate volume of growth medium such that there is an appropriate cell density for cell culture vessel.
9. Seed cells onto prepared Matrigel coated plates.

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Cyropreservation of Human iPSC

Inclusion ofY-27632, an inhibitor ofp160-Rbo-associated coiled-coil kinase (ROCK), in iPSC cryopreservation medium is important to viability after thaw.

1. Remove spent medium from culture and rinse with PBS.
2. Add 1 ml/well dispase and place in incubator for 5 minutes or until edges of colonies begin to roll up.
3. Aspirate enzyme solution.
4. Add 1 ml of warm mTeSR1 to cells.
5. Using cell scraper, gently dislodge cells from plate. Transfer the detached cell aggregates to a 15 ml conical tube containing mTeSR1. Rinse each well with an additional l ml of growth medium to collect any remaining aggregates. Add the rinse to conical tube containing cells.
6. Centrifuge conical tube containing cells at 228 ± 24 g for 2 min at room temperature.
7. Remove supernatant and resuspend cells in pre-cooled cryopreservation medium (1 ml for each well of 6 well plate to be harvested).
8. Transfer 1 ml of cell suspension to cryovials on ice.
9. Place cryovials in isopropanol freezing container and store at -80°C overnight.
10. Transfer vials to liquid nitrogen vapor.

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Table 1. Troubleshooting Tips

Problem	Observation of problem	Possible causes
Spontaneous differentiation	Morphology of differentiated cells can vary but is commonly characterized by 1. hypertrophic colonies 2. colonies without distinct borders 3. flattened cells	1. Low confluency or suboptimal passaging of cells (see "low viability after passage") 2. Poor or inappropriate matrigel quality-this can be prevented by testing matrigel prior to using in an experiment
Non-uniform distribution of colonies within culture vessel	Areas within culture vessel with highly confluent iPSC colonies AND areas with few or no iPSC colonies	Rock plates back and forth gently immediately following plating - usually rocking plates back and forth and then side to side produces a fairly uniform distribution
Low Viability after Passage	Little to no cell colonies are visible 24 hours after passage	During passaging, clusters may have been disrupted into a single cell suspension-decrease pipetting of cells during passaging
Low Viability after Recovery from Cryopreservation	Little to no cell colonies are visible within 4 days after recovery	Lack of or insufficient ROCK inhibitor in cryopreservation medium