



**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 o-rings.
- It is highly recommended that a small number of vials are cryopreserved as a master stock before beginning any experimentation.

**Media and Reagents**

**Matrigel®**

**Rho Kinase (ROCK) Inhibitor (Y-27632)**

**Table 1. Media**

<b>human iPSC growth medium OPTIONS</b>	<b>human iPSC cryopreservation medium</b>
mTeSR™1  OR  Essential 8™ Medium (E8)	90% Knock-Out Serum Replacement  10% DMSO

**Table 2. Dissociation Enzymes** – enzymes for dissociation should be chosen based on downstream assay or information supplied on the Certificate of Analysis supplied with each iPSC line.

<b>Enzyme</b>	<b>Concentration</b>	<b>Incubation Period</b>	<b>Stop Medium</b>
Versene (1X)	N/A	2 minutes or until edges of colonies begin to roll up.	growth medium
Dispase	10 µg/ml	5 minutes at 37° or until edges of colonies begin to roll up.	growth medium



### Coating Plates with Matrigel

1. Reconstitute aliquot of Matrigel with 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 rock plate to spread the Matrigel solution evenly across the plate.
4. Place in 37°C incubator for 30 minutes to 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 incubate for 30 minutes at 37°C.
5. Aspirate Matrigel and add warm mTeSR1 or E8 (culture medium).

### Thawing Human iPSC cells

Perform the following steps in a time-efficient manner to obtain optimal cell viability.

1. Remove iPSC from liquid nitrogen vapor or dry ice and immerse the cryovial in a 37°C water bath. Thaw quickly by gently swirling until only a small piece of frozen material remains. Spray the vial with 70% ethanol before transferring to a biological safety cabinet.
2. Gently add the thawed cell suspension dropwise to 10 ml warm culture medium in a sterile 15 ml conical tube and gently mix cells by swirling.
3. Centrifuge conical tube containing cells at 1100 rpm for 3 min at room temperature.
4. Aspirate supernatant and gently re-suspend cells into 2 ml of warm culture medium supplemented with 10µM Y-27632 Rho kinase (ROCK) inhibitor (Y-compound).
5. Plate cells in 1 well of 6-well plate coated with Matrigel and gently rock plate to evenly distribute cells.
6. Replace with fresh medium without Y-compound. It is important to remove the Y-compound from the medium within 20 hours.
7. Maintain cells by daily medium exchange. **WARNING:** Failure to replace medium daily can result in spontaneous differentiation.
8. Colonies should be observed within 1-5 days.

### 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. 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 1 ml/well of enzyme solution for specified amount of time or until edges of colonies begin to roll up (see Table 2 and refer to the CofA for each cell line)
3. Aspirate enzyme solution.
4. Wash each well with 1ml of growth medium (see Table 2).
5. Add 1 ml of warm growth medium to cells and, using cell scraper, gently dislodge cells from plate. We recommend working with a 5ml or a 2ml pipette but NOT micropipettes since small bore of pipette tips can break colony clumps to smaller size.
6. Transfer the detached cell aggregates to a 15 ml conical tube. Rinse each well with 1 ml of growth medium to collect any remaining aggregates and add to conical tube containing cells.
7. Re-suspend cells in appropriate volume of growth medium for the appropriate cell density for cell culture vessel. We recommend passaging the cells without centrifugation ('no spin' method).
8. Seed cells onto prepared Matrigel coated plates. Rock plate gently to achieve a uniform cell distribution.

### Cyropreservation of Human iPSC

1. Remove spent medium from culture and rinse with PBS.
2. Add 1 ml/well of enzyme solution for specified amount of time or until edges of colonies begin to roll up (see Table 2).
3. Aspirate enzyme solution.
4. Wash each well with 1ml of specified stop medium (see Table 2)..
5. Add 1 ml of warm growth medium to cells and, using cell scraper, gently dislodge cells from plate.



6. Transfer the detached cell aggregates to a conical tube. Rinse each well with 1 ml of growth medium to collect any remaining aggregates and add to conical tube containing cells.
7. Centrifuge conical tube containing cells at 1100 rpm for 3 min at room temperature.
8. Remove supernatant and re-suspend cells in pre-cooled cryopreservation medium from Table 1 (1 ml for each well of a 6 well plate to be harvested).
9. Transfer 1 ml of cell suspension to each cryovial on ice.
10. Place cryovials in isopropanol freezing container and store at -80°C overnight.
11. Transfer vials to liquid nitrogen vapor.

**Troubleshooting Tips**

<b>Problem</b>	<b>Observation of problem</b>	<b>Possible causes</b>
Spontaneous differentiation	<p>Morphology of differentiated cells can vary but is commonly characterized by</p> <ol style="list-style-type: none"> <li>1. hypertrophic colonies</li> <li>2. colonies without distinct borders</li> <li>3. flattened cells</li> </ol>	<ol style="list-style-type: none"> <li>1. Low confluency or suboptimal passaging of cells (see "low viability after passage")</li> <li>2. Poor or inappropriate Matrigel quality-this can be prevented by testing Matrigel prior to using in an experiment</li> </ol>
Non-uniform distribution of colonies within culture vessel	<p>Areas within culture vessel with highly confluent iPSC colonies AND areas with few or no iPSC colonies</p>	<p>Failure to properly rock plates after plating cells. Rock plates back and forth gently immediately following plating - usually rocking plates back and forth and then side to side and diagonally produces a fairly uniform distribution</p>



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 and decrease incubation time with Versene.
Low Viability after Recovery from Cryopreservation	Little to no cell colonies are visible within 4 days after recovery	Lack of or insufficient Rho kinase (ROCK) inhibitor in thawing medium.

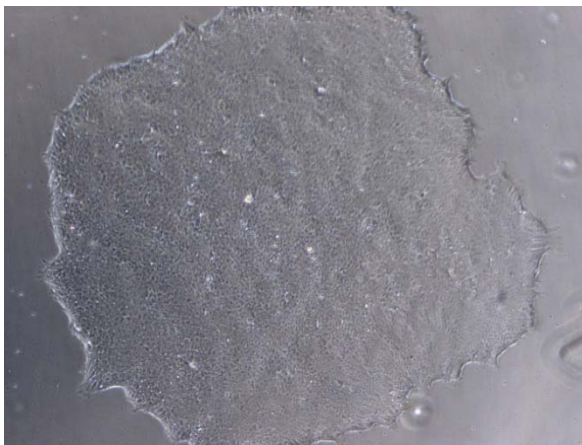


Image 1: Typical image of a human induced pluripotent stem cell (iPSC) colony. Note the distinct borders of the colony and morphology of the iPSCs.

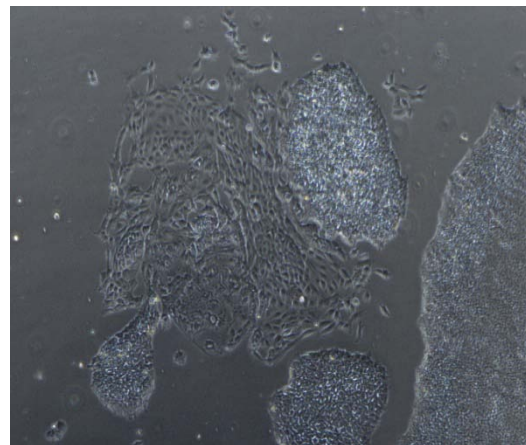


Image 2: Image of a differentiated colony. Note the flattened morphology of the cells and lack of distinct borders.