



Nov 24, 2025

iPSC Cell Culture Protocol

DOI

<https://dx.doi.org/10.17504/protocols.io.n2bvje7xpgk5/v1>

Isabelle Fisher¹, Sally Salomonsson¹, Claire Clelland^{1,2}

¹Weill Institute for the Neurosciences, University of California, San Francisco, CA, USA;

²Department of Neurology, University of California, San Francisco, CA, USA



Claire D Clelland

UCSF Weill Institute for Neurosciences

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.n2bvje7xpgk5/v1>

Protocol Citation: Isabelle Fisher, Sally Salomonsson, Claire Clelland 2025. iPSC Cell Culture Protocol. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.n2bvje7xpgk5/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working



Created: November 24, 2025

Last Modified: November 24, 2025

Protocol Integer ID: 233363

Keywords: ipsc cell culture protocol, ipsc cell culture protocol this protocol, induced pluripotent stem cell, pluripotent stem cell, ipsc, protocol this protocol

Abstract

This protocol describes regular maintenance of induced pluripotent stem cell (iPSC) cultures.

Image Attribution

Fig. 1. Cells on ROCK Inhibitor in colonies of 10-25 cells/colony. The spikey appearance of cells is due to RI. Dense/dark cells are cell debris/cell death and will clear over time. Imaged at 10x magnification.

Fig. 2. Cell Confluency Guide, Imaged at 10x magnification. A. 10% confluency B. 20% confluency C. 60% confluency D. 70% confluency

Fig 3. Example of spontaneous differentiation with spikey cells at edges of colony and lacunar or cobblestone appearance of cells within the colony. Imaged at 10x magnification.

Materials

Reagents:

- Matrigel Growth Factor Reduced (GFR) Basement Membrane Matrix, Phenol Red-free, LDEVfree (Corning, 356231)
- KnockOut DMEM (ThermoFisher, 10829018)
- mTeSR Plus (STEMCELL Technologies, 100-0276)
- ROCK Inhibitor (Y-27632 2HCl) (Selleckchem, S1049)
- PBS, pH 7.4 (ThermoFisher, 10010023)
- ReLeSR (STEMCELL Technologies, 05872)
- CryoStor CS10 (STEMCELL Technologies, 7930)
- DMSO (Sigma-Aldrich, D8418)
- Antibiotic-Antimycotic (100X) (ThermoFisher, 15240062)

Equipment:

- Thermo Scientific™ Nunc™ Cell-Culture Treated Multidishes (Thermo Scientific, 140685)
- Falcon™ 15 mL Conical Centrifuge Tubes (Falcon, 352196)
- CoolCell Containers (Corning, 432002)



Safety warnings

Infection:

1. Signs of bacterial infection:
 - a. Yellow, cloudy medium
 - b. Moving debris visible under the microscope, beyond Brownian motion
 - c. Increased cell death
2. Unless the plate is precious, spray all of the wells in the infected plate with 10% bleach, leave in the sink for at least 30 minutes, and throw away in the biohazard bin.
3. Sterilize the incubator and BSC hood. Notify the lab via email.
4. If the line is precious, it is sometimes possible to save other wells in the plate by treating with antibiotics (any severely infected well should be bleached as this is likely to spread to other wells and the incubator)
 - a. For example, make media with antibiotic-antimycotic 1:100, and feed the plate.
 - b. Effective antibiotics may vary.



Healthy iPSC Maintenance

- 1 Coat four wells of a six-well plate with matrigel solution and incubate at 37°C for at least 30 minutes.
To make matrigel solution, resuspend matrigel 1:100 in KnockOut DMEM.
- 2 Make thawing medium:
Add ROCK inhibitor (RI) 1:1000 to mTeSR Plus media (ex: 20 mL medium with 20 µL RI).
Warm medium in a bead bath.
- 3 Frozen cells should be kept on ice until ready to thaw. Thaw rapidly in a water bath or by holding tube in hand. Transfer cells suspended in freezing media to a 15 mL falcon tube with 2-5 mL of dPBS.
- 4 Centrifuge cells at 150-300 rcf for 3 minutes.
While cells are spinning, replace matrigel on six well plate with 2 mL warm thawing medium per well.
- 5 Carefully aspirate supernatant from centrifuged cells by tilting the tube to the side, avoiding close contact with the pellet at the bottom of the tube.
- 6 Gently resuspend the cell pellet in 1 mL thawing medium.
- 7 Transfer cell solution into the six well plate with media. Optionally, make a concentration gradient between wells, for example:
Add 400 µL, 300 µL, 200 µL, 100 µL of resuspended cell solution into each well, respectively.
- 8 Once cells are in colonies of at least 4-6 cells per colony, replace thawing media with mTeSR Plus without RI, ideally after about 24 hours and up to 48 hours.
- 9 Continue to feed every day, until 50-70% confluent.
If cells are single cell or very small colonies, may opt to feed every other day until 10-20% confluency or large colonies.

10

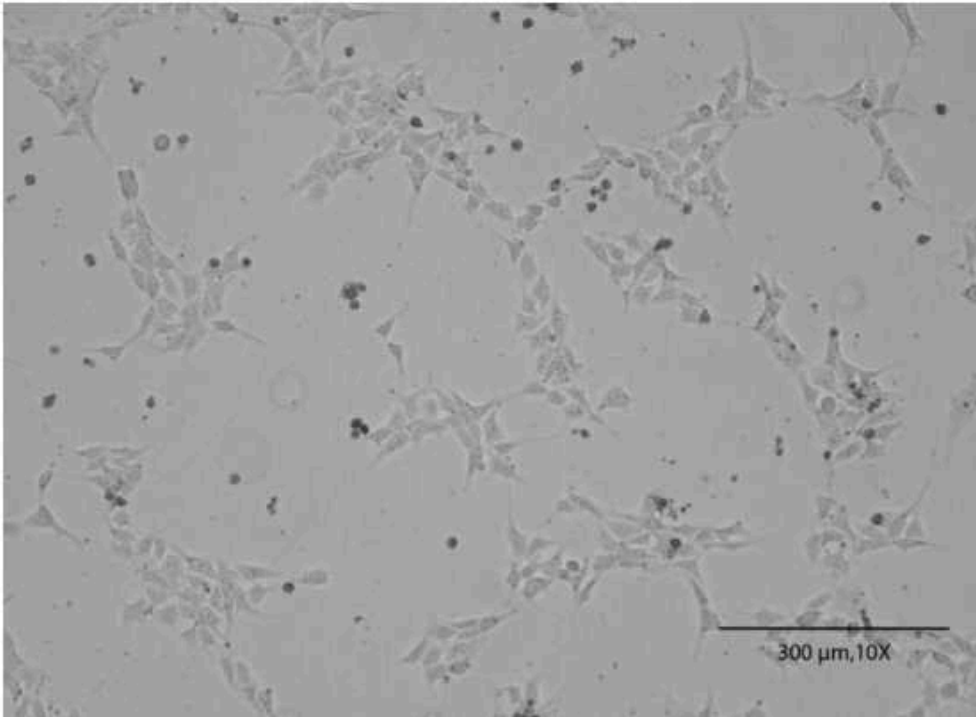


Fig. 1. Cells on ROCK Inhibitor in colonies of 10-25 cells/colony. The spikey appearance of cells is due to RI. Dense/dark cells are cell debris/cell death and will clear over time. Imaged at 10x magnification.

Passaging iPSCs

- 11 When cells are 50-70% confluent, they should be passaged.
tip: we 'split' or passage cells before their density outstrips the nutrient capacity of the culture media and while they are still in log growth phase. Once cells are approaching 100% confluency, they reach the plateau (stationary) phase where fewer than 10% of cells are actively dividing, and the cells are more susceptible to stress. This low rate of division makes it hard to maintain the line and increases the chances of unwanted mutations accumulating in the line over time.
- 12 Coat a six-well plate with matrigel solution and incubate at 37°C for at least 30 minutes. To make matrigel solution, resuspend matrigel 1:100 in KnockOut DMEM.
- 13 Aspirate medium from a well with healthy iPSCs at appropriate confluency.
- 14 Wash once with dPBS.



- 15 Add one mL of ReLeSR and incubate at room temperature in the hood for 30-40 seconds.
- 16 Aspirate ReLeSR.
- 17 Incubate dry well at 37°C for 3-9 minutes.
While incubating, replace matrigel with warm mTeSR Plus on the new plate (optionally add RI at 1:1000).
- 18 Resuspend cells with one mL mTeSR Plus.
To resuspend, pipette mTeSR Plus over the well to lift cells. Only go over the well 1-2 times, leaving behind cells that do not lift without mechanical force (the cells that do not lift are the unhealthy cells).
- 19 Distribute resuspended cells across 1-6 wells of your new plate.
- 20 Gently rock plate back and forth and side to side to evenly distribute cells across the wells.
- 21 Label plate with cell line, passage number, initials, and date.
- 22 Look at your cells under the microscope to confirm even distribution and check the quantity of cells passaged.
- 23 Leave plate in incubator.
- 24 After 48 hours, replace media with fresh mTeSR Plus without RI.
Continue to feed cells every 24 hours.
When the cells are 50-70% confluent (typically after about three days), passage into a new plate.

25

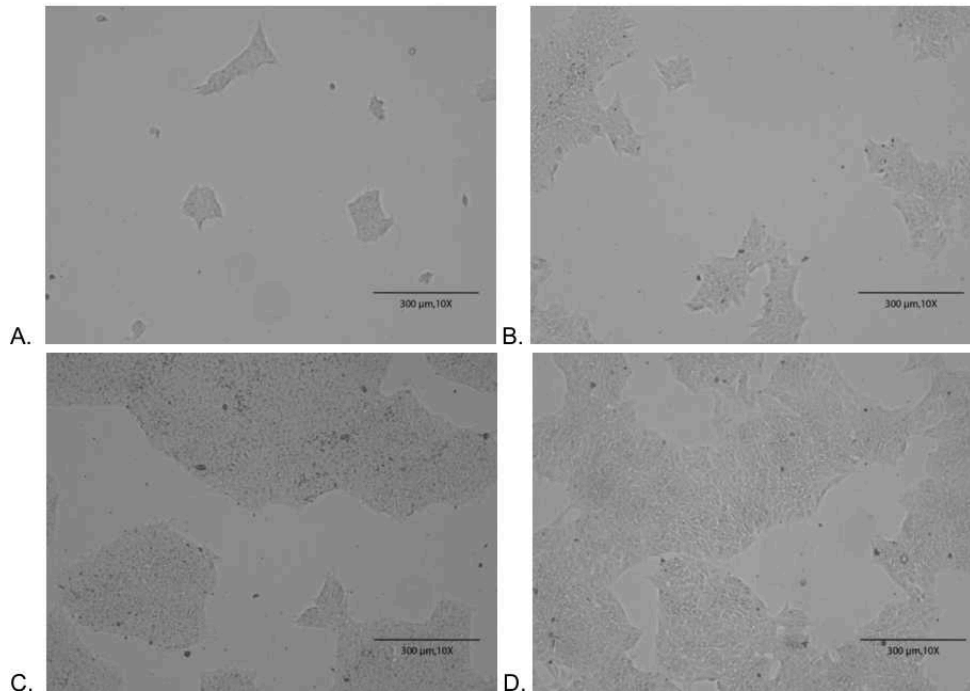


Fig. 2. Cell Confluency Guide, Imaged at 10x magnification. A. 10% confluency B. 20% confluency C. 60% confluency D. 70% confluency

Freezing Cells

- 26 Freeze cells when the well is at 70% confluency.
- 27 Make freezing media:
Make a solution of 90% mTeSR Plus and 10% DMSO
Add rock inhibitor 1:1000
Ex: 9 mL mTeSR Plus, 1 mL DMSO, 10 μ L RI
- 28 Label cryovials with cell line, passage number (freezing counts as a passage), initials, and date.
- 29 Aspirate medium from a well with healthy iPSCs at 70% confluency.
- 30 Wash once with dPBS.
- 31 Add one mL of ReLeSR and incubate in the hood for 40 seconds.

- 32 Aspirate ReLeSR.
- 33 Incubate dry well at 37°C for 3 minutes.
- 34 Resuspend cells with one mL freezing medium or Cryostor.
To resuspend, pipette medium over the well to lift cells. Only go over the well 1-2 times, leaving behind cells that do not lift without mechanical force (cells that remain stuck down are the unhealthy cells).
- 35 Transfer one mL of resuspended cell solution into a labeled cryovial.
- 36 Add cryovials to a Corning CoolCell and leave in the -80°C freezer for a minimum of 24 hours and a maximum of 72 hours.
- 37 Transfer vials to a box in the liquid nitrogen tank and update the liquid nitrogen spreadsheet.

38

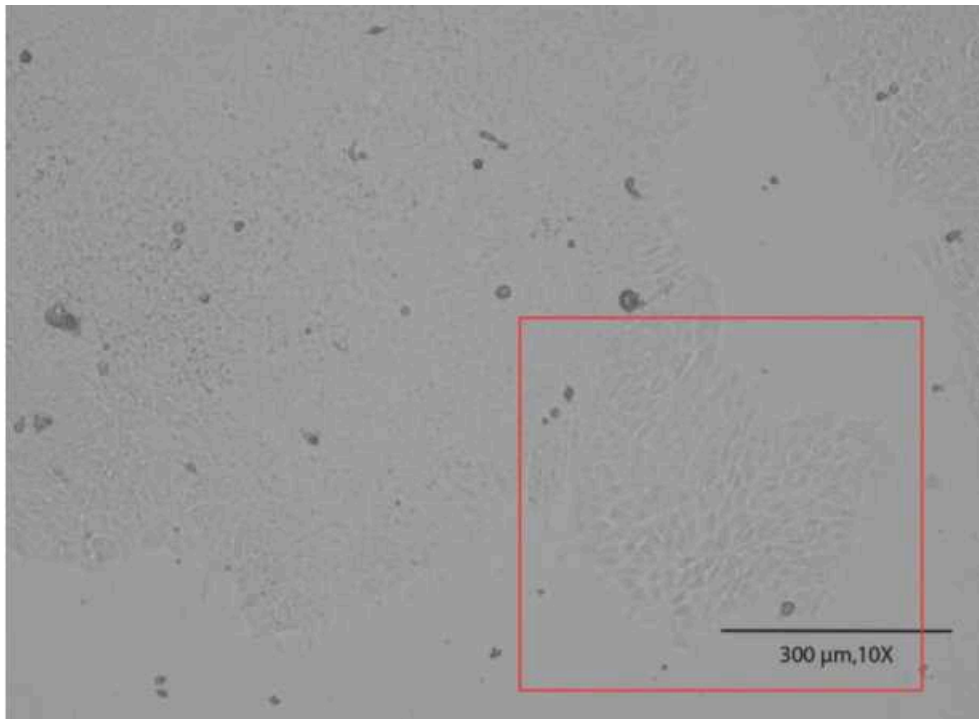


Fig 3. Example of spontaneous differentiation with spikey cells at edges of colony and lacunar or cobblestone appearance of cells within the colony. Imaged at 10x magnification.

Troubleshooting

- 39 Identify spontaneous differentiation
Signs of spontaneous differentiation
Cells on the edge of the colonies look "spikey"
Cells are growing slowly
The first line of treatment is to passage very lightly, being careful to only pick up healthy cells.

Infection

- 40 Signs of bacterial infection:
Yellow, cloudy medium
Moving debris visible under the microscope, beyond Brownian motion
Increased cell death
Unless the plate is precious, spray all of the wells in the infected plate with 10% bleach, leave in the sink for at least 30 minutes, and throw away in the biohazard bin.
Sterilize the incubator and BSC hood. Notify the lab via email.
If the line is precious, it is sometimes possible to save other wells in the plate by treating with antibiotics (any severely infected well should be bleached as this is likely to spread to other wells and the incubator)
For example, make media with antibiotic-antimycotic 1:100, and feed the plate.
Effective antibiotics may vary.