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Human iPSC Culture: Standard Operating Procedure

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We use this protocol and it's working

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Abstract

To ensure consistent and high-quality maintenance of induced pluripotent stem cells (iPSCs) by implementing standardized protocols and adhering to good cell culture practices. This includes minimizing inter- and intra-batch variability, preventing microbial and cross-contamination, and optimizing culture conditions such as media composition, passaging frequency, and confluency thresholds to support reproducible and robust downstream applications including differentiation, gene editing, and transcriptomic profiling.

Materials

1. Reagents
A. mTeSR1 (STEMCELL Technologies, #85851)
B. Y-27632 (Dihydrochloride) (STEMCELL Technologies, #72308)
C. Matrigel-Growth Factor Reduced (GFR) (Corning, #356231)
D. Dispase (STEMCELL Technologies, #07913)
E. Freezing media (10% DMSO in KSR)

Safety warnings

Note 1: Always disinfect the biosafety cabinet with 70% ethanol before use.
Note 2: To avoid cross-contamination, thaw only one cell line at a time and handle it carefully.
Note 3: It is recommended to thaw cells quickly and freeze them gradually to maintain optimal cell recovery.
Note 4: The incubation time with Dispase may vary depending on the cell line.

Reagent and Media Preparation

1 Mix both components thoroughly to prepare 500 mL of complete mTeSR1 medium.
Store at 4°C and use within the recommended time frame provided by the manufacturer.

2 Dissolve 50 mg of Y-27632 in 15.6 mL of sterile, nuclease-free water.
This yields a 10 mM stock solution, Store at −20°C.
Working concentration - 10 µM (1000x)

3 Thaw a 10 mL bottle overnight by placing it in ice and storing it at 4°C.
*Ensure the bottle is fully submerged to allow gradual thawing.
On ice, aliquot the thawed Matrigel into sterile microcentrifuge tubes, 100 µL per tube.
Store aliquots at −20°C.
When ready to use, resuspend one 100 µL aliquot in 6 mL of cold, filtered DMEM/F12.
Coat a 6-well plate by adding 1 mL of the diluted Matrigel solution per well.
*Incubate for at least 1 hour at 37°C before use.

4 Thaw 100 mL of Dispase (STEMCELL Technologies, #07913) if frozen – 5U/mL.
Aliquot 1 mL of Dispase into sterile microcentrifuge tubes.
Store aliquots at −20°C

5 Prepare freezing medium by mixing 10% DMSO in KnockOut Serum Replacement (KSR).
*For example: mix 100 µL DMSO with 900 µL KSR to make 1 mL of freezing medium.
Use 1 mL of freezing medium per well of a 6-well plate for cryopreservation.

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6 Prepare 3 mL of DMEM/F12 in a sterile 15 mL conical tube.
Prepare mTeSR1 medium supplemented with Y-27632 to a final concentration of 10 µM.
Retrieve the frozen cell vial and partially submerge it in a 37°C water bath.
*Do not fully immerse the vial; keep the cap above the water to avoid contamination.
Monitor the vial closely and remove it once the medium begins to thaw.
*This typically takes about 1 minute.
Using a P1000 pipette, transfer the thawed cell suspension (approximately 1 mL) into the prepared 15mL conical tube containing 3 mL of DMEM/F12.
Rinse the cryovial with 1 mL of fresh DMEM/F12, then collect and transfer the wash to the same 15mL conical tube to bring the total volume to approximately 5 mL.
Centrifuge the tube at 1,100 rpm for 2 minutes.
While the centrifuge is running, retrieve the pre-coated Matrigel 6-well plate from the incubator.
Label the plate with all necessary cell information (e.g., date, cell line ID, passage number).

Wash with 1x PBS, and then add 1 mL of prepared mTeSR1 containing Y-27632 to each well.
After centrifugation, carefully aspirate the supernatant without disturbing the cell pellet.
Gently resuspend the cell pellet in 1 mL of mTeSR1 medium supplemented with 10 µM Y-27632.
Seed onto Matrigel-coated plates pre-filled with the same medium.
*Generally, use one vial of thawed and plated into a single well of a 6-well plate.
Gently distribute the cells evenly, then place the plate in the incubator.
The following day, replace the medium with fresh mTeSR1 without Y-27632.

7 Allow mTeSR1 to equilibrate to room temperature before use.
Remove spent medium gently, avoiding disturbance of the colonies.
Add fresh medium (e.g., 1.5–2 mL per well of a 6-well plate).
Return the plate to the incubator promptly.
*Avoid letting the medium run dry or acidic (yellow), as this can induce spontaneous differentiation.
*Examine iPSC colonies daily under a phase-contrast microscope before changing media.
- Healthy iPSC colony features:
  i) Tight, compact colonies with well-defined borders
  ii) High nuclear-to-cytoplasmic ratio
  iii) Prominent nucleoli and uniform cell size
  iv) Smooth edges with no signs of differentiation
- Signs of spontaneous differentiation:
  i) Flattened cells at the colony edge
  ii) Irregular colony shape or borders
  iii) Increased granularity or vacuolation
  iv) Disrupted monolayer or mixed cell populations

8 Prepare Matrigel-coated 6-well plates in advance before passaging.
Allow mTeSR1 to equilibrate to room temperature before use.
*Ensure that the cells are approximately 70% confluent.
*Colonies should exhibit well-defined borders with minimal spontaneous differentiation.
*If extensive differentiation is observed, manually remove the affected areas under a microscope.
Wash with 1× PBS, then add 1 mL of DMEM/F12 to each well.
Add 166 µL of Dispase (0.83U) to each well.
Incubate the plate at 37°C for 7 minutes.
*Colony edges lift visibly, with rounded borders detaching slightly from the surface.
*This indicates the optimal time to proceed with gentle dissociation.
During the incubation, bring the pre-coated Matrigel 6-well plate to room temperature.
Label the plate with all necessary cell information (e.g., date, cell line ID, passage number).
Wash with 1× PBS, and then add 1.5 mL of prepared mTeSR1.

After 7 minutes, aspirate the DMEM/F12 with Dispase from the original plate.
wash with 1× PBS and add 1 mL of mTeSR1 to each well.
Gently scrape the colonies using a P1000 pipette tip.
Gently pipette up and down 1–2 times to break the colonies into small clumps.
Transfer the desired cell clumps into the prepared plate containing 1.5 mL of mTeSR1.
*The typical passaging ratio is 1:6, but this may vary depending on the downstream application.
Gently distribute the cells evenly, then place the plate in the incubator.
*Change the medium daily using fresh mTeSR1.
*Cells are typically passaged every 5 to 7 days, depending on confluency.

9 Prepare an appropriate amount of freezing medium (10% DMSO in KSR).
*Typically, 1 mL of freezing medium is used per well of a 6-well plate.
Wash with 1× PBS, then add 1 mL of DMEM/F12 to each well.
Add 166 µL of Dispase (0.83U) to each well.
Incubate the plate at 37°C for 7 minutes.
*Colony edges lift visibly, with rounded borders detaching slightly from the surface.
*This indicates the optimal time to proceed with gentle dissociation.
During the incubation, label cryogenic vials with all necessary cell information. (e.g., date, cell line ID, passage number)
After 7 minutes, aspirate the DMEM/F12 with Dispase from the original plate.
wash with 1× PBS and add 1 mL of freezing medium to each well.
Gently scrape the colonies using a P1000 pipette tip.
Transfer the cell suspension into the labeled cryogenic vials.
Place the vials into a Mr. Frosty™ freezing container and store at −80°C overnight.
The next day, move the vials from −80°C to liquid nitrogen for long-term storage.
*Ensure proper inventory and storage location are recorded.