iPSC Recovery, Expansion, and Cryopreservation Protocol

(Instructions for Use)

These instructions describe a recommended workflow to recover, expand, and cryopreserve human iPSCs shipped on dry ice. Upon receipt, store cryovials in liquid nitrogen until use. Prior to storage, confirm that the cryovial or tube identifier matches the corresponding identifier on the packing slip.

All steps should be performed using standard sterile tissue culture technique.

Important Note: Review CoA to determine recommended media and matrix combination for cell lines. If no media/ matrix combination is listed on the CoA, use StemFlex™ Medium with Cultrex Ultrimatrix.

Materials

Reagents - Recovery

- Cultrex Ultimatrix (R&D Systems, Cat# BME001-05), Matrigel® hESC-Qualified Matrix (Corning, Cat# 354277), or alternative matrix (eg. Geltrex™ Thermo Fisher Scientific, Cat# A4000046802)
- StemFlex™ Medium (Thermo Fisher Scientific / Life Technologies, Cat# A3349401) or mTeSR™ Plus (STEMCELL Technologies, Cat# 100-0276)
- CloneR™ (STEMCELL Technologies, Cat# 05888) or CloneR™2 (STEMCELL Technologies, Cat# 100-0691)
- Trypan Blue or equivalent vital dye (optional, for viability assessment)

Equipment - Recovery

- Centrifuge
- 15 mL conical tubes (Thermo Scientific, Cat# 339651 or equivalent)
- Cell counter or hemocytometer
- 12-well tissue culture-treated plate (Corning Cat# 3513 or equivalent)

Reagents - Expansion & Cryopreservation

- Cultrex Ultimatrix (R&D Systems, Cat# BME001-05), Matrigel® hESC-Qualified Matrix (Corning, Cat# 354277), or alternative matrix (eg. Geltrex™ Thermo Fisher Scientific , Cat# A4000046802)
- DMEM/F12 (Thermo Fisher Cat# 11320082)
- StemFlex™ Medium (Cat# A3349401), mTeSR™ Plus (STEMCELL Technologies, Cat# 100-0276)
- ROCK inhibitor for post-passaging plating (e.g., Thiazovivin, Stemgent Cat# 04-0017; or Y-27632, STEMCELL Cat# 72302)
- Accutase (StemPro Accutase, Thermo Fisher Cat# A11105-01)
- Cryopreservation medium (e.g., Synth-a-Freeze™, Thermo Fisher Cat# A12542-01; or equivalent)

Equipment - Expansion & Cryopreservation

- Centrifuge
- 15 mL conical tubes
- 6-well tissue culture-treated plate (Corning Cat# 3516 or equivalent)
- Cryovials of choice
- Controlled-rate freezing container (e.g., isopropanol-based freezing container)

Protocol A

Recovery of Cryopreserved iPSC (per cell line)

Before Starting:

- **Plate coating:** For coating of dishes, follow the manufacturer's recommended protocol. A typical working dilution is 1:100 of Ultimatrix™ stock in DMEM/F12. The working solution for Matrigel® is listed on the CoA from the manufacturer.
- **Recovery setup:** Coat one well of a 12-well plate with 1 mL of the diluted working stock matrix prepared above for each line to be recovered; pre-warm coated plates for at least 1 hour prior to use.
- **Medium preparation:** Prepare StemFlex™ Medium or mTeSR™ Plus Medium per the manufacturer instructions by adding the supplement to the provided basal medium. Use CloneR™ with StemFlex™ Medium and CloneR™2 with mTeSR™ Plus Medium.

1. Pre-warm complete culture medium (basal medium plus supplement) to 37°C and supplement the medium with 10% CloneR™ in StemFlex™ or 10% CloneR™ in mTeSR™ Plus Medium.
2. Pre-warm coated 12-well plates for at least 1 hour prior to use.
3. Keep the vial/tube of frozen iPSCs on dry ice until ready to thaw. Thaw cells in a 37°C water bath for 30 seconds to 1 minute and ensure a small amount of ice remaining.
4. Add 500 µL of StemFlex™ + 10% CloneR™ to the Matrix tube (total volume becomes 1 mL), gently mix by pipetting, and transfer the cell suspension to a 15 mL conical tube.
5. Rinse the tube once with 1 mL of StemFlex™ + 10% CloneR™ (or mTeSR ™ Plus + CloneR™2) and bring the final volume to 2–3 mL in the 15 mL conical tube.
6. Centrifuge at 130 RCF for 4 minutes.
7. Carefully aspirate the supernatant without disturbing the pellet and resuspend the pellet in 1 mL of fresh StemFlex™ + 10% CloneR™ (or mTeSR ™ Plus + CloneR™2).
8. *Optional:* Determine total cell number and viability using Trypan Blue and a cell counter or hemocytometer.
9. Aspirate matrix solution from the pre-warmed destination 12-well plate.
10. Add the entire 1 mL cell suspension into one well.
11. Distribute cells evenly with a very gentle side-to-side motion and place the plate in a 37°C / 5% CO2 incubator (Day 0). (See figure 1 for reference images)
12. Allow cells to recover for ~48 hours. Do not perform a media exchange the day after thaw (Day 1).
13. Starting ~48 hours after plating (Day 2), perform daily full media exchanges with 1 mL StemFlex™ or mTeSR™ Plus) without CloneR™/CloneR™2.
14. Wells typically reach >50% confluence within 7–10 days. Passage cells at 70–90% confluence; avoid reaching 100% confluence to reduce risk of spontaneous differentiation.

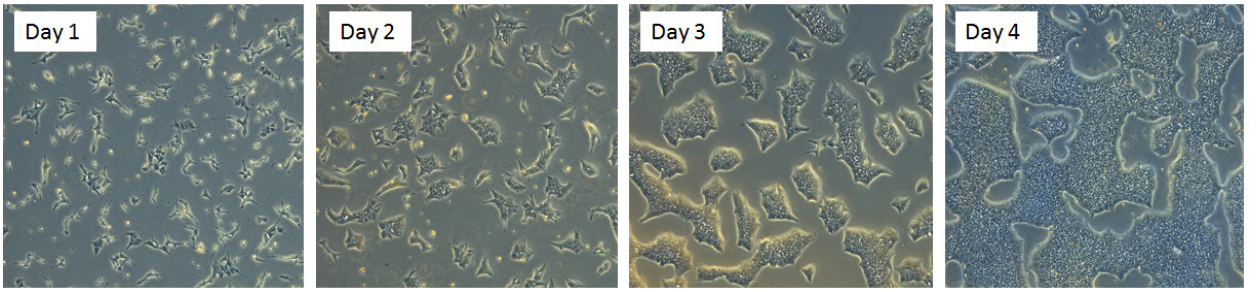


Figure 1: iPSC Morphology in Culture

Representative iPSC morphology after thaw. Cells may appear elongated after ROCK inhibitor exposure and typically revert to a normal colony morphology after inhibitor removal. Use morphology and confluence to guide feeding and passaging decisions.

Protocol B

Expansion/Passaging of iPSC (per cell line)

Before Starting:

- **Plate coating:** For coating of dishes, follow the manufacturer's recommended protocol. A typical working dilution is 1:100 of Ultimatrix™ stock in DMEM/F12. The working solution for Matrigel® is listed on the CoA from the manufacturer.
- Prepare StemFlex™ or mTeSR™ Plus (basal plus supplement) supplemented with ROCK inhibitor (e.g., 1 µM Thiazovivin) and warm to 37°C.
- Pre-warm Accutase to 37°C (1 mL per well for a 6-well plate; 500 µL per well for a 12-well plate).

1. Aspirate spent culture medium and add Accutase (1 mL per well for a 6-well plate; 500 µL per well for a 12-well plate).
2. Incubate at 37°C / 5% CO2 for 5–10 minutes, or until cells are freely floating.
3. Neutralize Accutase by adding 1 mL per well of StemFlex™ + ROCK inhibitor (or mTeSR™ Plus + ROCK Inhibitor).
4. Transfer the total volume to a 15 mL conical tube and rinse the well once with an additional 1 mL of StemFlex™ + ROCK inhibitor (or mTeSR™ Plus + ROCK Inhibitor).
5. Centrifuge at 130 RCF for 4 minutes.
6. Aspirate supernatant without disturbing the pellet and resuspend the pellet in an appropriate volume of StemFlex™ + ROCK inhibitor (or mTeSR™ Plus + ROCK Inhibitor).
7. *Optional:* Determine total cell number and viability using Trypan Blue and a cell counter or hemocytometer.
8. Seed cells onto matrix-coated 6-well plates at 300,000–400,000 cells per well in a final volume of 2 mL per well. Aspirate matrix solution from the destination plate immediately before seeding.
9. At ~24 hours after plating (Day 1), perform a half media exchange using your preferred medium without ROCK inhibitor.
10. At ~48 hours after plating (Day 2) and onward, perform daily full media exchanges with your preferred medium without ROCK inhibitor.
11. Passage again at 70–90% confluence; avoid reaching 100% confluence.

Protocol C

Cryopreservation (Freezing) of iPSCs (per cell line)

1. After steps 1-5 in protocol B above, aspirate the supernatant without disturbing the pellet and resuspend cells in cryopreservation medium (e.g., Synth-a-Freeze™) at a density consistent with the manufacturer recommendations.
2. *Optional:* Determine total cell number and viability using Trypan Blue and a cell counter or hemocytometer.
3. Aliquot the desired volume into labeled cryovials.
4. Slow-freeze using a controlled-rate freezing container and store at -80°C for ~24 hours.
5. Transfer cryovials to liquid nitrogen for long-term storage.

Important: Successful recovery and expansion depend on careful sterile technique, appropriate matrix handling, and passaging before over-confluence. If you have questions, contact JAX Technical Information Services using the email or phone numbers in the footer.