Thawing iPS cells on mTeSR1/Cultrex:

Media Preparation

mTeSR1 (500 mL): Stem Cell Technologies. Cat # 85850 (for one bottle of complete medium)
Contains FGF. Avoid heating. Aliquot and freeze or use within two weeks.

400 mL mTeSR1 basal media
+100 mL mTeSR1 5X supplement (thaw at 4°C overnight or room temperature)
+5 mL Penicillin-Streptomycin (10,000 U/mL) (optional)

Preparing Cultrex plates (alternatively Matrigel or Geltrex can be used)

Reagent: Cultrex. Trevigen, Cat # 3434-005-02

Aliquoting Cultrex:
Adapted from Trevigen Protocol: Thaw Cultrex overnight at 2-8°C. Refrigerator temperatures may vary; therefore, it is recommended to keep Cultrex on ice in a refrigerator during thawing process. Thawed Cultrex solidifies quickly at temperatures above 15°C; when aliquoting, keep all the vials on ice to prevent untimely gelling. Mix Cultrex by pipetting up and down and aliquot 250ul into cryovials and store at -20°C.

Plating cultrex

1. Thaw Cultrex aliquot on ice. Keep Cultrex cold at all times prior to plating. Cultrex will start to solidify above 15°C.
2. Prepare a tube with 25 mL of ice-cold DMEM/F12.
3. Clean Cultrex vial with 70% ethanol.
4. Pipet 500 µL of ice-cold DMEM/F12 into Cultrex aliquot, then add aliquot contents to tube of DMEM/F12. Pipet up and down to mix.
5. Plate Cultrex solution onto tissue culture-treated plates or dishes (1 mL per well of a 6-well plate; 6 mL per 10 cm dish). Ensure that the solution covers the entire bottom of the well or dish. Keep at room temperature for one hour.
6. *If not used immediately, seal plates or dishes with Parafilm and store at 4°C for up to one week. Allow refrigerated Cultrex-coated plates/dishes at least 30 minutes to reach room temperature right before use.*
7. Aspirate the excess solution just before plating cells.

Thawing hPSCs

Before beginning, prepare Cultrex coated plates or dishes.
Each cryovial should be thawed into 1 well of a 6-well plate.

1. Partially thaw the cryovial in a 37°C water bath until a small piece of ice remains.

2. Clean the cryovial with 70% ethanol.
3. Slowly add pre-warmed mTeSR1 media to the cryovial and transfer the liquid contents into a 15 mL conical tube. Repeat until no ice remains in the cryovial, typically 1-2 times. Minimize pipetting of thawed cells.
4. Slowly add mTeSR1 media to the conical tube to a volume of 8 mL.
5. Centrifuge at 200 x G (1,000 RPM) for 4 min.
6. Aspirate the supernatant.
7. Gently resuspend the pellet in mTeSR1 with 10 µM Y-27632 (ROCK inhibitor). Aspirate excess Cultrex from plate or dish and immediately plate cell suspension.
8. Position plates on incubator racks and move them forward and back, then side-to-side several times to evenly distribute the cells. Do not use a circular motion.