Protocol for the Differentiation of KOLF2.1J iPSCs into Cardiomyocytes

This protocol is designed for 6-well cell culture plates.

Preparing Matrigel Plates

1. Aliquot 12 mL of cold DMEM without glucose (Gibco™, Thermo Fisher Scientific; Catalog #11966) into a 15 mL Falcon tube.

2. Remove one aliquot (150 µL) of Matrigel (Corning®; catalog #354230) from -20°C, and quickly melt/thaw the aliquot by pipetting up and down with ~1 mL of DMEM without glucose from your 12 mL aliquot. Combine once thawed.

3. Add 1mL of Matrigel-DMEM without glucose mixture to each well in a 12-well plate (500 µL/well), ensure all wells are covered and incubate plate at 37°C for at least one hour.

Split KOLF2.1J iPSCs

1. Before splitting cells, prepare mixture of mTeSR™1 (STEMCELL Technologies; catalog #185581) containing 1:2000 ROCK-inhibitor (Y-27632 dihydrochloride) (TOCRIS; catalog #1254) (from stock conc 10 mM). Make sure to prepare enough mTeSR™1-ROCKi for your upcoming split plus 1 mL extra.

2. Take out KOLF 2.1J iPS cells and aspirate mTeSR™1 media from the split well and wash with 500 µL PBS per well.

3. Add 750 µL Accutase (EMD Millipore; catalog #SCR005) to the split well and incubate at 37°C for 5 min. While waiting, prepare inactivation tubes: add 2 mL fresh mTeSR™1 to a 15 mL Falcon tube.

4. After five minutes, remove the plate from the incubator and pipet the Accutase/cell solution to dislodge remaining cells. Spin KOLF2.1J iPS cells at 200G for 3 min at 4°C.

5. Aspirate supernatant, then resuspend cell pellet in 1 mL of the mTeSR™1-ROCKi solution.

6. Seed these cells at approx. 1million cells/well density (count using hemocytometer).

7. Place in 37°C incubator, with an additional round of rocking, for 24 hours.

8. Next 2-3 days, add new 1 mL mTESR™1 media (until cells becomes >90% confluent).

Cardiomyocyte Differentiation

Day 0: When iPS cells become >90% confluent, aspirate media and wash each well with 1 mL of PBS. Add RPMI-B27 without insulin (RPMI: Gibco™, Thermo Fisher Scientific; catalog #11875093; B-27™ Supplement without insulin: Gibco™, Thermo Fisher Scientific; catalog #A1895602) mixed with CHIR 99021 (TOCRIS; catalog #4423) concentration at 4 µM (from stock conc 12 mM). Incubate at 37°C for 48 hours.

Day 2: After 48 hours, aspirate media and wash each well with 1 mL of PBS and add 1 mL of RPMI-B27 without insulin media. Incubate at 37°C for 24 hours.

Day 3: Aspirate media, add RPMI-B27 without insulin mixed with IWP-4 (TOCRIS; catalog #5214) for a 2:1000 dilution (from stock conc 2.5 mM). Incubate at 37°C for 48 hours.

Day 5: After 48 hours, aspirate media and wash each well with 1 mL of PBS and add 1 mL of RPMI-B27 without insulin media. Incubate at 37°C for 48 hours.

Day 7: After 48 hours, aspirate media and add 1 mL of RPMI-B27 without insulin media. Incubate at 37°C for 48 hours.

Day 9 & 11: Aspirate media and add 1 mL of RPMI-B27 with insulin (B-27™ Supplement: Gibco™, Thermo Fisher Scientific; catalog #17504001) media. Incubate at 37°C for 48 hours. *You should be able to see beating cardiomyocytes by day 11.*

Day 13: After 48 hours, aspirate media and wash each well with 1 mL of PBS. Add DMEM without glucose mixed with 4 mM Lactate (Sigma-Aldrich; catalog #L7022) for an 8:1000 dilution (stock conc 500 mM). Incubate at 37°C for 24 hours.

Day 14: After 24 hours, aspirate media and wash each well with 1 mL of PBS. Add DMEM without glucose mixed with 4 mM Lactate for an 8:1000 dilution (stock concentration: 500 mM). Incubate at 37°C for 24 hours.

Day 15: Aspirate media and add 1 mL of RPMI-B27 with insulin media.

Day 16: Proceed for cardiomyocyte replating.

Cardiomyocyte Replating

Prepare Fibronectin Plate

1. Prepare fibronectin according to the manufacturer's instructions (Gibco™, Thermo Fisher Scientific, catalog #33016015). Dispense into 300 µL aliquots and store at -20°C.

2. Dilute fibronectin aliquot in up to 12 mL PBS and add 1 mL of the Fibronectin-PBS mixture to each well in a 6-well plate and incubate plate at 37°C for a minimum of 3 hours (overnight incubation works too).Once the Fibronectin-coated plate is ready, prepare a mixture of RPMI-B27 with insulin containing 2% FBS.

3. Remove the Fibronectin-coated plate from the incubator, aspirate liquid from each well and wash with 1 mL PBS per well.

4. Add 500 µl of 2% FCS-RPMI-B27 with insulin solution to each well and set plate aside at room temperature.

Replating Cardiomyocytes

1. Wash KOLF 2.1J iPSC-differentiated cardiomyocyte plate with 500 µL of PBS.

2. Add 300 µL Trypsin (Sigma-Aldrich; catalog #59417C) to each well and incubate at 37°C for 6-7 mins (or until cells begin to detach).

3. Remove the plate from incubation and pipet the Trypsin/cell solution to dislodge remaining cells. Add each well of Trypsin/cell solution to its own inactivation tube (Trypsin inactivates through antitrypsin in FCS). Use inactivation solution to wash each Trypsin-treated well. Spin at 200G for 3 min at 4°C.

4. *Carefully* aspirate supernatant, then resuspend each pellet in 500 µL existing 2% FCS-RPMI-B27 with insulin solution. Drip-add each cell resuspension (500 µL) to its own well in the Fibronectin plate.

5. Incubate at 37°C. Next day, add new RPMI-B27 with insulin media.

6. Replace media with RPMI-B27 with insulin every Monday, Wednesday, Friday.