hiPSC Culture Protocol:

1. Coating Culture Plates:

- Dilute growth factor-reduced Matrigel in DMEM-F12 medium according to the manufacturer's instructions.
- Coat culture plates with the appropriate substrate and incubate at 37°C for at least 2 hour to overnight
- Aspirate the coating solution before use.

2. Thawing and Seeding hiPSCs:

- Thaw frozen hiPSCs in a 37°C water bath.
- Transfer cells to a sterile tube containing pre-warmed mTeSR1 medium.
- Centrifuge at 200 × g for 5 minutes to pellet cells.
- Resuspend the cell pellet in mTeSR1 medium supplemented with 5 µM blebbistatin.
- Plate cells at a density of 1,000–1,500 cells per cm^2 on the coated plates.
- Place cells in a 10% CO_2/5% O_2 incubator at 37°C.

3. Daily Maintenance:

- Replace the medium with fresh mTeSR1 (without blebbistatin) two days after seeding.
- Change the medium daily thereafter, ensuring cells are not over-confluent.

4. Passaging hiPSCs:

- When colonies reach 70–80% confluency, prepare for passaging.
- Add 5 µM blebbistatin to mTeSR1 medium and incubate cells for 5–10 minutes.
- Aspirate the medium and wash cells gently with PBS.
- Add Accutase to the cells and incubate at 37°C for 5–10 minutes until colonies detach.
- Gently dissociate cell clumps into a single-cell suspension using a pipette.
- Transfer the cell suspension to a sterile tube and centrifuge at 200 × g for 5 minutes.
- Resuspend the cell pellet in mTeSR1 medium supplemented with 5 µM blebbistatin.
- Plate cells at a density of 1,000–1,500 cells per cm^2 on freshly coated plates.
- Return cells to the 10% CO_2/5% O_2 incubator.

5. Post-Passaging Maintenance:

- Two days after passaging, replace the medium with fresh mTeSR1 (without blebbistatin).
- Continue daily medium changes until the next passage.