# Induced pluripotent stem cell (iPSC)

## Materials

1. mTeSR+ medium

2. 0.5 mM EDTA in 1XPBS

3. 1XPBS

4. ROCK inhibitors (Y27632)

5. Matrigel

6. DME/F-12

Coated (Before passage)

1. Before passage or thaw iPSC cells, the cells should be prepared on plates coated with Matrigel in DME/F-12 medium (Matrigel 10 µl in DME/F-12 medium 1 ml)
2. Incubated at 37°C, for at least 30-60 mins.
3. Before plating cells, remove excess Matrigel from the plate.

## Thawing

1. iPSC cryogenic stock is submerged into water bath at 37°C (until small amount of ice pellet remains)

2. iPSC stock transfer into 15 ml conical tube containing 6-8 ml 0f DMEM F/12 (slightly resuspend beware do not break them into single cells.

3. Centrifuge at 500 g at 25°C in 5 minutes. While waiting centrifuge, add 5 µM ROCK inhibitors into mTeSR+ medium.

6. Remove supernatant and add 1 ml of mTeSR+ plus have ROCK inhibitor medium transfer into the 6 well (Drop them thoroughly into the well)

7. Incubate at 37°C hypoxia condition (5% O2 and 5% CO2)

8. Change the medium every other day (ROCK inhibitors is only included on the first day)

## Passage

1. When the cell confluence 70-80% discard old medium.

2. Wash with 1XPBS one time.

3. Passage the cell using 0.5 mM EDTA incubates at room temp 3-5 minutes.

4. Discard 0.5 mM EDTA.

5. Wash the cells gently with 1X PBS, using the lightest handling to avoid cell detachment.

6. Remove 1XPBS and resuspend with mTeSR+ medium and transfer to new well (in the well have mTeSR+ medium plus 5 µM ROCK inhibitor.

7. Incubate at 37°C hypoxia condition (5% O2 and 5% CO2)

8. Change the medium every other day (ROCK inhibitors is only included on the first day)

## Freezing

1. When the cell confluence 70-80% discard old medium.

2. Wash with 1XPBS one time.

3. Passage the cell using 0.5 mM EDTA (e.g., 1 ml/well of 6-well-plate) and incubate at room temp 5 minutes.

4. Remove 0.5 mM EDTA.

5. Wash the cells gently with 1X PBS, using the lightest handling to avoid cell detachment.

6. Remove 1XPBS and resuspend with cell-banker2 freezing solution pipette 2–3 times to break colonies into small clumps (avoid single-cell suspension).

7. Transfer to cryovial tube.

8. Cool cell with a Mr. Frosty at -80°C overnight.

9. Transfer cryovial to liquid nitrogen.