Cryopreserve and thaw iPSCs

Cryopreservation

1. Pre-warm the required volume of Essential 8™ Flex Medium Kit (Gibco, Thermofisher Scientific) at room temperature.
2. Prepare Essential 8™ Flex Freezing Medium: for every 1 mL of freezing medium, combine 0.9 mL Essential 8™ Flex Medium Kit with 0.1 mL DMSO. Keep it on ice.
3. Aspirate the spent medium from the cells and rinse them with DPBS.
4. Add 0.5 mM EDTA solution to the dish (1 mL to 6-well plate dishes) and swirl to coat the entire cell surface.
5. Incubate the dish at room temperature for 5-6 minutes until the cells start to separate and round up, and the colonies appear to have holes when viewed under a microscope.
6. Aspirate the EDTA, add Essential 8™ Flex Medium to the dish and pipete up-down to collect the cells
7. Centrifuge for 5 minutes at 200 x *g*
8. Aspirate the supernatant and resuspend the cells in Essential 8™ Flex Freezing Medium.
9. Aliquot 1 mL of the cell suspension into each cryovial.
10. Quickly place the cryovials in a cryofreezing container (e.g., Mr. Frosty) and freeze the cells by decreasing the temperature by 1°C per minute. Once frozen, transfer the cells to –80°C overnight.
11. After overnight storage at –80°C, transfer the cells to a liquid nitrogen tank vapor phase for long-term storage.

Thaw

1. Pre-warm the required volume of Essential 8™ Flex Medium Kit (Gibco, Thermofisher Scientific) at room temperature.
2. Prepare the required volume of Essential 8™ Flex Medium supplemented with RevitaCell™ (Gibco, ThermoFisher Scientific): for every 1 mL, combine 990 µL of Essential 8™ Flex Medium with 10 µL RevitaCell™ Supplement (100x)
3. To a 15 mL tube add 5 mL of Essential 8™ Flex Medium
4. Place the cryovial containing the iPSCs in a 37ºC water bath for 1-2 minutes, until only a small piece of ice is visible.
5. Decontaminate the cryovial surface with 70% etanol and transfer the cells to the 15 mL tube containing the Essential 8™ Flex Medium
6. Centrifuge for 5 minutes at 200 x *g*
7. Aspirate the supernatant, resuspend the cells in Essential 8™ Flex Medium supplemented with RevitaCell™ (1 mL for 6-well plate dishes) and seed the cells in vitronectin-coated dishes.
8. Incubate at 37 °C with 5% CO_2 (Binder CB 150) for 16-18 hours.
9. Change the media to Essential 8™ Flex Medium (2 mL for 6-well plate dishes)
10. Replace the medium every other day until cells reach a high confluency.