Standard Operating Procedure
for
FREEZING INDUCED PLURIPOTENT STEM CELLS

1.0 PURPOSE

This procedure describes how to freeze induced pluripotent stem cells (iPSC).

2.0 SCOPE (Should include which Cores this SOP applies to)

This procedure applies to all personnel who wish to freeze iPSC.

3.0 PROCEDURE

1. Prepare fresh 2× freezing medium (60% FBS, 20% DMSO, 20% hES medium) and place it on ice.
2. Aspirate the culture medium, wash each well of the 6-well plate with 1 mL DMEM/F12.
3. Add 0.5 mL dispase solution to each well of 6-well plate.
4. Incubate the dish for 3-5 minutes in 37°C incubator.
5. Aspirate the dispase solution and wash each well with 2 ml DMEM/F12.
6. Add 2 mL of hES medium and dislodge the cells using a glass pipet. Transfer the cells to a 15 mL tube. Wash the plate with another 1 ml hES medium and transfer it to the same tube.
7. Centrifuge the cells at 200 *g* for 3 minutes at room temperature.
8. Discard the supernatant, resuspend the cells in hES medium (usually, 1 ml for one well). Add an equal volume of 2× freezing medium to the resuspend cell and mix them gently.
9. Aliquot 1 mL of the cell suspension into each cryovial. Put the cryovials into a cryo freezing container and transfer them to –80°C overnight.
10. In the next day, transfer the cells to a liquid nitrogen tank for long term storage.

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