Freezing and thawing of hiPS cells

Cultivate the cells in wells on a 12-well plate.

Materials

Cryopreservation kit, #A24439SA, Gibco

Cryo tubes

Mr. Frosty or equivalent box for freezing cells in

Complete E8 medium

Laminin-521

4-well plates (NUNC) or any other 24-well plate

Methods

Media

Divide thawed PSC Cryopreservation Medium into usage-size aliquots and store at -20^{o}C, once thawed store the Cryopreservation medium at +4^{o}C until further use.

Divide the Growth Supplement into usage-size aliquots and store at -20^{o}C, once thawed the Growth Supplement can be stored at +4^{o}C.

For thawing, use complete E8 medium.

Freezing

One well of cells, 80% confluent, in a 12-well plate is generally sufficient for freezing in one or two vials.

Harvest the cells according to standard passaging protocol

Centrifuge the cells for 3 minutes at 1400 rpm.

Aspirate the medium and resuspend the cells in appropriate amount of cold Cryopreservation medium.

Dispense in one or more vials according to the amount of cells, a suitable concentration is 1-2 x 10^{6} cells/ml.

Place the vials in a suitable freezing box (Mr Frosty) and leave at -80^{o}C for at least 24 hours before moving them to long-term storage in liquid nitrogen.

Thawing

Dilute the Growth Supplement x100 in the amount of complete E8 medium needed, keep at room temperature

Quickly thaw the vial in a +37^{o}C waterbath until a small ice crystal remains

Add 500 µl of medium dropwise to the thawed ampoule

Transfer the cells to a 15 ml conical tube and add a further 2 ml of medium

Cfg for 3 minutes at 1400 rpm

Aspirate medium and resuspend in E8 with growth supplement; count the cells and check the viability.

Plate 200 000 cells/well in a Laminin coated 4-well/24well plate

Replace medium with unsupplemented medium after 18-24 hours