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Freezing and thawing of hiPS cells

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Introduction

This protocol covers cultivation in 12-well plates, for any other size the volumes need to be adjusted. For information of cultivation of the cells, see “Cultivation of hiPS cells on Laminin”.

Materials

Cryopreservation kit, #A2644401, LifeTechnologies.

Cryo tubes

Mr. Frosty or equivalent box for freezing cells in

Complete E8 medium

Laminin-521

12-well plates

Methods

Media

- Divide thawed iPSC Cryopreservation Medium into usage-size aliquots (10 ml) and store at -20°C, once thawed store the Cryopreservation medium at +4°C until further use.
- For thawing, use complete E8 medium (E8 medium with Growth Supplement)

Freezing

- One well of cells, 80% confluent, in a 12-well plate is generally sufficient for freezing in one or two vials. Aim for about 500 000/0.5 ml cryomedium.
- Harvest the cells according to standard passaging protocol.
- Centrifuge the cells for 3 minutes at 1400 rpm.
- Aspirate the medium and re-suspend 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°C for at least 24 hours before moving them to long-term storage in liquid nitrogen.

Thawing

- Quickly thaw the vial in a +37°C water bath until a small ice crystal remains

- Add 500 µl of E8 medium dropwise to the thawed ampoule
- Transfer the cells to a 15 ml conical tube and add a containing 1 ml of medium
- Centifuge for 3 minutes at 1400 rpm
- Aspirate medium and re-suspend in complete E8; count the cells and check the viability.
- Plate 100 000-150 000 cells/well in a Laminin521 coated well in a 12-well plate, in complete E8 medium. Add ROCK inhibitor 1:1000. Two or three wells in a 12-well plate are suitable to start with.
- Replace medium with complete E8 medium after 18-24 hours