Standard operational procedure

Start-up hiPS cells in TeSR-E8 from liquid nitrogen

LUMC iPSC line: _____
Passage number: _____
Mycoplasma status: _____

Goal: _____

Sticker or information on vial:

Materials:

- Vitronectin XF aliquot (SCT/07180) Date of coating: _____
- CellAdhere Dilution Buffer (SCT/07183) Lot: _____ exp.: _____
- Suspension Culture Plates (Greiner/657 185)
- TeSR-E8 *(iPS_SOP_0034.2)* Prepared on: _____
- Fasudil 5mM (Rock inhibitor) (LC Labs/F-4660) Prepared on: _____

Method:

- □ Coat one well of a 6-well plate with vitronectin according to manufacturer's protocol.
- □ Defrost hiPS ampoule quickly at 37°C.
- □ Add 1 ml TeSR-E8 (room temperature) slowly to the vial.
- □ Transfer to a 15ml tube.
- □ Rinse the vial with 1 ml TeSR-E8 and slowly add to the 15 ml tube while constantly shaking the tube.
- □ Slowly add an additional 6-8 ml TeSR-E8 with a 5 or 10 ml pipet while constantly shaking the tube. Careful not to break-up the pieces too much!
- □ Let the pieces sink to the bottom, when pieces are too small to sink, centrifuge for 2' at 100xg (~600rpm) maximal.

- □ Take off as much liquid as possible without disturbing the pellet.
- □ Very carefully reconstitute pellet into 2 ml TeSR-E8 with 2 µl Rock inhibitor (final concentration of 5µM).
- □ Wash vitronectin coated well with 1 ml CellAdhere Dilution Buffer and seed cells into the well.

Note: _____