Standard operating procedure

Passaging hiPS cells on mTeSR Plus + Vitronectin

Materials:

- Vitronectin coated 6-well plate (iPS_SOP_0032)
  Date of coating: _____
- mTeSR Plus (iPS_SOP_0099)
  Prepared on:
- GCDR (SCT/07174)
  Lot: _____ exp.: _____
- CellAdhere Dilution Buffer (SCT/07183)
  Lot: _____ exp.: _____
- Cell scraper (Greiner/541-070)
- 10 µl filter tips (Corning/4807)
- 15 ml tube (Greiner/188 271)
- 1000 µl filter tips (Corning/4809)

Important note:
Keep different lines in separate 6-well plates to avoid cross-contamination.
Be sure that all materials are at RT before starting the procedure.
Important: Check SCT mTESR Plus manual for details.

Method (based on one 6-well):

□ Remove differentiated parts of the colonies with a 10 µl pipet tip.

□ Aspirate the medium and treat colonies with 1 ml GCDR for 2-5 min.
NOTE: *Incubation time will differ per clone; colonies are ready when small wholes visible at 10x magnification start to appear within the colonies.*

□ Remove GCDR and add 1 ml mTeSR Plus.

□ Gently detach colonies by scraping with a cell scraper.

□ Transfer the detached cell aggregates to a 15 mL tube with a P1000.

□ Carefully breakup the colonies by pipetting the cell aggregate suspension up and down 2 - 3 times with a P1000. Avoid making single cells.

- □ Remove vitronectin from the vitronectin coated 6-well plate.
- □ Add 2 ml mTeSR Plus medium to each well.
- □ Add X µl of the cell aggregate suspension onto this new plate according the desired split ratio. Usual split ratio is between 1:20 – 1:100
- □ Place the plate in a 37°C incubator. Move the plate in several quick, short, back-and-forth and side-to-side motions to disperse cells across the surface of the well.
- □ Refresh every other day with 2 ml mTeSR Plus or 4 ml mTeSR Plus over the weekend.

Note: _____