HUMAN PLURIPOTENT STEM CELLS PASSAGING (Vitronectin-E8 media)

Materials:

- Vitronectin coated 6-well plate
- E8 (essential 8) (Life Technologies, A1517001)
- UltraPure™ 0,5 M EDTA*, pH 8,0 (Invitrogen,15575-038)
- DPBS -CaCl2, -MgCl2 (Gibco/14190)

Important note:
Be sure that all materials are at RT before starting the procedure.
Cells expanded twice a week (on Monday and Thursday)

Method (based on one 6-well):

- Dilute EDTA in PBS (1:1000 at final concentration 0.5 mM EDTA).
- Aspirate medium.
- Treat cells with 1 ml EDTA for 4-5 min.
  NOTE: colonies are ready when small wholes visible at 10x magnification start to appear within the colonies.
- Remove EDTA very gently and add 1 ml E8 medium.
- Gently detach colonies by pipetting (if the cells are difficult to dislodge, use a cell scraper).
- Transfer the detached cells to a 15 mL tube with a P1000 and add 1 ml/well of E8 medium to collect the rest of the cells into the same tube
- Carefully dissociate the clusters by pipetting the cell aggregate mixture up and down 2 - 3 times with a P1000, in order to get single cell suspension.
- Remove vitronectin from the vitronectin coated 6-well plate.
- Plate the cell suspension onto this new plate in the correct split ratio (1:6) and top up with E8 medium to 2ml
- Place the plate at 37 °C, 5% CO_2 in a humidified 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 daily with 2 ml E8 or 6 ml E8 over the weekend.