

Maintaining of hiPS-FS.2 cell

HiPS-FS.2 cell was cultured, without feeder cells, in 1:80 diluted matrigel (BD Biosciences, Heidelberg, Germany) coated flask 75 cm² (1:100) (BD Biosciences, Heidelberg, Germany) in essential 8 (E8) (Thermo Fisher Scientific, Darmstadt, Germany). The medium was changed every day. They were split using a ReLeSR dissociation reagent (Thermo Fisher Scientific) once they reached roughly 80-90 % confluency. The cells were dissociated using 3 ml of ReLeSR dissociation reagent for 7 minutes at 37 °C after first being washed with phosphate-buffered saline (PBS) free of calcium and magnesium. Following the collection of the cells using 5 ml pipettes, 3 ml of E8 media was added, and the cells were centrifuged at 900 rpm for 5 minutes. The cells were suspended in 12 milliliters of E8 media and plated on matrigel-coated 12-well plates for further differentiation following centrifugation and supernatant removal. During splitting of the cells, 10 µM of the ROCK inhibitor Y-27632 (Santa Cruz Biotechnologies, Santa Cruz, CA) was added to the E8 medium.