Maintaining of hiPS cell

HiPS cell was cultured, without feeder cells, in 1:80 diluted matrigel (BD Biosciences, Heidelberg, Germany) coated flask 75 cm^2 (1:100) (BD Biosciences, Heidelberg, Germany) in mTeSR™1 medium (STEMCELL technologies). 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 mTeSR™1 medium was added, and the cells were centrifuged at 900 rpm for 5 minutes. The cells were suspended in 12 milliliters of mTeSR™1 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 mTeSR™1 medium.