

In vitro three-germ-layer differentiation

The iPSCs colonies were dissociated using TrypLE™ Express (Gibco) for 6-8 min. The dissociated iPSCs were then cultured for 7 days as embryoid bodies (EBs) in ultra-low attachment 6-well plates in DMEM/F12/Glutamax medium (Gibco) supplemented with 20% KnockOut Serum Replacement (Gibco), 0.1 mM non-essential amino acids (Gibco), 0.1 mM 2-mercaptoethanol (Gibco) and 10 µM Y-27632 (Tocris). On day 8, EBs were dissociated and seeded on a Matrigel® (standard formulation, Corning)-coated 24-well plate and cultured in DMEM/F12/Glutamax media (Gibco) containing 10% fetal bovine serum (Biowest) and 1% Penicillin-Streptomycin (Gibco). After 7 days, the cells were fixed using 4% paraformaldehyde and stained for three germ-layer markers.