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Protocol for in vitro propagation of hTCEpi cells

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Reagents
PBS Trypsin-EDTA (Gibco, Cat# 25300054) Trypsin-Inhibitor (Gibco, Cat# R007100) KGM™-2 BulletKit™ (Lonza, Cat# CC-3107) without GA-1000 from this kit
Practical application
For detachment of cells remove and discard culture medium and wash cells once with PBS. Remove PBS completely. Then, add 0.05 % Trypsin-EDTA (1x) solution (room-temperature; 20 µl/cm²), make sure that all cells have been in contact with this solution and incubate the culture flask at 37°C for approximately 4 – 5 min. Observe cell detachment under an inverted microscope. Make sure that all cells are detached (cells typically detach as a cell layer which decomposes when cells are taken up and resuspended in medium). Add Trypsin-Inhibitor (20 µl/cm²). Thereafter, resuspend the cells in growth medium and centrifuge at 170 g for 5 min. Discard the supernatant, re-suspend the cell pellet in the remaining droplet and add growth medium (~160 µl/cm²). Then, add appropriate aliquots of the cell suspension to new culture vessels with growth medium (final volume of 240 µl/cm²). hTCEpi cells are grown in keratinocyte medium KGM™-2 BulletKit™ without GA-1000 at 37°C in a humidified atmosphere with 5 % CO_2. Cells are split with a ratio of 1:8 twice a week after having reached about 60 – 70 % confluence. Never allow the culture to become completely confluent!