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Protocol for in vitro propagation of HBEC3-KT cells

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Reagents
Gelatin from porcine skin (Sigma-Aldrich, Cat# G1890) Keratinocyte-SFM (1X) Kit (Gibco, Cat# 17005-042) PBS Trypsin-EDTA solution (Gibco, Cat# R-001-100) Fetal Bovine Serum (FBS) (Sigma-Aldrich, Cat# F7524)
Practical application
Propagation: Cells are grown in Keratinocyte-SFM (1X) Kit at 37°C in a humidified atmosphere with 5 % CO_2 in culture ware pre-coated with 0.1 % gelatin from porcine skin. For coating, the culture ware is treated with the gelatin for at least 4 hours (up to 1 week) at 37°C. After removal of the gelatin the culture ware can be used directly for seeding of cells. Subculturing: Cells are passaged after having reached about 80 - 90 % confluence. For detachment of the cells remove and discard the culture medium and wash the cells twice with PBS. Remove PBS completely. Then, add 0.025 % Trypsin-EDTA solution (room temperature), make sure that all cells have been in contact with this solution and incubate the culture flask at 37°C for approximately 2 - 3 min. Observe cell detachment under an inverted microscope. As soon as all cells are detached (if necessary agitate the cells by gently hitting the flask), Trypsin action is stopped by addition of PBS supplemented with 2 % FBS (140 µl/cm²) and the cell suspension is centrifuged for 5 minutes at 170 g. Discard the supernatant, resuspend the cell pellet in the remaining droplet and add growth medium (about 160 µl/cm²). Then, add appropriate aliquots of the cell suspension to gelatin coated culture vessels (see propagation) supplemented with growth medium (final volume of 240 µl/cm²). A split ratio of 1:4 twice a week is recommended.