

Protocol for in vitro propagation of NHEK/SVTERT3-5 cells

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Reagents
PBS Trypsin-EDTA (Gibco, Cat# 25300054) Trypsin-Inhibitor (Gibco, Cat# R007100) KeratinoUp ready-to-use-medium (Evercyte, Cat# MHT-011) which contains: <ul style="list-style-type: none"> • KGM™-2 BulletKit™ (Lonza, Cat# CC-3107) without GA-1000 from this kit and • 50 µg/ml G418 (InvivoGen, Cat# ant-gn)
Practical application
<p>For detachment of cells remove and discard the culture medium and wash the 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 5-7 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), add Trypsin-Inhibitor (20 µl/cm²). Thereafter, resuspend the cells in growth medium and centrifuge at 170 g for 5 min. Discard the supernatant, resuspend the cell pellet in the remaining droplet and add growth medium (about 160 µl/cm²). Add appropriate aliquots of the cell suspension to new culture vessels supplemented with growth medium (final volume of 240 µl/cm²).</p> <p>NHEK/SVTERT3-5 cells are grown in KeratinoUp ready-to-use medium at 37°C in a humidified atmosphere with 5 % CO₂. A split ratio of 1:3 to 1:4 twice a week is recommended (after having reached about 60 - 70 % confluence). Never allow the culture to become completely confluent!</p>
Related products
<ul style="list-style-type: none"> • KeratinoUp ready-to-use medium, 500 ml (Evercyte, Cat# MHT-011)