

Protocol for in vitro propagation of hTEC/SVTER24-B cells

Version: Oktober 2017

Reagents
<p>Collagen human, Bornstein and Traub Type I (Sigma Aldrich, Cat# C7624-5ML) PBS Trypsin-EDTA (Gibco, Cat# 25300054) Trypsin-Inhibitor (Gibco, Cat# R007100) ThymoUp ready-to-use-medium (Evercyte, Cat# MHT-010) which contains:</p> <ul style="list-style-type: none"> • OptiPRO SFM (Gibco, Cat# 12309-019) • 2 mM Glutamax (Gibco, Cat# 35050-038) • 50 µg/ml G418 (InvivoGen, Cat# ant-gn-5)
Practical application
<p>Culture flasks have to be pre-coated with human collagen. Therefore, the culture flasks are treated with human collagen solution I (diluted to 50 µg/mL in PBS; diluted to 50 µg/ml in PBS; 60 µl/cm²) at 37°C for at least 30 min. Before introducing cells, remove excess of human collagen solution I and rinse flask twice with PBS (160 µl/cm²).</p> <p>For detachment of the 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 1 - 2 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). Then, add appropriate aliquots of the cell suspension to new culture vessels supplemented with growth medium (final volume of 240 µl/cm²).</p> <p>Cells are grown in ThymoUp medium supplemented with G418 at 37°C in a humidified atmosphere with 5 % CO₂. A split ratio of 1:6 to 1:8 twice a week is recommended after having reached about 90 % confluence.</p> <p>Never allow the culture to become completely confluent!</p>
Related products
<ul style="list-style-type: none"> • ThymoUp ready-to-use medium, 500 ml (Evercyte, Cat# MHT-010)