

## Protocol for in vitro propagation of RPTEC/TERT1 cells

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Reagents
<p>PBS Trypsin-EDTA, (Gibco, Cat# 25300054) Trypsin-Inhibitor (Gibco, Cat# R007100) ProxUp ready-to-use-medium (Evercyte, Cat# MHT-003) which contains:</p> <ul style="list-style-type: none"> <li>• DMEM/Ham's F-12 (1:1) (Biochrom, Cat# F4815)</li> <li>• 10 mM HEPES-buffer (Biochrom, Cat# L1613)</li> <li>• 2 mM GlutaMAX™-I (Gibco, Cat# 35050-038)</li> <li>• 10 ng/ml hEGF (Sigma-Aldrich, Cat# E9644)</li> <li>• 5 pM 3,3',5-Triiodo-L-thyronine sodium salt (Sigma Aldrich, Cat# T6397)</li> <li>• 3.5 µg/ml L-Ascorbic Acid (Sigma-Aldrich, Cat# A4544)</li> <li>• 5 µg/ml Transferrin Holo (Merck Millipore, Cat# 616424)</li> <li>• 25 ng/ml Prostaglandine E1 (Sigma-Aldrich, Cat# P8908)</li> <li>• 25 ng/ml Hydrocortisone (Sigma-Aldrich, Cat# H0396)</li> <li>• 8.65 ng/ml Sodium-Selenite (Sigma-Aldrich, Cat# S5261)</li> <li>• 100 µg/ml G418 (InvivoGen, Cat# ant-gn-5)</li> <li>• 5 µg/ml Insulin (Sigma-Aldrich, Cat# I9278)</li> </ul>
Practical application
<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/cm<sup>2</sup>), make sure that all cells have been in contact with this solution and incubate the culture flask at 37°C for approximately 2 – 5 min. Observe the 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/cm<sup>2</sup>). 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/cm<sup>2</sup>). Then, add appropriate aliquots of the cell suspension to new culture vessels supplemented with growth medium (final volume of 240 µl/cm<sup>2</sup>).</p> <p>Cells are cultivated at 37°C in a humidified atmosphere with 5 % CO<sub>2</sub>. A split ratio of 1:2 twice a week is recommended (after having reached about 90 - 95 % confluence).</p>
Related products
<ul style="list-style-type: none"> <li>• ProxUp ready-to-use medium, 500 ml (Evercyte, Cat# MHT-003)</li> <li>• ProxUp basal medium, 500 ml (Evercyte, Cat# MHT-003-B)</li> <li>• ProxUp supplements, 9 vials (Evercyte, Cat# MHT-003-S)</li> </ul>