

## Protocol for passaging of RPTEC/TERT1

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Evercyte Ord. No.:	CHT-003-0002
Designation:	RPTEC/TERT1, human renal proximal tubular epithelial cells
Growth medium:	<p>The ProxUp medium for cultivation of RPTEC/TERT1 cells can either be ordered from Evercyte as ready-to-use medium (Cat# MHT-003) or as basal medium (Cat# MHT-003-B) plus supplements (Cat# MHT-003-S).</p> <p>The medium can also be prepared by mixing the following components:</p> <p>DMEM/F12 (1:1) (PAN-Biotech, Cat# P04-41154)          10 mM HEPES-buffer (Sigma-Aldrich, Cat# H0887, ready-to-use)          10 ng/ml hEGF (Sigma-Aldrich, Cat# E9644)          5 pM 3,3',5-Triiodo-L-thyronine sodium salt (T3, Sigma-Aldrich, Cat# T6397)          3.5 µg/ml L-Ascorbic Acid (Sigma-Aldrich, Cat# A4544)          5 µg/ml Transferrin Holo (Merck Millipore, Cat# 616424)          25 ng/ml Prostaglandine E1 (Sigma-Aldrich, Cat# P8908)          25 ng/ml Hydrocortisone (Sigma-Aldrich, Cat# H0396)          8.65 ng/ml Sodium-Selenite (Sigma-Aldrich, Cat# S5261)          5 µg/ml Insulin (Sigma-Aldrich, Cat# I9278, ready-to-use)          100 µg/ml G418 (InvivoGen, Cat# ant-gn-5, ready-to-use)</p> <ul style="list-style-type: none"> <li>- take one bottle of DMEM/F12 (1:1) (500 ml)</li> <li>- add 5 ml of Hepes (1M, ready-to-use)</li> <li>- add 250 µl of hEGF stock (20 µg/ml, prepared in cell culture grade water)</li> <li>- add 250 µl of T3 stock (10 nM, prepared in NaOH, PBS)</li> <li>- add 250 µl of Ascorbic acid stock (7 mg/ml, prepared in cell culture grade water)</li> <li>- add 250 µl of Transferrin Holo stock (10 mg/ml, prepared in cell culture grade water)</li> <li>- add 250 µl of Prostaglandine E1 stock (50 µg/ml, prepared in basal medium)</li> <li>- add 250 µl of Hydrocortisone Stock (50 µg/ml, prepared in cell culture grade water)</li> <li>- add 250 µl of Sodium-Selenite stock (100 µM, prepared in cell culture grade water)</li> <li>- add 250 µl of Insulin (10 mg/ml, ready-to-use)</li> <li>- add 500 µl of G418 stock (100 mg/ml, ready-to-use)</li> <li>- mix properly</li> <li>- store at 4°C for 4 weeks</li> <li>- temper the medium to room temperature before use</li> </ul>
Additional reagents:	<p>0.05% Trypsin-EDTA (Gibco, Cat# 25300-054, ready-to-use, stored at 4°C after thawing)          Defined Trypsin Inhibitor (Gibco, Cat# R007100, ready-to-use, stored at 4°C after thawing)          PBS (Sigma-Aldrich, Cat# D8537, ready-to-use, stored at RT)</p>
Passaging of cells:	<ul style="list-style-type: none"> <li>- remove and discard the culture medium</li> </ul>

- wash the cells once with PBS (each 160  $\mu\text{l}/\text{cm}^2$ ), remove PBS completely
- add Trypsin-EDTA solution (20  $\mu\text{l}/\text{cm}^2$ ), make sure that all cells have been in contact with this solution
- incubate the culture flask at 37°C for 2-5 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 Defined Trypsin Inhibitor (20  $\mu\text{l}/\text{cm}^2$ )
- resuspend the cells in growth medium (about 160  $\mu\text{l}/\text{cm}^2$ ) and aspirate the cells by pipetting
- centrifuge at 170 g for 5 min
- discard the supernatant, resuspend the cell pellet in the remaining droplet and add growth medium
- transfer appropriate aliquots of the cell suspension to new roux flasks supplemented with growth medium (final volume of 240  $\mu\text{l}/\text{cm}^2$ )
- a split ratio of 1:2 to 1:3 twice a week is recommended (after having reached about 95 %), the split ratio should not exceed 1:4
- perform a medium change after 3 days if cells have not reached the required cell density, do not passage the cells before having reached about 95% confluence
- cultivate cells at 37°C in a humidified atmosphere with 5% CO<sub>2</sub>

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Related products: ProxUp ready-to-use medium, 500 ml (Evercyte, Cat# MHT-003)  
ProxUp basal medium, 500 ml (Evercyte, Cat# MHT-003-B)  
ProxUp supplements (Evercyte, Cat# MHT-003-S)

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