

Protocol for passaging of RPTEC/TERT1

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Version: October 2024

Evercyte Ord. No.:	CHT-003-0002
Designation:	RPTEC/TERT1, human renal proximal tubular epithelial cells
Growth medium:	<p>The ProxUp2 medium for cultivation of RPTEC/TERT1 cells can either be ordered from Evercyte as ready-to-use medium (Cat# MHT-003-2) or as basal medium (Cat# MHT-003-B) plus supplements (Cat# MHT-003-2-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) 0.5 % FBS (e.g. PAN-Biotech, Cat# P30-3031, 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">- take one bottle of DMEM/F12 (1:1) (500 ml)- add 5 ml of HEPES-buffer (1M, ready-to-use)- add 2.5 ml of FBS (ready-to-use)- add 250 µl of hEGF stock (20 µg/ml, prepared in cell culture grade water)- add 250 µl of T3 stock (10 nM, prepared in NaOH, PBS)- add 250 µl of Ascorbic acid stock (7 mg/ml, prepared in cell culture grade water)- add 250 µl of Transferrin Holo stock (10 mg/ml, prepared in cell culture grade water)- add 250 µl of Prostaglandine E1 stock (50 µg/ml, prepared in basal medium)- add 250 µl of Hydrocortisone Stock (50 µg/ml, prepared in cell culture grade water)- add 250 µl of Sodium-Selenite stock (100 µM, prepared in cell culture grade water)- add 250 µl of Insulin (10 mg/ml, ready-to-use)- add 500 µl of G418 stock (100 mg/ml, ready-to-use)- mix properly- store at 4°C for 4 weeks- temper the medium to room temperature before use
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# R007-100, ready-to-use, stored at 4°C after thawing)</p>

PBS (Sigma-Aldrich, Cat# D8537, ready-to-use, stored at RT)

- Passaging of cells:
- remove and discard the culture medium
 - 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 once a week is recommended (after having reached about 95 %),
 - perform a medium change after 3 days, do not passage the cells before having reached about 95% confluence
 - cultivate cells at 37°C in a humidified atmosphere with 5% CO₂
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- Related products:
- ProxUp2 ready-to-use medium, 500 ml (Cat# MHT-003-2)
 - ProxUp2 Kit consisting of basal medium (Cat# MHT-003-B) and supplements (Cat# MHT-003-2-S)
 - ProxUp3 (Cat# MHT-003-3, for US customers), this medium contains all components of ProxUp2 but FBS; **before use, 2.5 ml FBS must be added to ProxUp3 medium to give rise to ready-to-use ProxUp2 medium**
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