

## Protocol for preparation of ProxUp2 medium

Version: October 2024

Evercyte Ord. No.:	MHT-003-2
Designation:	RPTEC/TERT1, human renal proximal tubular epithelial cells
Reagents:	<p>The ProxUp2 medium contains the herein described components and is prepared as described in the following:</p> <p>DMEM/F12 (1:1) (PAN-Biotech, Cat# P04-41154) 10 mM HEPES-buffer (Sigma-Aldrich, Cat# H0887) 0.5 % FBS (e.g. PAN-Biotech, Cat# P30-3031) 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) 100 µg/ml G418 (InvivoGen, Cat# ant-gn-5)</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 2.5 ml of FBS (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 (not 37°C) before use</li></ul>
Related products:	<ul style="list-style-type: none"><li>- ProxUp2 ready-to-use medium, 500 ml (Cat# MHT-003-2)</li><li>- ProxUp2 Kit consisting of basal medium (Cat# MHT-003-B) and supplements (Cat# MHT-003-2-S)</li></ul>

- 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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