Protocol for passaging of RPTEC/TERT1

Version: January 2023

Evercyte Ord. No.:	CHT-003-0002
Designation:	RPTEC/TERT1, human renal proximal tubular epithelial cells
Growth medium:	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-003-2-B) plus supplements (Cat# MHT-003-2-S). The medium can also be prepared by mixing the following components: DMEM/F12 (1:1) (PAN-Biotech, Cat# P04-41154) 10 mM HEPES-buffer (Sigma-Aldrich, Cat# H0887, ready-to-use) 0.5 % FBS (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) - 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 E1stock (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:	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)

EVERCYTE GmbH
Leberstraße 20, 1110 Vienna, AUSTRIA
office@evercyte.com | www.evercyte.com

	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 µl/cm²), remove PBS completely - add Trypsin-EDTA solution (20 µl/cm²), 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 µl/cm²) - resuspend the cells in growth medium (about 160 µl/cm²) 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 µl/cm²) - 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_2
Related products:	ProxUp2 ready-to-use medium, 500 ml (Evercyte, Cat# MHT-003-2) ProxUp2 basal medium, 500 ml (Evercyte, Cat# MHT-003-2-B) ProxUp2 supplements (Evercyte, Cat# MHT-003-2-S)

EVERCYTE GmbH
Leberstraße 20, 1110 Vienna, AUSTRIA
office@evercyte.com | www.evercyte.com