Protocol for cryopreservation of RPTEC/TERT1

Version: October 2024

Evercyte Ord. No.:	CHT-003-0002
Designation:	RPTEC/TERT1, human renal proximal tubular epithelial cells
Freezing medium:	Cryostor® cell cryopreservation medium CS10 (Sigma-Aldrich, Cat# C2874, ready-to-use)
Additional reagents:	0.05 % Trypsin-EDTA (Gibco, Cat# 25300-054, ready-to-use, stored at 4°C) Defined Trypsin Inhibitor (Gibco, Cat# R007-100, ready-to-use, stored at 4°C) PBS (Sigma, Cat# D8537, ready-to-use, stored at RT) ProxUp2 medium (Evercyte, Cat# MHT-003-2)
Freezing cells:	- detach the cells from the culture vessel by using Trypsin-EDTA and Defined Trypsin Inhibitor as described in protocol *Passaging of RPTEC/TERT1 cells* - resuspend the detached cells in growth medium and centrifuge at 170 g for 5 min - discard the supernatant - resuspend the resulting cell pellet in the remaining droplet - add freezing medium (tempered to 4°C) to reach a cell density of about 1.5-2 x 10^6 cells/ml (for thawing in a 25 cm^2 culture flask) - add 1 ml of this cell suspension to each pre-cooled cryovial and immediately transfer the cells to -80°C - after 24 hours transfer the vials to the liquid nitrogen tank
Thawing cells:	**When you start cultivating the cells, please transfer the content of the original Evercyte vial containing RPTEC/TERT1 cells into a T25 roux flask as described in the following:** - add 6 ml of growth medium to a 25 cm^2 culture flask and place the culture flask in the incubator for at least 30 min to allow the medium to reach 37°C and its normal pH - take a vial of frozen cells, rinse it outside with ethanol and pre-warm in the hand until one last piece of frozen cells is seen - then, immediately transfer the content of the vial to a 15 ml centrifugation tube pre-filled with 9 ml of medium pre-cooled to 4°C and centrifuge for 5 min at 170 g - discard the supernatant and resuspend the cell pellet in the remaining droplet - add 1 ml of the pre-warmed medium to the cells, transfer the cells to the prepared culture flask and incubate at 37°C in a suitable incubator - perform a medium change 24 hours after thawing - if the cells are already confluent at this point, they must be passaged as described in protocol *Passaging of RPTEC/TERT1 cells* - for the first passages after thawing, we recommend a split ratio of 1:2 or lower - after thawing, cells generally need 2-3 days before they can be passaged, do not split the cells before having reached about 95% confluence
Related products:	- ProxUp2 ready-to-use medium, 500 ml (Cat# MHT-003-2)

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- ProxUp2 Kit consisting of basal medium (MHT-003-B) and supplements (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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