

Protocol for cryopreservation of RPTEC/TERT1

Version: January 2023

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:	<ul style="list-style-type: none">- detach the cells from the culture vessel by using Trypsin-EDTA and Defined Trpsin Inhibitor as described in protocol <i>Passaging of RPTEC/TERT1 cells</i>- 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 $1.5-2 \times 10^6$ cells/ml (for thawing in a 25 cm² 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:	<p>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:</p> <ul style="list-style-type: none">- add 6 ml of growth medium to a 25 cm² 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 <i>Passaging of RPTEC/TERT1 cells</i>- 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 (Evercyte, Cat# MHT-003-2) ProxUp2 basal medium, 500 ml (Evercyte, Cat# MHT-003-2-B)

ProxUp2 supplements (Evercyte, Cat# MHT-003-2-S)