Protocol for cryopreservation of hTEC/SVTERT24-B

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Evercyte Ord. No.:	CLHT-010-0024-B
Designation:	hTEC/SVTERT24-B, human thymic epithelial cells
Freezing medium:	CryoStor® cell cryopreservation medium CS10 (Sigma-Aldrich, Cat# C2874, ready-to-use)
Additional reagents:	OptiPRO™ SFM medium (Evercyte, Cat# MHT-033-3) supplemented with GlutaMAX™-I and G418 (for preparation please refer to protocol *Preparation of hTEC/SVTERT24-B medium*) 50 µg/ml Collagen I solution (Sigma-Aldrich, Cat# C2249), for preparation please refer to protocol *Passaging of hTEC/SVTERT24-B cells* 0.05 % Trypsin-EDTA (Gibco, Cat# 25300-054, ready-to-use, stored at 4°C after thawing) Defined Trypsin Inhibitor (Gibco, Cat# R007100, ready-to-use, stored at 4°C after thawing) PBS (Sigma-Aldrich, Cat# D8537, ready-to-use, stored at RT)
Freezing cells:	- detach the cells from the culture vessel by using Trypsin-EDTA solution as described in protocol *Passaging of hTEC/SVTERT24-B 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 x 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:	**When you start cultivating the cells, please transfer the content of the original Evercyte vial containing hTEC/SVTERT24-B cells into a T25 roux flask as described in the following:** - pre-coat a 25 cm² culture flask with Collagen I as described in protocol *Passaging of hTEC/SVTERT24-B cells* - add 6 ml of growth medium to the Collagen I coated 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 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

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

- 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 90 % confluent at this point, they have to be passaged as described in protocol *Passaging of hTEC/SVTERT24-B cells*

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