

## Protocol for cryopreservation of hTCEpi

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Evercyte Ord. No.:	CHT-045-0237
Designation:	hTCEpi, human corneal epithelial cells
Freezing medium:	CryoStor® 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# R007100, ready-to-use, stored at 4°C) PBS (Sigma-Aldrich, Cat# D8537, ready-to-use, stored at RT)
Freezing cells:	<ul style="list-style-type: none"><li>- detach the cells from the culture vessel by using Trypsin-EDTA solution as described in protocol <i>Passaging of hTCEpi cells</i></li><li>- resuspend the detached cells in growth medium and centrifuge at 170 g for 5 min</li><li>- discard the supernatant</li><li>- resuspend the resulting cell pellet in the remaining droplet</li><li>- add freezing medium (tempered to 4°C) to reach a cell density of about <math>1 \times 10^6</math> cells/ml (for thawing in a 25 cm<sup>2</sup> culture flask)</li><li>- add 1 ml of this cell suspension to each pre-cooled cryovial and immediately transfer the cells to -80°C</li><li>- after 24 hours transfer the vials to the liquid nitrogen tank</li></ul>
Thawing cells:	<p><b>When you start cultivating the cells, please transfer the content of the original Evercyte vial containing hTCEpi cells into a T25 roux flask as described in the following:</b></p> <ul style="list-style-type: none"><li>- add 6 ml of growth medium to a 25 cm<sup>2</sup> 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</li><li>- 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</li><li>- 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</li><li>- discard the supernatant and resuspend the cell pellet in the remaining droplet</li><li>- 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</li><li>- perform a medium change 24 hours after thawing, if the cells are already 60 - 70% confluent at this point, they have to be passaged as described in protocol <i>Passaging of hTCEpi cells</i></li></ul>
Related products:	HCEC-1CT, colonic epithelial progenitor cells (Evercyte, Cat# CkHT-039-0229) PODO/TERT256, kidney tissue-derived podocytes (Evercyte, Cat# CHT-033-0256) PODO/SVTERT152, urine-derived podocytes (Evercyte, Cat# CLHT-033-0152)