

Protocol for cryopreservation of HCEC-1CT

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Evercyte Ord. No.:	CkHT-039-0229
Designation:	HCEC-1CT, human colonic epithelial progenitor cells
Freezing medium:	<p>The freezing medium is prepared by mixing the following components:</p> <p>ColoUp medium (Evercyte, Cat# CHT-039), for preparation please refer to protocol <i>Preparation of ColoUp medium</i></p> <p>10 % DMSO (Sigma-Aldrich, Cat# D2650, ready-to-use, stored at RT)</p> <p>10 % Cosmic Calf Serum (Hyclone, Cat# SH30087, ready-to-use, stored at -20°C)</p> <p>Preparation of 10 ml freezing medium:</p> <ul style="list-style-type: none"> - take 8 ml ColoUp medium and transfer to 15 ml centrifugation tube - add 1 ml DMSO and mix carefully - add 1ml of Cosmic Calf Serum and mix carefully - store at 4°C until use, use freshly prepared freezing medium for cryopreservation
Additional reagents:	<p>ColoUp medium (Evercyte, Cat# MHT-039), for preparation please refer to protocol <i>Preparation of ColoUp medium</i></p> <p>0.05% Trypsin-EDTA (Gibco, Cat# 25300-054, ready-to-use, stored at 4°C after thawing)</p> <p>Defined Trypsin Inhibitor (Gibco, Cat# R007100, ready-to-use, stored at 4°C after thawing)</p> <p>PBS (Sigma, Cat# D8537, ready-to-use, stored at RT)</p>
Freezing cells:	<ul style="list-style-type: none"> - detach the cells from the culture vessel by using Trypsin-EDTA and Defined Trypsin Inhibitor as described in protocol <i>Passaging of HCEC-1CT 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 - 2 x 10⁶ 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 HCEC-1CT 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 reach to 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 have to be passaged as described in protocol *Passaging of HCEC-1CT cells*

Related products: ColoUp ready-to-use medium, 500 ml (Evercyte, Cat# MHT-039)
ColoUp basal medium, 500 ml (Evercyte, Cat# MHT-039-B)
ColoUp supplements (Evercyte, Cat# MHT-039-S)
HCEC-1CT, human colon epithelial cells (Evercyte, Cat# CkHT-039-0229)