

Protocol for passaging of HCEC-1CT

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Evercyte Ord. No.:	CkHT-039-0229
Designation:	HCEC-1CT, human colonic epithelial progenitor cells
Growth medium:	<p>The ColoUp medium contains the herein described components and is prepared as described in the following:</p> <p>DMEM (Gibco, Cat # 61965-026) / M199 (Gibco, Cat# 31150022) (4+1) 2 % Cosmic Calf Serum (e.g. Hyclone, Cat# SH30087, ready-to-use) 20 ng/ml hEGF (Sigma-Aldrich, Cat# E9644) 10 µg/ml Insulin (Sigma-Aldrich, Cat# I9278, ready-to-use) 2 µg/ml Apo-Transferrin (Sigma-Aldrich, Cat# T2036) 5 nM Sodium-Selenite (Sigma-Aldrich, Cat# S5261) 1 µg/ml Hydrocortisone (Sigma-Aldrich, Cat# H0396)</p> <ul style="list-style-type: none">- take one bottle (500 ml) of DMEM and discard 100 ml- add 100 ml of M199 and mix properly- discard 11.6 ml DMEM/M199 mixture- add 10 ml Cosmic Calf Serum (ready-to-use)- add 500 µl of hEGF stock (20 µg/ml, prepared in cell culture grade water)- add 500 µl of Insulin (10 µg/ml, ready-to-use)- add 100 µl of Apo-Transferrin stock (10 mg/ml, prepared in M199)- add 25 µl of Sodium-Selenite stock (100 µM, prepared in cell culture grade water)- add 500 µl Hydrocortisone stock (1 mg/ml, prepared in cell culture grade water)- mix properly and store at 4°C for up to 1 month- temper the medium to room temperature (not 37°C) before use
Additional reagents:	<ul style="list-style-type: none">- 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)
Passaging of cells:	<ul style="list-style-type: none">- remove and discard the culture medium- wash the cells once with PBS (each 160 µl/cm²), remove PBS completely- add Trypsin-EDTA solution (20 µl/cm²), make sure that all cells have been in contact with this solution- incubate the culture flask at 37°C for approximately 2-3 min- observe cell detachment under an Inverted microscope- as soon as all cells are detached (if necessary, agitate the cells by gently hitting the flask), add Defined Trypsin Inhibitor (20 µl/cm²)

- resuspend the cells in growth medium (160 $\mu\text{l}/\text{cm}^2$) and aspirate the cells by pipetting
- centrifuge at 170 g for 5 min
- discard the supernatant, resuspend the cell pellet in the remaining droplet and add growth medium
- transfer appropriate aliquots of the cell suspension to new culture vessels supplemented with growth medium (final volume of 240 $\mu\text{l}/\text{cm}^2$)
- a split ratio of 1:16 twice a week is recommended (after cells have reached about 85-95 % confluence)
- cultivate cells at 37°C in a humidified atmosphere with 5% CO₂

Related products:

- ColoUp ready-to-use medium, 500 ml (Cat# MHT-039)
 - ColoUp Kit consisting of basal medium (Cat# MHT-039-B) and supplements (Cat# MHT-039-S)
 - ColoUp2 (Cat# MHT-039-2, for US customers), this medium contains all components of ColoUp but Cosmic Calf Serum (CCS); **before use, 10 ml CCS must be added to ColoUp2 medium to give rise to ready-to-use ColoUp medium**
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