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:	The ColoUp medium for cultivation of HCEC-1CT cells can either be ordered from Evercyte as ready-to-use medium (Cat# MHT-039) or as basal medium (Cat# MHT-039-B) plus supplements (Cat# MHT-039-S). The medium can also be prepared by mixing the following components: DMEM (Gibco, Cat # 10566016) / M199 (Gibco, Cat# 31150022) (4+1) 2 % Cosmic Calf Serum (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) - 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 mg/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:	- 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:	- 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

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	- 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 µl/ cm²) 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 µl/cm²) - 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% CO_2
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)

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