Product-Data-Sheet for HCEC-1CT

Version: May 2021

Evercyte Ord. No.:	CkHT-039-0229
Designation:	HCEC-1CT
Biosafety Level:	1
Shipped:	Frozen on dry ice
Medium:	ColoUp (Evercyte, Cat# MHT-039): 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)
Growth:	Adherent
Organism:	Homo sapiens (human)
Morphology:	Irregular cuboidal after seeding, spindle-shaped morphology when reaching confluence Cuboidal, epithelial morphology when differentiation is induced
Source:	Human colonic biopsies
Cell Type:	Human colonic epithelial progenitor cells
Antigen Expression:	Positive for Mucin-1, antigen A33, Villin
Ethical statement:	Approved by Institutional Review Board (IRB) in accordance with the Declaration of Helsinki.
Comments:	HCEC-1CT was developed from human colonic epithelial cells by transduction with retroviral vectors containing cdk-4 and hTERT gene. The cell line was continuously cultured for more than 200 population doublings without showing signs of growth retardation or replicative senescence. Growing cells show markers of mesenchymal cells, which can be differentiated towards colonic epithelial cells expressing typical markers such as Mucin-1, antigen A33 and Villin.
Propagation:	Cells are grown in above described ColoUp medium at 37°C in a humidified atmosphere with 5 % CO_2.

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Subculturing:	For detachment of the cells remove and discard the culture medium and wash the cells once with PBS. Remove PBS completely. Then, add 0.05 % Trypsin-EDTA solution (RT, 20 µg/ cm², Gibco, Cat# 25300-054), make sure that all cells have been in contact with this solution and 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; Gibco, Cat# R007-100). Thereafter, resuspend the cells in growth medium (about 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. Then, add appropriate aliquots of the cell suspension to the new culture flasks supplemented with growth medium (final volume of 240 µl/cm²). A split ratio of 1:16 twice a week is recommended (after having reached about 85-95% confluence). Cultivate cells at 37°C in a humidified atmosphere with 5 % CO_2.
Preservation:	Freezing medium: ColoUp medium (Evercyte, Cat# MHT-039) 10 % DMSO (Sigma-Aldrich, Cat# D2650) 10 % Cosmic Calf Serum (Hyclone, Cat# SH30087) Storage temperature: liquid nitrogen
Freezing and thawing procedure:	Freezing of cells: Detach the cells from the culture vessel by using Trypsin-EDTA and Defined Trypsin Inhibitor as described above, resuspend the detached cells in growth medium and centrifuge at 170 g for 5 min. Then, discard the supernatant, resuspend the cell pellet in the remaining droplet and add freezing medium (tempered to 4°C) to reach a cell density of about 1-2 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 transferred the cells to -80°C. After 24 hours transfer the vials to the liquid nitrogen tank for long-term storage. Thawing of cells: **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**: 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 to reach 37°C and its normal pH. Take a vial of frozen cells, rinsed 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 pre-warmed medium to the cells, transfer them 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 (see above or protocol *Passaging of HCEC-1CT cells*).
Doubling Time:	18 - 24 hours
Virus Testing	Cells have been tested negative for HAV and Parvo B19 with Roche DPX-PCR (cobas® TaqScreen DPX-Test), for HBV and HCV nucleic acids with Roche-Multiplex-PCR (cobas® TaqScreen MPX Test, v2.0). The presence of HIV was excluded using a reverse transcriptase (PERT)-assay and a p24 ELISA.
Other Analytical Data:	Cells are negative for Mycoplasma contaminations as tested using MycoAlertTM Mycoplasma Detection Kit from Lonza. Cells are negative for bacterial and fungal contaminations as tested according to Ph. Eur. 2.6.1. / USP <71>. STR profile has been analysed and is as expected.

Please Note:

The classification of biosafety level is based on Austrian Legislation (Gentechnikbuch; Systemverordnung) and on recommendations of the Central Committee on Biological Safety (ZKBS). While Evercyte undertakes all reasonable measures to test for absence of a selected panel of known human pathogenic viruses, there is currently no test procedure available that guarantees for complete absence of infectious pathogens. The use of state-of-the art infectious virus assays or viral antigen assays may leave open the possible existence of a latent viral genome, even if a negative test result is obtained. Therefore, we recommend that all human cell lines should be handled with caution such as an organism of ACDP Hazard Group 2.
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