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Product-Data-Sheet for HCEC-1CT

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Evercyte Ord. No.:	CkHT-039-0229
Designation:	HCEC-1CT
Biosafety Level:	1
Shipped:	Frozen on dry ice
Medium:	ColoUp (Evercyte, Cat# MHT-039): DMEM / Medium 199 Earle's, 4+1 (Biochrom Cat# F0435 and Cat# FG0615) 4 mM GlutaMAX™-1 (100X), (Gibco, Cat# 35050-038) 2 % Cosmic Calf Serum (Hyclone, Cat# SH30087) 20 ng/ml EGF (Sigma Aldrich, Cat# E9644) 10 µg/ml Insulin (Sigma Aldrich, Cat# I9278) 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 on Primaria™ culture flasks (Corning, Cat# 353808 and Cat# 353810)
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.

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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 so far 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 medium at 37°C in a humidified atmosphere with 5 % CO_2 and 2 - 5 % oxygen in Primaria culture ware (see above).
Subculturing:	Cells are passaged after having reached about 85 - 95 % confluence. For detachment the cell culture medium is removed and the cells are washed once with PBS. Then, Trypsin-EDTA solution (0.05 %, Gibco Cat# 25300-054, 20 µl/cm²) is added and the culture flasks are incubated at 37°C for approximately 2 - 3 minutes. Cell detachment is observed under an inverted microscope. As soon as all cells are detached (if necessary agitate the cells by gently hitting the flask), Trypsin action is halted by addition of Defined Trypsin Inhibitor (Gibco, Cat# R007-100, 20 µl/cm²). Growth medium is then added to the cells and the cell suspension is centrifuged for 5 minutes at 170 g. Then, the supernatant is discarded, the cell pellet is resuspended in the remaining droplet and growth medium (about 160 µl/cm²) is added. Then, appropriate aliquots of the cell suspension are transferred 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.
Preservation:	Freezing medium: Growth medium (see above) + 10 % DMSO + 10 % cosmic calf serum; storage temperature: liquid nitrogen
Freezing and thawing procedure:	Freezing of cells: Cells are detached from the culture vessel by using Trypsin-EDTA and Trypsin-Inhibitor as described above and centrifuged at 170 g for 5 min. Then, the supernatant is discarded, the cell pellet is resuspended in the remaining droplet and freezing medium (pre-cooled to 4°C) is added to reach a cell density of about 1 - 2 x 10^6 cells/ml (for thawing in a 25 cm² culture flask). Then, 1 ml of this cell suspension is added to each pre-cooled cryovial which are then immediately transferred to -80°C. After 24 hours the vials are transferred to liquid nitrogen for long-term storage. Thawing of cells: 6 ml of growth medium are added to a 25 cm² culture flask, which is transferred for at least 30 min to a humidified incubator to allow the medium to reach 37°C and its normal pH. Then, a

	vial of frozen cells is taken, rinsed outside with Ethanol and pre-warmed in the hand until one last piece of frozen cells is seen. Thereafter, the content of the vial is immediately transferred to a 15 ml centrifugation tube pre-filled with 9 ml of medium pre-cooled to 4°C and centrifuged for 5 min at 170 g. Then, the supernatant is discarded, the cell pellet is resuspended in the remaining droplet and 1 ml of the pre-warmed medium is added to the cells. This cell suspension is then transferred to the prepared culture flask and incubated at 37°C in a suitable incubator. After 24 h a medium change is performed. If the cells are already confluent at this point, they should be passaged (see subculturing).
Doubling Time:	About 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 MycoAlert™ 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 analyzed and is as expected.

Please Note:

The classification of biosafety level is based on the directive 2000/54/EG of the European Parliament and of the Council on the protection of workers from risks related to exposure to biological agents at work. 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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