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Product-Data-Sheet for RPTEC/TERT1

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Evercyte Ord. No.:	CHT-003-0002
Designation:	RPTEC/TERT1
Biosafety Level:	1
Shipped:	Frozen on dry ice
Medium:	ProxUp (Evercyte, Cat# MHT-003): DMEM/Ham's F-12 (1:1) (Biochrom, Cat# AG F4815) 10 mM HEPES-buffer (Biochrom, Cat# AG L1613) 2 mM GlutaMAX™-I (Gibco, Cat# 35050-038) 10 ng/ml hEGF (Sigma Aldrich, Cat# E9644) 5 pM 3,3',5-Triiodo-L-thyronine sodium salt (Sigma Aldrich, Cat# T6397) 3.5 µg/ml L-Ascorbic Acid (Sigma Aldrich, Cat# A4544) 5 µg/ml Transferrin Holo (Merck Millipore, Cat# 616424) 25 ng/ml Prostaglandine E1 (Sigma Aldrich, Cat# P8908) 25 ng/ml Hydrocortisone (Sigma Aldrich, Cat# H0396) 8.65 ng/ml Sodium-Selenite (Sigma Aldrich, Cat# S5261) 100 µg/ml G418 (InvivoGen, Cat# ant-gn-5) 5 µg/ml Insulin (Sigma Aldrich, Cat# I9278)
Growth:	Adherent
Organism:	Homo sapiens (human)
Morphology:	Epithelial-like
Source:	Human kidney cortex
Cell Type:	Proximal tubular epithelial cells

Antigen Expression:	Positive for CD13, E-Cadherin, ZO-1
Ethical statement:	Approved by Institutional Review Board (IRB) in accordance with the Declaration of Helsinki.
Comments:	The RPTEC/TERT1 cell line was developed from human proximal tubular epithelial cells by transduction with a retroviral expression vector (pLXSN) containing the hTERT gene. The cell line was continuously cultured for more than 60 population doublings after cryoconservation without showing signs of growth retardation so far or replicative senescence whereas the parental cells senesced after having reached 24 population doublings. The cells show morphological as well as biochemical markers of proximal tubular epithelial cells as they are characterized by the typical cobblestone appearance, dome formation when grown to high cell density and gamma-glutamyl transferase activity. Furthermore, RPTEC/TERT1 cells respond to parathyroid hormone (PTH) but not arginine vasopressin (AVP) treatment by enhancing cAMP production.
Propagation:	Cells are grown in ProxUp medium (see above) at 37°C in a humidified atmosphere with 5 % CO_2.
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 (1x) solution (room-temperature; 20 µl/cm²; Gibco, Cat# 25300054), make sure that all cells have been in contact with this solution and incubate the culture flask at 37°C for approximately 2 – 5 min. Observe the cell detachment under an inverted microscope. As soon as all cells are detached (if necessary agitate the cells by gently hitting the flask), add Trypsin-Inhibitor (20 µl/cm²; Gibco, Cat# R007100). Thereafter, resuspend the cells in growth medium and centrifuge at 170 g for 5 min. Discard the supernatant, resuspend the cell pellet in the remaining droplet and add growth medium (about 160 µl/cm²). Then, add appropriate aliquots of the cell suspension to new culture vessels supplemented with growth medium (final volume of 240 µl/cm²). A split ratio of 1:2 twice a week is recommended (after having reached about 90 - 95 % confluence).
Preservation:	Freezing medium: CryoStor® cell cryopreservation medium CS10 (Sigma Aldrich, Cat# C2874) Storage temperature: liquid nitrogen
Freezing and thawing procedure:	Freezing of cells: Detach the cells from the culture vessel by using Trypsin and 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 in the remaining droplet and

	add freezing medium (4°C) to reach a cell density of about 1.5 - 2 x 10^6 cells/ml (for thawing in a 25 cm² culture flask). Transfer 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 of cells: 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. Take a vial of frozen cells, rinse outside with Ethanol and pre-warm in 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. Then, discard the supernatant and resuspend the cells in the remaining droplet. Add 1 ml of pre-warmed medium to the cells, transfer the cell suspension 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 should be passaged (see subculturing).
Doubling Time:	About 72 – 96 hours
Virus Testing	Cells have been tested negative for HAV and Parvo B19 with Roche DPX-PCR (cobas® TaqScreen DPX-Test), for HBV, HCV, HIV nucleic acids with Roche-Multiplex-PCR (cobas® TaqScreen MPX Test, v2.0).
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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