

## Product-Data-Sheet for RPTEC/TERT1

Version: May 2021

Evercyte Ord. No.:	CHT-003-0002
Designation:	RPTEC/TERT1
Biosafety Level:	1
Shipped:	Frozen on dry ice
Growth medium:	<p>ProxUp (Evercyte, Cat# MHT-003)</p> <p>DMEM/F12 (1:1) (PAN-Biotech, Cat# P04-41154)            10 mM HEPES-buffer (Sigma-Aldrich, Cat# H0887)            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)</p>
Growth:	Adherent
Organism:	Homo sapiens (human)
Morphology:	Epithelial, cobblestone morphology
Source:	Human kidney cortex, male donor
Cell Type:	Renal 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:	<p>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.</p> <p>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. Dome formation at high cell density is enhanced by addition of 0.5% FBS to the medium.</p>

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Furthermore, RPTEC/TERT1 cells respond to parathyroid hormone (PTH) but not arginine vasopressin (AVP) treatment by enhancing cAMP production.

The cell line was continuously cultured for more than 60 population doublings without showing signs of growth retardation or replicative senescence whereas the parental cells senesced after having reached 24 population doublings. Cells readily recover from cryopreservation and no changes in growth characteristics have been observed after thawing

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**Propagation:** Cells are grown in ProxUp medium (see above) at 37°C in a humidified atmosphere with 5 % CO<sub>2</sub>.

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**Subculturing:** For detachment of the cells remove and discard the culture medium and wash the cells once with PBS (about 160 µl/cm<sup>2</sup>). Remove PBS completely. Then, add 0.05 % Trypsin-EDTA solution (room-temperature, 20 µl/cm<sup>2</sup>, 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 Defined Trypsin Inhibitor (20 µl/cm<sup>2</sup>; 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/cm<sup>2</sup>). Then, add appropriate aliquots of the cell suspension to new culture vessels supplemented with growth medium (final volume of 240 µl/cm<sup>2</sup>). A split ratio of 1:2 to 1:3 twice a week is recommended (after having reached about 95 % confluence). The split ratio should not exceed 1:4. In order to maintain the epithelial character of the cells, it is better not to passage the cells before having reached a density of about 95%.

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**Preservation:** Freezing medium:  
  
CryoStor® cell cryopreservation medium CS10 (Sigma-Aldrich, Cat# C2874)  
  
Storage temperature: liquid nitrogen

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**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 (4°C) to reach a cell density of about 1.5-2 x 10<sup>6</sup> cells/ml (for thawing in a 25 cm<sup>2</sup> 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:

**When you start cultivating the cells, please transfer the content of the original Evercyte vial containing RPTEC/TERT1 cells into a T25 roux flask as described in the following:**

Add 6 ml of growth medium to a 25 cm<sup>2</sup> 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, 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 culture medium pre-cooled to 4°C and centrifuge for 5 min at 170 g.

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 have to be passaged (see above or protocol *Passaging of RPTEC/TERT1 cells*).

For the first passages after thawing we recommend a split ratio of 1:2 or lower. After thawing, cells generally need 2-3 days before they can be passaged. Do not split the cells before having reached about 95% confluence.

Doubling Time:	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 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. People who work with our cells must follow national regulations and safety precautions. The laboratories must be equipped with a security level according to the classification of the cells / products. Evercyte assumes no liability whatsoever in connection with the receipt, handling or the consequences of improper use of our products.

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