Product-Data-Sheet for hTCEpi

Version: July 2024

Evercyte Ord. No.:	CHT-045-0237
Designation:	hTCEpi
Biosafety Level:	1
Shipped:	Frozen on dry ice
Growth medium:	KGM™ Gold Keratinocyte Growth Medium BulletKit™ (Lonza, Cat# 00192060): Final components: KBM™ Gold™ Basal Medium (Lonza, Cat# 00192151) Components of KGM™ Gold™ SingleQuots™ supplements (Lonza, Cat# 00192152: BPE, hEGF, Insulin, Hydrocortisone, Transferrin, Epinephrine, **without GA**)
Growth:	Adherent
Organism:	Homo sapiens (human)
Morphology:	Epithelial
Source:	Human limbal region of corneal tissue
Cell Type:	Corneal epithelial cells
Antigen Expression:	Positive for ZO-1, expression of KRT3 after induction of differentiation
Ethical statement:	Approved by Institutional Review Board (IRB) in accordance with the Declaration of Helsinki
Comments:	hTCEpi cell line was developed from human limbal corneal epithelial cells by ectopic expression of the catalytic subunit of human telomerase (hTERT). Cells express ZO-1 and when grown to high cell densities cells start to express KRT3 (keratin 3). In an air-lift-culture, cells form multi-layered cell sheets showing stratification and expression of the cornea specific KRT3 (Robertson et al., Invest Ophthalmol Vis Sci. 2005 Feb; 46(2):470-8). The cell line was continuously cultured in low calcium conditions for more than 200 population doublings without showing signs of replicative senescence. When cultivated in the presence of high calcium concentrations cells cease to grow and start to differentiate. Cells readily recover from cryopreservation as shown by longevity studies performed post thawing. No changes of the growth characteristics have been observed after thawing.
Propagation:	Cells are grown in KGM™ Gold Keratinocyte Growth Medium BulletKit™ without GA-1000 (see above) at 37°C in a humidified atmosphere with 5 % CO_2.

EVERCYTE GmbH
Leberstraße 20, 1110 Vienna, AUSTRIA
office@evercyte.com | www.evercyte.com

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 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 4-5 min. Observe cell detachment under an inverted microscope. Make sure that all cells are detached (if necessary, carefully shake the flask for complete detachment) and add Defined Trypsin Inhibitor (20 µl/cm², Gibco, Cat# R007100). Cells typically detach as a cell layer which decomposes when cells are taken up and resuspended in medium. 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, 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 1:6-1:8 twice a week is recommended (after having reached about 60-70 % confluence). Never allow the culture to become completely confluent!
Preservation:	Freezing medium: CryoStor® CS10 (Sigma-Aldrich, Cat# C2874, ready-to-use) 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 resulting cell pellet in the remaining droplet and add freezing medium (tempered to 4°C) to reach a cell density of about 1 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 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 hTCEpi 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, rinse 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. Then, discard the supernatant and resuspend the cell pellet in the remaining droplet. Add 1 ml of the 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 60-70 % confluent at this point, they must be passaged (see above or protocol *Passaging of hTCEpi cells*).
Doubling Time:	24-32 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 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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