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Product-Data-Sheet for hTCEpi

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Evercyte Ord. No.:	CHT-045-0237
Designation:	hTCEpi
Biosafety Level:	1
Shipped:	Frozen on dry ice
Medium:	KGM-2 Bullet Kit (Lonza, Cat# CC-3107) without GA-1000 from this kit
Growth:	Adherent
Organism:	Homo sapiens (human)
Morphology:	Epithelial-like
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). 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 express ZO-1 and

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	when grown to high cell densities cells start to express KRT3 (keratin 3). In an air-lift-culture, cells form multilayered cell sheets showing stratification and expression of the cornea specific KRT3 (Robertson et al., Invest Ophthalmol Vis Sci. 2005 Feb; 46(2):470-8). 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 keratinocyte medium KGM-2 Bullet Kit without GA-1000 (see above) at 37°C in a humidified atmosphere with 5 % CO_2.
Subculturing:	For detachment of cells remove and discard culture medium and wash 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 4 – 5 min. Observe cell detachment under an inverted microscope. Make sure that all cells are detached (cells typically detach as a cell layer which decomposes when cells are taken up and resuspended in medium). 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, re-suspend the cell pellet in the remaining droplet and add growth medium (~160 µl/cm²). Then, add appropriate aliquots of the cell suspension to new culture vessels with growth medium (final volume of 240 µl/cm²). Cells are split with a ratio of 1:8 twice a week after having reached about 60 – 70 % confluence. Never allow the culture to become completely confluent!
Preservation:	Freezing medium: CryoStor® cell cryopreservation medium CS10 (Sigma Aldrich, Cat# C2874) Storage temperature: liquid nitrogen
Freezing and thawing procedure:	Freezing of cells: Detach cells from culture vessel by using trypsin and trypsin-inhibitor as described above, resuspend detached cells in growth medium and centrifuge at 170 g for 5 min. Then, discard the supernatant, resuspend the cells in the remaining droplet and add freezing medium (4°C) to reach a cell density of 5 x 10^5 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 liquid nitrogen. 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 to warm the medium and to allow the medium to reach 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 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 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 should be passaged (see subculturing).
Doubling Time:	About 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 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 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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