Product-Data-Sheet for HBEC3-KT

Version: May 2021

Evercyte Ord. No.:	CkHT-004-0230
Designation:	HBEC3-KT
Biosafety Level:	1
Shipped:	Frozen on dry ice
Growth medium:	Keratinocyte-SFM Kit (Gibco, Cat# 17005-042): Final components: Keratinocyte-SFM basal medium (Gibco, Cat# 17005042) Bovine Pituitary Extract (Gibco, Cat# 17005042) EGF (Gibco, Cat# 17005042)
Growth:	Adherent
Organism:	Homo sapiens (human)
Morphology:	Epithelial
Source:	Lung bronchiole (female donor)
Cell Type:	Bronchial epithelial progenitor cells
Antigen Expression:	Positive for CC10 (Clara cells 10 kDa secretory protein), p63
Ethical statement:	Approved by Institutional Review Board (IRB) in accordance with the Declaration of Helsinki
Comments:	HBEC3-KT was developed from human bronchiole epithelial cells by transduction with retroviral vectors containing the cdk-4 and hTERT gene. The cells show markers and functions of different types of lung epithelial cells. The cell line was continuously cultured for more than 90 population doublings without showing signs of growth retardation or replicative senescence. Cells readily recover from cryopreservation and no changes in growth characteristics have been observed after thawing.
Propagation:	Cells are grown on Gelatin coated culture flasks in Keratinocyte-SFM medium (see above) at 37°C in a humidified atmosphere with 5 % CO_2.
Subculturing:	The new culture flasks have to be pre-coated with porcine Gelatin (Sigma-Aldrich, Cat# G1890; diluted to 0.1 % in PBS). Therefore, the culture flasks are treated with Gelatin solution (80 µl/cm²) at 37°C for 4 hours (up to one week). Before introducing cells,

	remove excess of Gelatin solution. Use the pre-coated flasks immediately for seeding of cells, the surface must not dry out. For detachment of the cells remove and discard the culture medium and wash the cells twice with PBS (each 160 µl/cm²). Remove PBS completely. Then, add 0.025 % 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 2-3 min. Observe cell detachment 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 2 % FCS in PBS (220 µl/cm²). Centrifuge at 170 g for 5 min. Then, discard the supernatant, resuspend the cell pellet in the remaining droplet and add growth medium (about 160 µl/cm²). Transfer appropriate aliquots of the cell suspension to Gelatin-coated culture vessels supplemented with growth medium (final volume of 240 µl/cm²). A split ratio of 1:4 twice a week is recommended (after cells have reached about 80-90 % confluence). Cultivate cells at 37°C in a humidified atmosphere with 5 % CO_2.
Preservation:	Freezing medium: Growth medium for HBEC3-KT cells (see above) 10% FBS (Sigma-Aldrich, Cat# F7524) 10% DMSO (Sigma-Aldrich, Cat# D2650) Storage temperature: liquid nitrogen
Freezing and thawing procedure:	Freezing of cells: Detach the cells from the culture vessel by using Trypsin-EDTA 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 HBEC3-KT cells into a T25 roux flask as described in the following:** Pre-coat a 25 cm² culture flask with Gelatin (see above or protocol *Passaging of HBEC3-KT cells*). Add 6 ml of growth medium to a 25 cm² culture flask and place it 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 80-90 % confluent at this point, they have to be passaged (see above or protocol *Passaging of HBEC3-KT cells*).
Doubling Time:	36-48 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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