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Product-Data-Sheet for HBEC3-KT

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Evercyte Ord. No.:	CkHT-004-0230
Designation:	HBEC3-KT
Biosafety Level:	1
Shipped:	Frozen on dry ice
Medium:	Keratinocyte-SFM (1X) Kit (Gibco, Cat# 17005-042)
Growth:	Adherent
Organism:	Homo sapiens (human)
Morphology:	Epithelial-like, cobblestone appearance
Source:	Human central lung bronchiole, female donor
Cell Type:	Human bronchial epithelial cells
Antigen Expression:	Positive for CC10 (Clara cells 10kDa secretory protein), p63 (as tested within Evercyte)
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 (Ramirez R.D., et al. 2004, [PubMed ID 15604268]). The cell line was continuously cultured for more than 90 population doublings without showing signs of growth retardation or replicative senescence. The cells show markers and functions of different types of lung epithelial cells (Ramirez R.D., et al. 2004).

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Propagation:	Cells are grown in Keratinocyte-SFM (1X) Kit (Gibco, Cat# 17005-042) at 37°C in a humidified atmosphere with 5 % CO_2 in culture ware pre-coated with 0.1 % pig skin gelatine (Sigma Aldrich, Cat# G1890). For coating, the culture ware is treated with the gelatine for at least 4 hours (up to 1 week) at 37°C. After removal of the gelatine the culture ware can be used directly for seeding of cells.
Subculturing:	Cells are passaged after having reached about 80 - 90 % confluence. For detachment of the cells remove and discard the culture medium and wash the cells twice with PBS. Remove PBS completely. Then, add 0.025 % Trypsin-EDTA solution (room temperature, Gibco, Cat# R-001-100, 20 µl/cm²), 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 stopped by addition of PBS supplemented with 2 % FBS (220 µl/cm²) and the cell suspension is centrifuged for 5 minutes at 170 g. 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 gelatin coated culture vessels (see propagation) supplemented with growth medium (final volume of 240 µl/cm²). A split ratio of 1:4 twice a week is recommended.
Preservation:	Freezing medium: Keratinocyte-SFM (1X) Kit (Gibco, Cat# 17005-042) + 10 % DMSO + 10 % FBS (Sigma Aldrich, Cat# F7524) Storage temperature: liquid nitrogen
Freezing and thawing procedure:	Freezing of cells: Detach the cells from the culture vessel by using Trypsin-EDTA solution as described above 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: Add 6 ml of growth medium to a pre-coated 25 cm² culture flask (see propagation) and place the culture flask in the incubator for at least 30 min 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 cell pellet in the remaining droplet. Add 1 ml of the pre-warmed medium to the cells, transfer the cells 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 should be passaged (see subculturing).
Doubling Time:	About 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). Cells have been tested negative for production of infectious retroviral particles using PERT assay.
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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