Product-Data-Sheet for fHDF/TERT166

Version: May 2021

Evercyte Ord. No.:	CHT-031-0166
Designation:	fHDF/TERT166
Biosafety Level:	1
Shipped:	Frozen on dry ice
Medium:	DMEM/F12 (1:1), w: stable Glutamine, w: 1.2 g/L $NaHCO_3$ (PAN Biotech, Cat# P04-41150) 10 % FBS (Sigma-Aldrich, Cat# F7524, ready to use, stored at 4°C after thawing) 100 µg/ml G418 (InvivoGen, Cat# ant-gn5, ready-to-use, stored at -20°C)
Growth:	Adherent
Organism:	Homo sapiens (human)
Morphology:	Fibroblastoid morphology
Source:	Human foreskin
Cell Type:	Human foreskin fibroblasts
Antigen Expression:	Positive for Vimentin
Ethical statement:	Approved by Institutional Review Board (IRB) in accordance with the Declaration of Helsinki
Comments:	fHDF/TERT166 was developed from human foreskin fibroblasts by transduction with a retroviral expression vector (pLXSN) containing the hTERT gene. The cell line was continuously cultured for more than 60 population doublings without showing signs of growth retardation or replicative senescence. Cells readily recover from cryopreservation as shown by longevity studies performed post thawing. No changes in growth characteristics have been observed after thawing.
Propagation:	Cells are grown in above described medium at 37°C in a humidified atmosphere with 5 % CO_2.
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 2-3 min. Observe cell detachment under an inverted microscope. As soon as all cells are detached (if necessary carefully shake the flask for complete detachment), add growth medium (about 160 µl/cm²) and aspirate the cells by pipetting. Add appropriate aliquots of the cell suspension

	to new culture vessels supplemented with growth medium (final volume of 240 µl/cm²). A split ratio of 1:4 twice a week is recommended (after having reached about 90 % confluence).
Preservation:	Freezing medium: DMEM/Ham´s F12 (1:1) (PAN-Biotech Cat# P04-41150) 10 % FBS (Sigma-Aldrich, Cat# F7524, ready-to-use, stored at 4°C after thawing) 10 % DMSO (Sigma-Aldrich, Cat# D2650, ready-to-use) Storage temperature: liquid nitrogen
Freezing and thawing procedure:	Freezing of cells: Detach cells from culture vessel by using Trypsin-EDTA as described above, resuspend 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 5 x 10^5 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 liquid nitrogen. Thawing of cells: **When you start cultivating the cells, please transfer the content of the original Evercyte vial containing fHDF/TERT166 cells into a T25 roux flask as described in the following**: Add 6 ml of cultivation 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 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 medium (pre-warmed to 37°C), transfer 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 be passaged (see subculturing).
Doubling Time:	Approximately 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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