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Product-Data-Sheet for HDF/TERT1

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For questions please contact office@evercyte.com

Evercyte Cat.No.:	CHT-008-0012
Designation:	HDF/TERT1
Biosafety Level:	1
Shipped:	Frozen on dry ice
Medium:	FibroUp (Evercyte, Cat# MHT-008): DMEM/Ham's F-12 (1:1) (Biochrom, Cat# F4815) 10 % Fetal Bovine Serum (FBS) (Sigma Aldrich, Cat# F7524) 2 mM GlutaMAX™-I (Gibco, Cat# 35050-038) 100 µg/mL G418 (InvivoGen, Cat# ant-gn5)
Growth:	Adherent
Organism:	Homo sapiens (human)
Morphology:	Fibroblastoid
Source:	Human skin
Cell Type:	Dermal fibroblasts
Approved by:	Approved by Institutional Review Board (IRB) in accordance with the Declaration of Helsinki
Comments:	HDF/TERT1 was developed from human dermal fibroblasts by transduction with a retroviral expression vector (pLXSN) containing the hTERT gene. HDF/TERT1 cells can be grown for a minimum of 50 PDs without showing signs of growth retardation. Cells readily recover from cryopreservation as shown by longevity studies performed post thawing. No changes in growth characteristics have been observed after thawing.

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Propagation:	Cells are grown in FibroUp (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 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), add growth medium (about 160 µl/cm²) and aspirate 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 1:2 twice a week is recommended (after having reached about 90 % confluence).
Preservation:	Freezing medium: DMEM/Ham´s F12 (1:1) (Biochrom, Cat# AG F4815) + 10 % FBS (Sigma Aldrich, Cat# F7524) + 10 % DMSO Storage temperature: liquid nitrogen
Freezing and thawing procedure:	Freezing of cells: Detach cells from culture vessel by using trypsin as described above, resuspend detached cells in growth medium and centrifuge at 170 g for 5 min. Then, discard supernatant, resuspend 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: Add 5 ml of cultivation medium to a 25 cm² culture flask and place the culture flask in the incubator for at least 20 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 10 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 supernatant and resuspend 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 should be passaged (see subculturing).
Doubling Time:	About 72 - 96 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 Eur.Ph.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.

Notices and disclaimers: Evercyte products are intended for laboratory research purposes only. They are not intended for use in humans.

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