Product-Data-Sheet for HDMVEC/TERT164-B

Version: May 2021

Evercyte Ord. No.:	CHT-013-0164-B
Designation:	HDMVEC/TERT164-B
Biosafety Level:	1
Shipped:	Frozen on dry ice
Growth medium:	Endopan MV kit (PAN Biotech, Cat# P04-0020K) supplemented with G418: Final components: Endopan MV basal medium (PAN Biotech, Cat# P04-0020B) Endopan MV supplements (PAN Biotech, Cat# P04-0020S) 20 µg/ml G418 (InvivoGen, Cat# ant-gn5, 100 mg/ml stock solution, ready-to-use)
Growth:	Adherent
Organism:	Homo sapiens (human)
Morphology:	Endothelial morphology
Source:	Human skin (female donor, 52 years)
Cell Type:	Microvascular endothelial cells, lymphatic
Antigen Expression:	Positive for vWF, CD31, CD105, podoplanin
Ethical statement:	Approved by Institutional Review Board (IRB) in accordance with the Declaration of Helsinki
Comments:	HDMVEC/TERT164-B cell line was developed from human dermal microvascular endothelial cells isolated from skin biopsies transduced with a retroviral expression vector (pLXSN) containing the hTERT gene. The cells show the typical endothelial morphology and express typical endothelial cell markers and functions such as formation of tubule-like structures when inoculated onto Matrigel-matrix. The cell line was continuously cultured for more than 50 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 in Endopan MV medium supplemented with G418 (see above) at 37°C in a humidified atmosphere with 5 % CO_2.

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Subculturing:	The new culture flasks have to be pre-coated with Gelatin (Sigma Aldrich, Cat# G1393; diluted to 0.1 % in PBS). Therefore, the culture flasks are treated with Gelatin solution (80 µl/cm²) at 37°C for at least 10 min (10-60 min). Before introducing cells, remove excess of Gelatin solution. 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 0.05 % 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 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 Defined Trypsin Inhibitor (20 µl/cm²; Gibco, Cat# R007100). Thereafter, resuspend the cells in growth medium (room temperature; about 160 µl/cm²) and aspirate the cells by pipetting, centrifuge at 170 g for 5 min. Discard the supernatant, resuspend the cell pellet in the remaining droplet and add growth medium. Then, add 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 1:2 to 1:3 twice a week is recommended (after having reached about 90 % confluence).
Preservation:	Freezing medium: Growth medium for HDMVEC/TERT164-B cells (see above) 10 % FBS (Sigma Aldrich, Cat# F7524, ready-to-use, stored at 4°C) 10 % DMSO (Sigma Aldrich, Cat# D2650, ready-to-use, stored at RT) Storage temperature: liquid nitrogen
Freezing and thawing procedure:	Freezing of cells: Detach the cells from the culture vessel by using Trypsin-EDTA and Defined Trypsin Inhibitor 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 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 the liquid nitrogen tank. Thawing of cells: **When you start cultivating the cells, please transfer the content of the original Evercyte vial containing HDMVEC/TERT164-B 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 HDMVEC/TERT164-B 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 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 % confluent at this point, they have to be passaged (see above or protocol *Passaging of HDMVEC/TERT164-B* cells).
Doubling Time:	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 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.
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