EVERCYTE GmbH
Leberstrasse 20
1110 Vienna, AUSTRIA
office@evercyte.com
www.evercyte.com

Product-Data-Sheet for HUVEC/TERT66

Version: January 2020

For questions, please contact *office@evercyte.com*

Evercyte Ord. No.:	CHT-006-0066
Designation:	HUVEC/TERT66
Biosafety Level:	1
Shipped:	Frozen on dry ice
Medium:	**Serum-free growth medium for HUVEC-TERT66 consists of** - Endopan 300 SL kit (PAN Biotech, Cat# P04-0065K) w/o GA supplemented with - Serum substitute Panexin SL-S (PAN Biotech, Cat# P04-0065S, additional aliquot[1]) - G418 (20 µg/ml, InvivoGen, Cat#ant-gn-5)
Growth:	Adherent
Organism:	Homo sapiens (human)
Morphology:	Epithelial-like
Source:	Umbilical vein
Cell Type:	Human umbilical vein endothelial cells
Antigen Expression:	Positive for vWF, CD31
Ethical statement:	Approved by Institutional Review Board (IRB) in accordance with the Declaration of Helsinki

[1] Endopan 300SL kit (serum-free complete medium for human endothelial cells, Kit with 9 supplements) contains 1 aliquot of serum substitute Panexin SL-S, for proper growth of HUVEC-TERT66 cells an additional aliqot of Panexin SL-S has to be added to the final medium

Comments:	HUVEC/TERT66 was developed from human umbilical vein endothelial cells by transduction with a retroviral expression vector (pLXSN) containing the hTERT gene. The cells can be grown under serum-free cell culture conditions. The cells form neoangiogenic webs on Matrigel.
Propagation:	Cells are grown in a modified Endopan 300 SL medium (see above) at 37°C in a humidified atmosphere with 5 % CO_2.
Subculturing:	The culture flasks have to be pre-coated with gelatin (Sigma Cat # G1393; diluted to 0.1 % in PBS). Therefore, the culture flasks are treated with gelatin solution (60 µl/cm²) at 37°C for at least 10 min (10 – 60 min). Before introducing cells, remove excess of gelatin solution. For detachment of cells remove and discard culture medium and wash cells twice 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 3 - 4 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 Trypsin-Inhibitor (20 µl/cm²; Gibco, Cat# R007100). Thereafter, resuspend the cells in growth medium (about 160 µl/cm²) and 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 twice a week is recommended (after having reached about 90 – 95 % confluence).
Preservation:	Freezing medium: CryoStor® cell cryopreservation medium CS10 (Sigma Aldrich, Cat# C2874) Storage temperature: liquid nitrogen
Freezing and thawing procedure:	Freezing of cells: Detach the cells from the culture vessel by using Trypsin-EDTA solution and 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: Pre-coat a 25 cm² culture flask with gelatine (see subculturing). 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 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 supernatant and resuspend cells in the remaining droplet. Add 1 ml of 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 confluent at this point, they should be passaged (see subculturing).
Doubling Time:	About 72 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 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.

While Evercyte uses reasonable efforts to include accurate and up-to-date information on this site, no warranties or representations are made to its accuracy. Citations from scientific literature and patents are provided for informational purposes only and no warranties are made that such information has been confirmed to be accurate.