

## Product-Data-Sheet for HUVEC/TERT2

Version: October 2017

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Evercyte Ord. No.:	CHT-006-0008
Designation:	HUVEC/TERT2
Biosafety Level:	1
Shipped:	Frozen on dry ice
Medium:	EndoUp2 (Evercyte, Cat# MHT-006-2): EBM Basal Medium 500 mL (Lonza Cat# CC-3121) with selected supplements from EGM SingleQuot Kit (Lonza, Cat# CC-4133) – namely BBE (bovine brain extract), HEGF, hydrocortisone solution and ascorbic acid solution – and 10 % FBS (Sigma Aldrich, Cat# F7524) 20 µg/ml G418 (InvivoGen, Cat# ant-gn5)
Growth:	Adherent
Organism:	Homo sapiens (human)
Morphology:	Epithelial-like
Source:	Umbilical vein, female newborn donor
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.
Comments:	HUVEC/TERT2 was developed from human umbilical vein endothelial cells by transduction with a retroviral expression vector (pLXSN) containing the hTERT gene. The cell line was continuously cultured for more than 70 population doublings

	<p>without showing signs of growth retardation or replicative senescence whereas the parental cells senesced after having reached 33 population doublings. The cells respond to VEGF treatment and form neoangiogenic webs on Matrigel.</p>
Propagation:	<p>Cells are grown in EndoUp2 medium (see above, Evercyte, Cat# MHT-006-2) at 37°C in a humidified atmosphere with 5 % CO<sub>2</sub>.</p>
Subculturing:	<p>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/cm<sup>2</sup>) at 37°C for at least 10 min (10 – 60 min). Before introducing cells, remove excess of gelatin solution and rinse flask once with PBS (160 µl/cm<sup>2</sup>). 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/cm<sup>2</sup>; 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/cm<sup>2</sup>) and aspirate cells by pipetting. Add appropriate aliquots of the cell suspension to gelatin coated culture vessels supplemented with growth medium (final volume of 240 µl/cm<sup>2</sup>). A split ratio of 1:4 to 1:6 twice a week is recommended (after having reached about 90 – 95 % confluence).</p>
Preservation:	<p>Freezing medium: EndoUp2 (see above) + 10 % DMSO Storage temperature: liquid nitrogen</p>
Freezing and thawing procedure:	<p><b>Freezing of cells:</b> 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 in the remaining droplet and add freezing medium (4°C) to reach a cell density of about 5 x 10<sup>5</sup> cells/ml (for thawing in a 25 cm<sup>2</sup> 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.</p> <p><b>Thawing of cells:</b> Pre-coat a 25 cm<sup>2</sup> culture flask with gelatine (see subculturing). Add 6 ml of growth medium to a 25 cm<sup>2</sup> 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</p>

	<p>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.</p> <p>Perform a medium change 24 hours after thawing. If the cells are already confluent at this point, they should be passaged (see subculturing).</p>
Doubling Time:	About 28-36 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.

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