# Product-Data-Sheet for hTEC/SVTERT24-B

Version: May 2021

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| Evercyte Ord. No.: | CLHT-010-0024-B |
| Designation: | hTEC/SVTERT24-B |
| Biosafety Level: | 1 |
| Shipped: | Frozen on dry ice |
| Growth medium: | OptiPRO™ SFM (Gibco, Cat# 12309-019) supplemented with GlutaMAX™-I and G418:<br><br>Final components:<br>OptiPRO™ SFM (Gibco, Cat# 12309-019)<br>2 mM GlutaMAX™-I (Gibco, Cat# 35050-038, ready-to-use, stored at RT)<br>50 µg/ml G418 (InvivoGen, Cat# ant-gn5, 100 mg/ml stock solution, ready-to-use, stored at -20°C) |
| Growth: | Adherent |
| Organism: | Homo sapiens (human) |
| Morphology: | Epithelial morphology |
| Source: | Human thymic tissue (female donor) |
| Cell Type: | Thymic epithelial cells |
| Antigen Expression: | Positive for KRT5, KRT8, E-Cadherin, ZO-1<br>Expression of Thymopoietin, Prolactin receptor, Growth Hormone Receptor, HOXA3 on RNA level |
| Ethical statement: | Approved by Institutional Review Board (IRB) in accordance with the Declaration of Helsinki |
| Comments: | hTEC/SVTERT24-B was developed from primary epithelial cells isolated from human thymic tissue by transfection with a plasmid encoding SV40 early region followed by transduction with a retroviral expression vector (pLXSN) containing the hTERT gene.<br><br>The cells show the typical epithelial morphology and express typical marker cell markers.<br><br>The cell line was continuously cultured for more than 55 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. |

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| Propagation: | Cells are grown on Collagen I coated culture flasks in OptiPRO™ SFM medium supplemented with GlutaMAX™-I and G418 (see above) at 37°C in a humidified atmosphere with 5 % $CO_2$. |
| Subculturing: | Culture flasks have to be pre-coated with human collagen I. Therefore, the culture flasks are treated with Collagen I solution (Sigma-Aldrich, Cat# C2249, diluted to 50 µg/ml in PBS; 80 µl/cm²) at 37°C for at least 30 min. Before introducing cells, remove excess of Collagen I solution and rinse flask once with PBS (160 µl/cm²).<br>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# 25300-054), make sure that all cells have been in contact with this solution and incubate the culture flask at 37°C for approximately 1-3 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 and centrifuge at 170 g for 5 min. Discard the supernatant, resuspend the cell pellet in the remaining droplet and add growth medium (about 160 µl/cm²). Then, 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 1:6 to 1:8 twice a week is recommended after having reached about 90 % confluence. Never allow the culture to become completely confluent! |
| Preservation: | Freezing medium:<br><br>CryoStor® cell cryopreservation medium CS10 (Sigma-Aldrich, Cat# C2874, ready-to-use)<br><br>Storage temperature: liquid nitrogen |
| Freezing and thawing procedure: | Freezing of cells:<br><br>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 1 x $10^6$ 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.<br><br>Thawing of cells:<br>**When you start cultivating the cells, please transfer the content of the original Evercyte vial containing hTEC/SVTERT24-B cells into a T25 roux flask as described in the following:**<br><br>Pre-coat a 25 cm² culture flask with Collagen I (see above or protocol *Passaging of hTEC/SVTERT24-B cells*). Add 6 ml of growth medium to the Collagen I coated culture flask and place the culture flask in the incubator for at least 30 min to allow the medium to |

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| | 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.<br>Perform a medium change 24 hours after thawing. If the cells are already 90 % confluent at this point, they have to be passaged (see above or protocol *Passaging of hTEC/SVTERT24-B cells*). |
| Doubling Time: | Approximately 30 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.<br>Cells are negative for bacterial and fungal contaminations as tested according to Ph. Eur. 2.6.1. / USP <71>.<br>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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