EVERCYTE GmbH
the pharmacocellomics™ company
Muthgasse 18
1190 Wien
office@evercyte.com
www.evercyte.com

Product-Data-Sheet for hTEC/SVTERT24-B

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For questions please contact *office@evercyte.com*

Evercyte Ord. No.:	CLHT-010-0024-B
Designation:	hTEC/SVTERT24-B
Biosafety Level:	1
Shipped:	Frozen on dry ice
Medium:	ThymoUp (Cat# MHT-010) OptiPRO SFM (Gibco, Cat# 12309-019) 2 mM Glutamax (Gibco, Cat# 35050-038) 50 µg/ml G418 (InvivoGen, Cat# ant-gn)
Growth:	Adherent
Organism:	Homo sapiens (human)
Morphology:	Epithelial Morphology
Source:	Human thymic tissue
Cell Type:	Thymic epithelial
Antigen Expression:	Positive for KRT5, KRT8, E-Cadherin, ZO1 RNA: Thymopoietin, Prolactin receptor, Growth hormone receptor, HOXA3
Ethical statement:	Approved by Institutional Review Board (IRB) in accordance with the Declaration of Helsinki.

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Comments:	hTEC/SVTERT24-B was developed from primary cells of human thymic tissue by transfection with a plasmid encoding SV40 early region. The resulting cell strain shows an extended life span but is not immortal. Immortalization was achieved subsequently by transduction with a retroviral expression vector (pLXSN) containing the hTERT gene. The cells show an epithelial morphology and express typical markers. The cell line was continuously cultured for more than 55 population doublings (after thawing) without showing signs of growth retardation or replicative senescence whereas the parental cells cannot be propagated in vitro. Cells readily recover from cryopreservation as shown by longevity studies performed post thawing. No changes in growth characteristics have been observed after thawing.
Propagation:	Cells are grown in ThymoUp (see above) supplemented with 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# C7624-5ML, diluted to 50 µg/mL in PBS; 60 µl/cm²) at 37°C for at least 30 min. Before introducing cells, remove excess of collagen I solution and rinse flask twice with PBS (160 µl/cm²). 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 (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 1 - 2 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 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: Cryostor CS10 (Sigma Aldrich, Cat# C2874) Storage temperature: liquid nitrogen
Freezing and	Freezing of cells:

thawing procedure:	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 1×10^6 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. Thawing of cells: Pre-coat a 25 cm² culture flask with collagen I (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 the supernatant and resuspend the cell pellet in the remaining droplet. Add 1 ml of the pre-warmed medium to the cells, transfer the cells 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 90 % confluent at this point, they should be passaged (see subculturing).
Doubling Time:	About 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. 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.

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