Product-Data-Sheet for TB/SVTERT350

Version: April 2025

Evercyte Ord. No.:	CLHT-070-0350
Designation:	TB/SVTERT350
Biosafety Level:	1
Shipped:	Frozen on dry ice
Medium:	TrophoUp (Evercyte, Cat# MHT-070): 80% TS medium (see "*Protocol preparation of TS medium*", stored at 4°C) 20% IMDM-FBS medium (see "*Protocol preparation of IMDM-FBS medium*", stored at 4°C) 100 µg/ml G418 (Invivogen, Cat# ant-gn-5, stored at 4°C)
Growth:	Adherent
Organism:	Homo sapiens (human)
Morphology:	Epithelial, cobblestone morphology
Source:	Human placental tissue
Cell Type:	Human trophoblasts
Antigen Expression:	Positive for Desmoplakin, Keratin-7, ZO-1
Ethical statement:	Approved by Institutional Review Board (IRB) in accordance with the Declaration of Helsinki
Comments:	TB/SVTERT350 cell line was developed from human trophoblast cells by transfection with plasmids carrying the catalytic subunit of human telomerase (hTERT) and SV40 largeT antigen. 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 as shown by longevity studies performed post thawing. No changes in growth characteristics have been observed after thawing.
Propagation:	Cells are grown in above described medium at 37°C in a humidified atmosphere with 5 % CO_2.
Subculturing:	The new culture flasks must be pre-coated with Collagen I. Therefore, the culture flasks are treated with Collagen I solution (80 µg/cm^2; Stemcell Technologies, Cat# 07005, 3 mg/ml in 0.01 N HCl) at room temperature for at least 1hour. Before introducing cells, remove excess of Collagen I solution and wash the flasks once with PBS (160 µl/cm^2). For detachment of the cells remove and discard the culture medium and wash the cells twice with PBS. Remove PBS completely. Then, add a 1:1 mixture of Trypsin-EDTA (Gibco, Cat# 25300054) and Trypsin 0.25% (Gibco, Cat# 15090046, diluted 1:10 in PBS)(10 µl/cm^2

	of each solution), make sure that all cells have been in contact with this solution and incubate the culture flask at 37°C for approximately 10 min. Observe cell detachment under an inverted microscope. As soon as all cells are detached (if necessary, carefully shake the flask for complete detachment), add Trypsin-Inhibitor (20 µl/cm^2, Gibco, Cat# R007100) and growth medium (about 160 µl/cm²) and aspirate the cells by pipetting. Then, transfer the cell suspension to a centrifugation tube and centrifuge for 5 min at 180 g. Discard the supernatant, resuspend the cell pellet in the remaining droplet and add growth medium. Thereafter, transfer appropriate aliquots of the cell suspension to new Collagen I pre-coated culture vessels supplemented with growth medium (final volume of 240 µl/cm²). A split ratio of 1:4 to 1:6 twice a week is recommended (after having reached about 80-90 % confluence).
Preservation:	Freezing medium: CryoStor® Cell Cryopreservation Cedium CS10 (Sigma Aldrich, Cat# C2874, ready-to-use, stored at 4°C) Storage temperature: liquid nitrogen
Freezing and thawing procedure:	Freezing of cells: Detach cells (80-90% confluence) from the culture vessel by using Trypsin-EDTA and Trypsin 0.25% as described above, resuspend detached cells in Trypsin-Inhibitor and growth medium and centrifuge at 180 g for 5 min. Then, discard the supernatant, resuspend the cell pellet in the remaining droplet and add freezing medium (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 liquid nitrogen. Thawing of cells: **When you start cultivating the cells, please transfer the content of the original Evercyte vial containing TB/SVTERT350 cells into a T25 roux flask as described in the following**: Pre-coat a 25 cm2 culture flask with Collagen I (see above or protocol *Passaging of TB/SVTER350 cells*). Add 6 ml of cultivation medium to the pre-coated 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 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 180 g. Then, discard the supernatant and resuspend the cells in the remaining droplet. Add 1 ml of medium (pre-warmed to 37°C), transfer 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 must be passaged (see above or protocol *Passaging of TB/SVTERT350 cells*).
Doubling Time:	Approximately 45 hours

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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.
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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