

## Product-Data-Sheet for P-EP/SVTERT344

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Version: June 2024

Evercyte Ord. No.:	CLHT-053-0344
Designation:	P-EP/SVTERT344
Biosafety Level:	1
Shipped:	Frozen on dry ice
Medium:	49.5 % EpiLife (Gibco, Cat# MEPI500CA) 0.5 % S7 Supplement (Gibco, Cat# S0175) 40 % DMEM (1x) + Glutamax (Gibco, Cat# 61965-026) 10 % FBS (PAN Biotech, Cat# P30-3031, ready-to-use, stored at 4°C after thawing) 100 µg/ml G418 (InvivoGen, Cat# ant-gn5, 100 mg/ml, ready-to-use, stored at -20°C)
Growth:	Adherent
Organism:	Homo sapiens (human)
Morphology:	Epithelial morphology
Source:	Placental amnion
Cell Type:	Human amniotic epithelial cells (hAEC), placental region
Antigen Expression:	Positive for E-Cadherin, ZO-1
Ethical statement:	Approved by Institutional Review Board (IRB) in accordance with the Declaration of Helsinki
Comments:	<p>P-EP/SVTERT344 was developed from human amniotic epithelial cells by transfection with a plasmid carrying the catalytic subunit of human telomerase (hTERT) and a plasmid carrying the SV40 early region.</p> <p>The cells show an epithelial, cobblestone appearance and expression of typical epithelial cells (expression of E-Cadherin and ZO-1) The cell line was continuously cultured for more than 50 population doublings without showing signs of growth retardation or replicative senescence.</p> <p>Cells readily recover from cryopreservation as shown by longevity studies. No changes in growth characteristics have been observed after thawing.</p>
Propagation:	Cells are grown in above described medium at 37°C in a humidified atmosphere with 5 % CO <sub>2</sub> .
Subculturing:	The new culture flasks have to be pre-coated with Collagen I (StemCell Technologies, Cat# 07005, 3 mg/ml, diluted 1:200 in 0.01 N HCl). Therefore, the culture flasks are incubated with Collagen I solution (80 µl/cm <sup>2</sup> ) at room temperature for at least 1 hour. Before

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introducing cells, remove excess of coating solution and wash the flask once with PBS (160  $\mu\text{l}/\text{cm}^2$ ). Remove PBS completely and use culture flask immediately for seeding of cells, the surface must not dry out.

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 (room-temperature, 20  $\mu\text{l}/\text{cm}^2$ , 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 5-7 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 growth medium (about 160  $\mu\text{l}/\text{cm}^2$ ) and aspirate the cells by pipetting. Add appropriate aliquots of the cell suspension to new culture vessels supplemented with growth medium (final volume of 240  $\mu\text{l}/\text{cm}^2$ ). A split ratio of 1:6-1:8 twice a week is recommended (after having reached about 70-90 % confluence).

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**Preservation:****Freezing medium:**

DMEM (Gibco, Cat# 61965-026)  
10 % FBS (PAN Biotech, Cat# P30-3031, ready-to-use, stored at 4°C after thawing)  
10 % DMSO (Sigma-Aldrich, Cat# D2650, ready-to-use, stored at RT)

Storage temperature: liquid nitrogen

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**Freezing and thawing procedure:****Freezing of cells:**

Detach cells from culture vessel by using Trypsin-EDTA as described above, resuspend detached cells in 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  $1 \times 10^6$  cells/ml (for thawing in a 25  $\text{cm}^2$  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 P-EP/SVTERT344 cells into a T25 roux flask as described in the following:**

Pre-coat a 25  $\text{cm}^2$  culture flask with Collagen I (see above or protocol *passaging of P-EP/SVTERT344*). Add 6 ml of cultivation medium to a 25  $\text{cm}^2$  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 have to be passaged (see subculturing).

Doubling Time:	24-37 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 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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