

Product-Data-Sheet for NHEK/SVTERT3-5

Version: February 2024

Evercyte Ord. No.:	CLHT-011-0026-5
Designation:	NHEK/SVTERT3-5
Biosafety Level:	1
Shipped:	Frozen on dry ice
Growth medium:	KGM™ Gold Keratinocyte Growth Medium BulletKit™ (Lonza, Cat# 00192060) supplemented with G418: <u>Final components:</u> KBM™ Gold™ Basal Medium (Lonza, Cat# 00192151) Components of KGM™ Gold™ SingleQuots™ supplements (Lonza, Cat# 00192152: BPE, hEGF, Insulin, Hydrocortisone, Transferrin, Epinephrine, without GA) 50 µg/ml G418 (InvivoGen, Cat# ant-gn5, 100 mg/ml stock solution, ready-to-use)
Growth:	Adherent
Organism:	Homo sapiens (human)
Morphology:	Epithelial
Source:	Human skin
Cell Type:	Epidermal keratinocytes
Antigen Expression:	Positive for Keratin 1, Keratin 2, Keratin 10 and Loricrin in a 3D skin model
Ethical statement:	Approved by Institutional Review Board (IRB) in accordance with the Declaration of Helsinki
Comments:	NHEK/SVTERT3-5 was developed from human epidermal keratinocytes by transfection with a plasmid encoding SV40 early region followed by transduction with a retroviral expression vector (pLXSN) containing the hTERT gene. The cells show an epithelial, cobblestone appearance and expression of typical keratinocyte markers and characteristics (differentiation and stratification in a 3D skin model with expression of Keratin 1, Keratin 2, Keratin 10 and Loricrin). The cell line was continuously cultured for more than 100 population doublings without showing signs of growth retardation or replicative senescence. Cells readily recover from cryopreservation as shown by longevity studies. No changes in growth characteristics have been observed after thawing.

Propagation:	Cells are grown in KGM™ Gold Keratinocyte Growth Medium BulletKit™ supplemented with G418 (see above) at 37°C in a humidified atmosphere with 5 % CO ₂ .
Subculturing:	<p>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 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 4-5 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), resuspend the cells in growth medium (about 160 µl/cm²) and pass cells through EASY strainer 100µm (Greiner, Cat# 542000). Centrifuge at 170 g for 5 min. Discard the supernatant, resuspend the cell pellet in the remaining droplet and add growth medium. 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 1:4 to 1:6 twice a week is recommended (after having reached about 60-70 % confluence). Never allow the culture to become confluent.</p>
Preservation:	<p>Freezing medium:</p> <p>CryoStor® cell cryopreservation medium CS10 (Sigma-Aldrich, Cat# C2874, ready-to-use, stored at 4°C)</p> <p>Storage temperature: liquid nitrogen</p>
Freezing and thawing procedure:	<p>Freezing of cells:</p> <p>Detach the cells (60-70% confluence) 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 5-7 x 10⁵ 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.</p> <p>Thawing of cells:</p> <p>When you start cultivating the cells, please transfer the content of the original Evercyte vial containing NHEK/SVERT3-5 cells into a T25 roux flask as described in the following:</p> <p>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 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.</p>

Recovery from cryopreservation will take a few days. Perform a medium change 24 hours after thawing. If the cells are already about 60-70 % confluent at this point, they must be passaged (see above or protocol *Passaging of NHEK/SVERT3-5* cells).

Doubling Time:	48-60 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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