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Product-Data-Sheet for NHEK/SVTERT3-5

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Evercyte Cat. No.:	CLHT-011-0026-5
Designation:	NHEK/SVTERT3-5
Biosafety Level:	1
Shipped:	Frozen on dry ice
Medium:	KeratinoUp (Evercyte, MHT-011) KGM™-2 BulletKit™ (Lonza, Cat# CC-3107) without GA-1000 from this kit supplemented with 50 µg/ml G418 (InvivoGen, Cat# ant-gn)
Growth:	Adherent
Organism:	Homo sapiens (human)
Morphology:	Epithelial-like
Source:	Human skin
Cell Type:	Human epidermal keratinocytes
Ethical statement:	Approved by Institutional Review Board (IRB) in accordance with the Declaration of Helsinki.
Antigen Expression:	Positive for Keratin 1, Keratin 2, Keratin 10 and Loricrin in a 3D skin model
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.

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	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 performed post thawing. No changes in growth characteristics have been observed after thawing.
Propagation:	Cells are grown in keratinocyte medium KGM™-2 BulletKit™ without GA-1000 supplemented with G418 (see above) at 37°C in a humidified atmosphere with 5 % CO_2.
Subculturing:	For detachment of 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 5-7 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). 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 1:3 to 1:4 twice a week is recommended (after having reached about 60-70 % confluence). Never allow the culture to become completely confluent!
Preservation:	Freezing medium: KGM™-2 BulletKit™ without GA-1000 + 10 % DMSO Storage temperature: liquid nitrogen
Freezing and thawing procedure:	Freezing of cells: Detach the cells from culture vessel by using Trypsin-EDTA and Trypsin-Inhibitor as described above, resuspend detached cells in growth medium and centrifuge at 170 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: Add 6 ml of cultivation 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 cells in the remaining droplet. Add 1 ml of pre-warmed medium to the cells, transfer them 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 should be passaged (see subculturing).
Doubling Time:	About 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 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.

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