

Product-Data-Sheet for NHEK/SV3

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Evercyte Ord. No.:	CLT-011-0026
Designation:	NHEK/SV3
Biosafety Level:	2
Shipped:	Frozen on dry ice
Medium:	KGM-2 Bullet Kit (Lonza, Cat# CC-3107)
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.
Comments:	NHEK/SV3 was developed from human epidermal keratinocytes by transfection with a plasmid encoding SV40 early region. The cells show an epithelial, cobblestone appearance, expression of typical keratinocyte markers and characteristics (differentiation and stratification in the Epidermal model).
Propagation:	Cells are grown in keratinocyte medium KGM-2 at 37°C in a humidified atmosphere with 5 % CO ₂ .

Subculturing:	<p>For detachment of cells remove and discard culture medium and wash cells once with PBS. Remove PBS completely. Then, add 0,25 % Trypsin-EDTA (1x) solution (room-temperature; 20 µl/cm²; Gibco Cat# 25200056), make sure that all cells have been in contact with this solution and incubate the culture flask at 37°C for approximately 2-3 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 (8 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 1:3 to 1:4 twice a week is recommended (after having reached about 60 - 70 % confluence). Never allow the culture to become completely confluent!</p>
Preservation:	<p>Freezing medium: KGM-2 + 10 % DMSO Storage temperature: liquid nitrogen</p>
Freezing and thawing procedure:	<p>Freezing of cells: Detach cells from culture vessel by using trypsin and trypsin-inhibitor as described above, resuspend detached cells in growth medium and centrifuge at 170g for 5 min. Then, discard supernatant, resuspend in the remaining droplet and add freezing medium (4°C) to reach a cell density of about 5 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 liquid nitrogen.</p> <p>Thawing of cells: Add 5 ml of growth medium to a 25cm² culture flask and place the culture flask in the incubator for at least 20 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 10ml centrifugation tube pre-filled with 9 ml of medium pre-cooled to 4°C and centrifuge for 5 min at 170g. Then, discard supernatant and resuspend 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).</p>
Doubling Time:	<p>About 48 hours</p>

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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