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Product-Data-Sheet for RS

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Evercyte Ord. No.:	CLT-003-0014
Designation:	RS
Biosafety Level:	1
Shipped:	Frozen on dry ice
Medium:	ProxUp3 (Evercyte, MHT-003-3) OptiPro™ SFM (Gibco, Cat#12309-019) supplemented with 4 mM GlutaMAX™-I (Gibco, Cat#35050-038)
Growth:	Adherent
Organism:	Homo sapiens (human)
Morphology:	Epithelial-like
Source:	Human kidney cortex
Cell Type:	Proximal tubular epithelial cells
Antigen Expression:	Positive for CD13, E-Cadherin, ZO-1
Ethical statement:	Approved by Institutional Review Board (IRB) in accordance with the Declaration of Helsinki.
Comments:	RS was developed from normal human proximal tubular epithelial cells by transfection with a plasmid encoding SV40 early region. Spontaneous reactivation of endogenous telomerase around population doubling level 40 led to immortalization. The cell line was continuously cultured for more than 95 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 have to be pre-coated with human collagen I solution. Therefore, the culture flasks are treated with collagen I solution (80 µl/cm²; Sigma Aldrich, Cat#C7624, 50 µg/ml in PBS) at 37°C for at least 30 min. Before introducing cells, remove excess of collagen I solution and rinse flask once with PBS (160 µl/cm²). Confluent cell monolayers are detached using Trypsin-EDTA solution (0.05%; Gibco, Cat#25300054). Therefore, the culture flask is washed once with PBS, followed by incubation with Trypsin-EDTA (20 µl/cm^2) at 37°C for 1 - 2 min. Thereafter, Trypsin action is halted by addition of Trypsin inhibitor (20 µl/cm² Gibco, Cat#R007100). Cells are centrifuged at 170 g for 5 min and the resulting cell pellet is resuspended in culture medium and transferred to new pre-coated culture flasks with a split ratio of 1:3 to 1:4 twice a week. After three days cells have to be passaged or a medium change has to be performed.
Preservation:	Freezing medium: CryoStor® cell cryopreservation medium CS10 (Sigma Aldrich, Cat# C2874) Storage temperature: liquid nitrogen
Freezing and thawing procedure:	Freezing of cells: Detach cells from the culture vessel by using Trypsin and Trypsin-inhibitor as described above, resuspend the detached cells in cultivation medium and centrifuge at 170 g for 5 min. Then, discard the supernatant, resuspend the cells 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 the liquid nitrogen tank. Thawing of cells: Add 6 ml of growth medium to a pre-coated 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 the 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 36 – 48 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 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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