Product-Data-Sheet for PODO/TERT256

Version: May 2021

Evercyte Ord. No.:	CHT-033-0256
Designation:	PODO/TERT256
Biosafety Level:	1
Shipped:	Frozen on dry ice
Medium:	PodoUp3 (Evercyte, Cat# MHT-033-3): MCDB131 (Pan Biotech, Cat# P04-80057) 1.6 mM GlutaMAX-I (Gibco, Cat# 35050-038, ready-to-use, stored at RT) 9.6 µg/mL BBE (Lonza, Cat# CC-4098, ready-to-use, stored at-20°C) 8 ng/ml hEGF (Sigma-Aldrich, Cat# E9644, stored at -20°C) 20 ng/ml Hydrocortisone (Sigma-Aldrich, Cat# H0396, stored at -20°C) 20% FBS (Sigma-Aldrich, Cat# F7524, ready-to-use, stored at 4°C after thawing) 100 µg/ml G418 (InvivoGen, Cat# ant-gn-5, ready-to-use, stored at -20°C)
Growth:	Adherent
Organism:	Homo sapiens (human)
Morphology:	Typical podocyte morphology, cytoplasmic extensions partially with arborized appearance
Source:	Kidney tissue, female donor
Cell Type:	Podocytes, visceral epithelial cells
Antigen Expression:	Positive for Nephrin, WT-1, Synaptopodin
Ethical statement:	Approved by Institutional Review Board (IRB) in accordance with the Declaration of Helsinki.
Comments:	PODO/TERT256 was developed from human kidney tissue derived podocytes by transduction with a retroviral expression vector (pLXSN) containing the hTERT gene. Sub-confluent cultures show flat cytoplasmatic protrusions and occasional elongated processes. Within the culture rare interspersed multinuclear cells with an arborized appearance are present. The cells readily recover from cryopreservation and can be continuously cultured for a minimum of 50 population doublings after thawing. No changes in growth characteristics have been observed after thawing.
Propagation:	Cells are grown in PodoUp3 medium (see above) at 37°C in a humidified atmosphere with 5 % CO_2.
Subculturing:	New culture flasks have to be pre-coated with human Collagen I. Therefore, the culture flasks are treated with Collagen I solution (Sigma-Aldrich, Cat# C2249, diluted to 50 µg/mL in PBS; 80 µl/cm²) 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). 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 3-4 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 growth medium (about 160 µl/cm²) and aspirate cells by pipetting. Add appropriate aliquots of the cell suspension to collagen I pre-coated culture vessels supplemented with growth medium (final volume of 240 µl/cm²). Cells should be split every 3-4 days (after having reached not more that 80 % confluence) with a split ratio of 1:4 to 1:6. Never allow the culture to become confluent! Cultivate cells at 37°C in a humidified atmosphere with 5 % CO_2.
Preservation:	Freezing medium: PodoUp3 medium (Evercyte, Cat# MHT-033-3) 10 % DMSO (Sigma-Aldrich, Cat# D2650, ready-to-use, stored at RT) Storage temperature: liquid nitrogen
Freezing and thawing procedure:	Freezing of cells: Detach the cells from the culture vessel by using Trypsin-EDTA solution 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 7 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: **When you start cultivating the cells, please transfer the content of the original Evercyte vial containing PODO/TERT256 cells into a T25 roux flask as described in the following**: Pre-coat a 25 cm² culture flask with Collagen I (see subculturing). Then, add 6 ml of growth medium to the prepared culture flask and transfer it to the incubator for at least 20 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 170 g. Then, discard the supernatant and resuspend the cell pellet in the remaining droplet. Add 1 ml of pre-warmed medium to the cells, transfer the cells 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 near confluent at this point, they have to be passaged (see subculturing).
Doubling Time:	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 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.
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