Protocol for passaging of PODO/SVTERT152

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Evercyte Ord. No.:	CLHT-033-0152
Designation:	PODO/SVTERT152, human urine-derived podocytes
Medium:	The PodoUp3 medium for cultivation of PODO/SVTERT152 cells can either be ordered from Evercyte as ready-to-use medium (Cat# MHT-033-3) or as basal medium (Cat# MHT-033-3-B) plus supplements (Cat# MHT-033-3-S). The medium can also be prepared by mixing the following components: MCDB131 (Pan Biotech, Cat# P04-80057) 1,6 mM GlutaMAX-I (Gibco, Cat# 35050-038, ready-to-use) 9,6 µg/ml BBE (Lonza, Cat# CC-4098, ready-to-use) 8 ng/ml hEGF (Sigma-Aldrich, Cat# E9644) 20 ng/ml Hydrocortisone (Sigma-Aldrich, Cat# H0396) 20 % FBS (Sigma-Aldrich, Cat# F7524, ready-to-use) 100 µg/ml G418 (InvivoGen, Cat# ant-gn-5, ready-to-use) – take one bottle (500 ml) of MCDB131 – add 5 ml GlutaMAX-I (200 mM stock, ready-to-use), mix properly – discard 105 ml from MCDB131 / GlutaMAX-I mixture – add 533 µl BBE (9 mg/ml stock, ready-to-use) – add 200 µl hEGF stock (20 µg/ml, prepared in cell culture grade water) – add 200 µl hydrocortisone stock (50 µg/ml, prepared in cell culture grade water) – add 100 ml FBS (ready-to-use) – add 500 µl G418 stock (100 mg/ml, ready-to-use) – mix properly and store at 4°C for up to 1 month – temper the medium to room temperature (not 37°C) before use
Coating solution:	50 µg/ml Collagen I solution The coating solution is prepared by mixing the following components: Collagen I (Sigma-Aldrich, Cat# C2249, 3 mg/ml) Phosphate buffered saline (PBS) (Sigma-Aldrich, Cat# D8537) – take 29.5 ml PBS and transfer to 50 ml centrifugation tube – add 0.5 ml of Collagen I solution (3 mg/ml stock solution) – mix carefully For coating of a T25 cell culture flask proceed as follows:

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	– pipette 2 ml of diluted Collagen I solution (50 µg/ml) to a T25 roux flask – completely wet the surface of the culture flask (80 µl/cm^2) – incubate the culture flask for a minimum of 30 min at 37°C – remove excess of Collagen I solution – rinse culture flask once with PBS (160 µl/cm^2) – use culture flask immediately for seeding of cells, the surface must not dry out
Additional reagents:	Phosphate buffered saline (PBS) (Sigma-Aldrich, Cat# D8537) 0.05 % Trypsin-EDTA (Gibco, Cat# 25300-054)
Passaging of cells:	– remove and discard the culture medium and wash the cells twice with PBS (160 µl/cm^2), remove PBS completely – then, add 0.05% Trypsin-EDTA solution (20 µl/cm^2), 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/cm^2) 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/cm^2) – cells should be split every 3-4 days (after having reached not more than 80 % confluence) with a split ratio of 1:6 to 1:8 (population doubling time is 24 - 32 hours) – never allow the culture to become confluent! – cultivate cells at 37°C in a humidified atmosphere with 5 % CO_2
Related products:	PodoUp3 ready-to-use medium, 500 ml (Evercyte, Cat# MHT-033-3) PodoUp3 basal medium, 500 ml (Evercyte, Cat# MHT-033-3-B) PodoUp3 supplements (Evercyte, Cat# MHT-033-3-S) PODO/SVTERT152, human urine-derived podocytes (Evercyte, Cat# CLHT-033-0152) PODO/TERT256, human kidney tissue-derived podocytes (Evercyte, Cat# CHT-033-0256)

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