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 533.6 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: — pipette 2 ml of diluted Collagen I solution (50 µg/ml) to a T25 roux flask

EVERCYTE GmbH
Leberstrasse 20, 1110 Vienna, AUSTRIA
office@evercyte.com | www.evercyte.com

	— 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 that 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)