Protocol for 2D differentiation of podocytes

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Evercyte Protocol No.:	Diff-2D-podo-V2
Cells:	E.g. PODO/TERT256 (Evercyte, Cat# CHT-033-0256)
Reagents, material:	PBS (Sigma Aldrich, Cat# D8537, ready-to-use, stored at RT) PodoUp3 medium (Evercyte, Cat# MHT-033) or *Protocol for preparation of PodoUp3 medium* 0.05 % Trypsin-EDTA (1x) solution (Gibco, Cat# 25300054) 0.3 mg/ml Collagen IV (Sigma Aldrich, Cat# C6745, stored in aliquots at -20°C) HBSS (Sigma Aldrich, Cat# H8264, ready-to-use, stored at RT) PodoUp3 medium with 0.5 % FBS (= starvation medium), follow *Protocol for preparation of PodoUp3 medium* - take 100 ml of ready-to-use basal medium MCDB131 - add 1 ml of ready-to-use GlutaMAX-I (200 mM stock) and mix properly - discard 1,8 ml of the MCDB131-GlutaMAX-I mixture - add 106,7 µl of BBE (9 mg/ml stock) - add 40 µl of hEGF (20 µg/ml stock) - add 40 µl of hydrocortisone (50 µg/ml stock) - add 0,5 ml of fetal bovine serum - add 100 µl of G418 (100 mg/ml stock) - mix properly and store at 4°C
Coating of cell culture plates	10 µg/ml Collagen IV solution, 120 µl/cm^2 of coating solution are needed The coating solution is prepared by mixing the following components: Collagen IV (Sigma Aldrich, Cat# C6745, 0.3 mg/ml, stored in aliquots at -20°C) HBSS (Sigma Aldrich, Cat# H8264, ready-to-use, stored at RT) - thaw the required amount of Collagen IV stock solution and dilute 1:30 with HBSS - e.g. take 9 ml of HBSS and transfer to a 15 ml centrifugation tube - add 300 µl of Collagen IV stock solution - mix carefully for coating of plates / flasks proceed as follows: - pipette 120 µl of coating solution (10 µg/ml) / cm^2 cell culture plate / flask - incubate at 37°C for 2 hours - rinse culture flasks / plates twice with HBSS (about 160 µl/cm^2)

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	- use culture flasks / plates immediately for seeding of cells, the surface must not dry out
Induction of differentiation:	- harvest podocytes in growth medium as described in the respective *Protocol for passaging of cells* and determine the cell number using a hemocytometer - prepare a cell suspension containing the required number of cells (8 x 10^3 cells are needed per cm^2) - centrifuge the cell suspension for 5 min at 180 g - discard the supernatant and resuspend the cell pellet in starvation medium - seed 8 x 10^3 cells / cm^2 into appropriate plates / flasks - incubate at 37°C for 3-5 days - monitor and document morphological changes of the cells towards differentiated cells and use the cells for your experiments as soon as the cells show the typical flattened morphology of differentiated podocytes