

Protocol for passaging of PODO/TERT256

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Evercyte Ord. No.:	CHT-033-0256
Designation:	PODO/TERT256, human kidney tissue derived podocytes
Medium:	<p>The PodoUp3 medium for cultivation of PODO/TERT256 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).</p> <p>The medium can also be prepared by mixing the following components:</p> <p>MCDB131 (Pan Biotech, Cat# P04-80057) 1,6 mM GlutaMAX-I (Gibco, Cat# 35050-038, ready-to-use) 9,6 µg/mL bovine brain extract (Lonza, Cat# CC-4098, ready-to-use) 8 ng/ml hEGF (Sigma-Aldrich, Cat# E9644) 20 ng/ml Hydrocortisone (Sigma-Aldrich, Cat# H0396) 20 % fetal bovine serum (Sigma-Aldrich, Cat# F7524, ready-to-use) 100 µg/ml G418 (InvivoGen, Cat# ant-gn-5, ready-to-use)</p> <ul style="list-style-type: none">– 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 fetal bovine serum (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 (no 37°C) before use
Coating solution:	<p>50 µg/ml Collagen I solution</p> <p>The coating solution is prepared by mixing the following components:</p> <p>Collagen I (Sigma-Aldrich, Cat# C2249, 3 mg/ml) Phosphate buffered saline (PBS) (Sigma, Cat# D8537)</p> <ul style="list-style-type: none">– 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 <p>For coating of a T25 cell culture flask proceed as follows:</p> <ul style="list-style-type: none">– 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 $\mu\text{l}/\text{cm}^2$)
- 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 $\mu\text{l}/\text{cm}^2$)
- use culture flask immediately for seeding of cells, the surface must not dry out

Additional reagents: PBS (Sigma-Aldrich, Cat# D8537, ready-to-use, stored at room temperature)
0.05 % Trypsin-EDTA (Gibco, Cat#25300-054, ready-to-use, aliquots stored at RT after thawing)

- Passaging of cells:
- remove and discard the culture medium and wash the cells once with PBS (160 $\mu\text{l}/\text{cm}^2$), remove PBS completely
 - then, add 0.05% Trypsin-EDTA solution (20 $\mu\text{l}/\text{cm}^2$), 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 $\mu\text{l}/\text{cm}^2$) 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 $\mu\text{l}/\text{cm}^2$)
 - cells should be split every 3-4 days (after having reached not more than 80 % confluence) with a split ratio of 1:4 to 1:6 (population doubling time is 36-48 hours)
 - never allow the culture to become confluent!
 - cultivate cells at 37°C in a humidified atmosphere with 5 % CO₂

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)
