

## 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)<br/>1,6 mM GlutaMAX-I (Gibco, Cat# 35050-038, ready-to-use)<br/>9,6 µg/ml bovine brain extract (Lonza, Cat# CC-4098, ready-to-use)<br/>8 ng/ml hEGF (Sigma-Aldrich, Cat# E9644)<br/>20 ng/ml Hydrocortisone (Sigma-Aldrich, Cat# H0396)<br/>20 % fetal bovine serum (Sigma-Aldrich, Cat# F7524, ready-to-use)<br/>100 µg/ml G418 (InvivoGen, Cat# ant-gn-5, ready-to-use)</p> <ul style="list-style-type: none"><li>– take one bottle (500 ml) of MCDB131</li><li>– add 5 ml GlutaMAX-I (200 mM stock, ready-to-use), mix properly</li><li>– discard 105 ml from MCDB131 / GlutaMAX-I mixture</li><li>– add 533 µl BBE (9 mg/ml stock, ready-to-use)</li><li>– add 200 µl hEGF stock (20 µg/ml, prepared in cell culture grade water)</li><li>– add 200 µl hydrocortisone stock (50 µg/ml, prepared in cell culture grade water)</li><li>– add 100 ml fetal bovine serum (ready-to-use)</li><li>– add 500 µl G418 stock (100 mg/ml, ready-to-use)</li><li>– mix properly and store at 4°C for up to 1 month</li><li>– temper the medium to room temperature (no 37°C) before use</li></ul> |
| 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)<br/>Phosphate buffered saline (PBS) (Sigma, Cat# D8537)</p> <ul style="list-style-type: none"><li>– take 29.5 ml PBS and transfer to 50 ml centrifugation tube</li><li>– add 0.5 ml of Collagen I solution (3 mg/ml stock solution)</li><li>– mix carefully</li></ul> <p>For coating of a T25 cell culture flask proceed as follows:</p> <ul style="list-style-type: none"><li>– pipette 2 ml of diluted Collagen I solution (50 µg/ml) to a T25 roux flask</li></ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |

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|                      | <ul style="list-style-type: none"><li>– completely wet the surface of the culture flask (80 <math>\mu\text{l}/\text{cm}^2</math>)</li><li>– incubate the culture flask for a minimum of 30 min at 37°C</li><li>– remove excess of Collagen I solution</li><li>– rinse culture flask once with PBS (160 <math>\mu\text{l}/\text{cm}^2</math>)</li><li>– use culture flask immediately for seeding of cells, the surface must not dry out</li></ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Additional reagents: | <p>PBS (Sigma-Aldrich, Cat# D8537, ready-to-use, stored at room temperature)</p> <p>0.05 % Trypsin-EDTA (Gibco, Cat# 25300-054, ready-to-use, aliquots stored at RT after thawing)</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| Passaging of cells:  | <ul style="list-style-type: none"><li>– remove and discard the culture medium and wash the cells twice with PBS (160 <math>\mu\text{l}/\text{cm}^2</math>), remove PBS completely</li><li>– then, add 0.05% Trypsin-EDTA solution (20 <math>\mu\text{l}/\text{cm}^2</math>), 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</li><li>– 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 <math>\mu\text{l}/\text{cm}^2</math>) and aspirate cells by pipetting</li><li>– add appropriate aliquots of the cell suspension to collagen I pre-coated culture vessels supplemented with growth medium (final volume of 240 <math>\mu\text{l}/\text{cm}^2</math>)</li><li>– 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)</li><li>– never allow the culture to become confluent!</li><li>– cultivate cells at 37°C in a humidified atmosphere with 5 % <math>\text{CO}_2</math></li></ul> |
| Related products:    | <p>PodoUp3 ready-to-use medium, 500 ml (Evercyte, Cat# MHT-033-3)</p> <p>PodoUp3 basal medium, 500 ml (Evercyte, Cat# MHT-033-3-B)</p> <p>PodoUp3 supplements (Evercyte, Cat# MHT-033-3-S)</p> <p>PODO/SVTERT152, human urine-derived podocytes (Evercyte, Cat# CLHT-033-0152)</p> <p>PODO/TERT256, human kidney tissue-derived podocytes (Evercyte, Cat# CHT-033-0256)</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |