Protocol for cryopreservation of PODO/SVTERT152

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Evercyte Ord. No.:	CLHT-033-0152
Designation:	PODO/SVTERT152, human urine-derived podocytes
Freezing medium:	The freezing medium is prepared by mixing the following components: PodoUp3 medium (Evercyte, Cat# MHT-033-3), for preparation please refer to protocol *Preparation of PodoUp3 medium* 10% DMSO (Sigma-Aldrich, Cat# D2650, ready-to-use, stored at RT) Preparation of 10 ml freezing medium: — take 9 ml PodoUp3 medium and transfer to 15 ml centrifugation tube — add 1 ml DMSO and mix carefully — store at 4°C until use, use freshly prepared freezing medium for cryopreservation
Additional reagents:	PodoUp3 medium (Evercyte, Cat# MHT-033-3), for preparation please refer to protocol *Preparation of PodoUp3 medium* 50 µg/ml Collagen I solution (Sigma-Aldrich, Cat# C2249), for preparation please refer to protocol *Passaging of PODO/SVTERT152 cells* 0.05 % Trypsin-EDTA (Gibco, Cat#25300-054) Phosphate buffered saline (PBS) (Sigma-Aldrich, Cat# D8537)
Freezing of cells:	— detach the cells from the culture vessel by using Trypsin-EDTA solution (please refer to protocol *Passaging of PODO/SVTERT152 cells*) — resuspend the detached cells in PodoUp3 medium and centrifuge at 170 g for 5 min — then, discard the supernatant, resuspend the resulting cell pellet in the remaining droplet — add freezing medium (tempered to 4°C) to reach a cell density of about 8 x 10^5 cells/ml (for thawing in a 25 cm² culture flask) — add 1 ml of this cell suspension to each pre-cooled cryovial and immediately transfer the cells to -80°C — after 24 hours transfer the vials to the liquid nitrogen tank
Thawing of cells:	**When you start cultivating the cells, please transfer the content of the original Evercyte vial containing PODO/SVTERT152 cells into a T25 roux flask as described in the following:** — pre-coat a 25 cm² culture flask with collagen I (please refer to protocol *Passaging of PODO/SVTERT152 cells*) — then, add 6 ml of growth medium to the prepared culture flask and transfer it to the incubator for at least 30 min to allow the medium to reach 37°C and its normal pH

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	— take a vial of frozen cells, rinse outside with ethanol and pre-warm in hand until one last piece of frozen cells is seen — immediately transfer the content of the vial to a 15 ml centrifugation tube pre-filled with 9 ml of PodoUp3 medium pre-cooled to 4°C and centrifuge for 5 min at 170 g. — discard the supernatant and resuspend the cell pellet in the remaining droplet — add 1 ml of pre-warmed medium to the cells, transfer the cells to the prepared culture flask — incubate at 37°C in a suitable incubator — perform a medium change 24 hours after thawing. If the cells are already near confluent at this point, they have to be passaged (please refer to protocol *Passaging of PODO/SVTERT152 cells)*
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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