Protocol for cryopreservation of HUVEC/TERT66

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Evercyte Ord. No.:	CHT-006-0066
Designation:	HUVEC/TERT66, umbilical vein endothelial cells
Freezing medium:	CryoStor® cell cryopreservation medium CS10 (Sigma-Aldrich, Cat# C2874, ready-to-use)
Additional reagents:	PBS (Sigma-Aldrich, Cat# D8537, ready-to-use, stored at RT) 0.1 % Gelatin (Sigma-Aldrich, Cat# G1393, 2 %), diluted in PBS 0.05 % Trypsin-EDTA (Gibco, Cat#25300-054, ready-to-use, stored at 4°C after thawing) Defined Trypsin Inhibitor (Gibco, Cat# R007100, ready-to-use, stored at 4°C after thawing)
Freezing cells:	- detach the cells (90-95 % confluence) by using 0.05 % Trypsin-EDTA solution as described in protocol *Passaging of HUVEC/TERT66 cells* - resuspend the detached cells in growth medium and centrifuge at 170 g for 5 min - 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 5 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 cells:	**When you start cultivating the cells, please transfer the content of the original Evercyte vial containing HUVEC/TERT66 cells into a T25 roux flask as described in the following:** - pre-coat a 25 cm² culture flask with Gelatin solution (Sigma-Aldrich, Cat# G1393; diluted to 0.1 % in PBS) as described in protocol *Passaging of HUVEC/TERT66 cells* - therefore, the culture flasks are treated with 0.1 % Gelatin solution (80 µl/cm²) at 37°C for 10-60 min; before introducing cells, remove excess of Gelatin solution - add 6 ml of growth medium to the pre-coated 25 cm² culture flask and place the culture flask in the incubator for at least 30 min to allow the medium to reach 37°C and its normal pH - take a vial of frozen cells, rinse it outside with ethanol and pre-warm in the hand until one last piece of frozen cells is seen - then, immediately transfer the content of the vial to a 15 ml centrifugation tube pre-filled with 9 ml of 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 the pre-warmed medium to the cells, transfer the cells to the prepared culture flask and incubate at 37°C in a suitable incubator - perform a medium change 24 hours after thawing, if the cells are already 90 % confluent at this point, they have to be passaged as described in protocol *Passaging of HUVEC/TERT66 cells*

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Related products: HUVEC/TERT2, umbilical vein endothelial cells (Evercyte, Cat# CHT-006-0008)

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