Protocol for cryopreservation of HDMVEC/TERT164-B

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Evercyte Ord. No.:	CHT-013-0164-B
Designation:	HDMVEC/TERT164-B, human dermal microvascular endothelial cells
Freezing medium:	HDMVEC/TERT164-B growth medium (see protocol *Passaging of HDMVEC/TERT164-B*) 10 % FBS (PAN Biotech Cat# P30-3031 or US. Approved Sigma Aldrich, Cat# F2442) 10 % DMSO (Sigma Aldrich, Cat# D2650) Preparation of 10 ml freezing medium, prepare just before use: - take 8 ml of HDMVEC/TERT164 growth medium, transfer to 15 ml centrifugation tube - add 1 ml of FBS - add 1 ml of DMSO - mix properly and store at 4°C
Additional reagents:	0.1 % Gelatin (Sigma Aldrich, Cat# G1393, 2 %) 0.05 % Trypsin-EDTA (Gibco, Cat# 25300-054) Defined Trypsin Inhibitor (Gibco, Cat# R007100) PBS (Gibco, Cat# 14190-144)
Freezing cells:	- detach the cells from the culture vessel by using Trypsin-EDTA solution as described in protocol *Passaging of HDMVEC/TERT164-B 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 HDMVEC/TERT164-B cells into a T25 roux flask as described in the following:** - pre-coat a 25 cm² culture flask with Gelatin solution as described in protocol *Passaging of HDMVEC/TERT164-B cells* - add 6 ml of growth medium to a 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 filled with 9 ml of medium pre-cooled to 4°C and centrifuge for 5 min at 170 g

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- 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 80 % confluent at this point, they must be passaged as described in protocol *Passaging of HDMVEC/TERT164-B cells*

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

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