Protocol for passaging of HDMVEC/TERT164-B

Version: May 2021

Evercyte Ord. No.:	CHT-013-0164-B
Designation:	HDMVEC/TERT164-B, human dermal microvascular endothelial cells
Growth medium:	Endopan MV kit (PAN Biotech, Cat# P04-0020K) supplemented with G418:

Final components:
Endopan MV basal medium (PAN Biotech, Cat# P04-0020B)
Endopan MV supplements (PAN Biotech, Cat# P04-0020S)
20 µg/ml G418 (InvivoGen, Cat# ant-gn5, 100 mg/ml stock solution, ready-to-use)

- take one bottle of Endopan MV basal medium (500 ml)
- add 30 ml of FBS (Endopan MV supplements)
- add 500 µl of Acorbic Acid (Endopan MV supplements)
- add 500 µl of FGF-2 (Endopan MV supplements)
- add 100 µl Hydrocortisone (Endopan MV supplements)
- add 500 µl of R3-IGF-1 (Endopan MV supplements)
- add 500 µl of EGF (Endopan MV supplements)
- add 100 µl of G418 stock solution

- mix properly, aliquot complete Endopan MV medium
- store at 4°C for a maximum of 4 weeks
- temper the medium to room temperature (not 37°C) before use

Coating: 0.1 % Gelatin solution

The coating solution is prepared by mixing the following components:
Gelatin (Sigma Aldrich, Cat# G1393, 2 %, stored at 4°C)
PBS (Sigma Aldrich, Cat# D8537, ready-to-use, stored at RT)

- liquify the Gelatin solution at 37°C
- add 38 ml PBS to 2 ml Gelatin stock solution
- mix carefully, store diluted Gelatin solution (0.1 %) in aliquots at 37°C

For coating of a T25 roux flask proceed as follows:
- transfer 2 ml of Gelatin solution (0.1 %) to a T25 roux flask (final 80 µl/cm^2)
- completely wet the surface of the culture flask
- incubate the culture flask at 37°C between 10-60 min
- remove excess of Gelatin solution

	- use culture flask immediately for seeding of cells, the surface must not dry out
Additional reagents:	0.05 % Trypsin-EDTA (Gibco, Cat# 25300-054) Defined Trypsin Inhibitor (Gibco, Cat# R007100) PBS (Sigma Aldrich, Cat# D8537) 0.1 % Gelatin (Sigma Aldrich, Cat# G1393, 2 %), diluted in PBS
Passaging of cells:	- remove and discard the culture medium - wash the cells twice with PBS (each 160 µl/cm²), remove PBS completely - add Trypsin-EDTA solution (20 µl/cm²), make sure that all cells have been in contact with this solution - incubate the culture flask at 37°C for approximately 3 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 Defined Trypsin Inhibitor (20 µl/cm²) - resuspend the cells in growth medium (about 160 µl/cm²) and aspirate the cells by pipetting - centrifuge at 170 g for 5 min - discard the supernatant, resuspend the cell pellet in the remaining droplet and add growth medium - transfer appropriate aliquots of the cell suspension to Gelatin pre-coated culture vessels supplemented with growth medium (final volume of 240 µl/cm²) - a split ratio of 1:2 to 1:3 twice a week is recommended (after having reached about 90 %) - perform a medium change after 2-3 days if cells have not reached required cell density - cultivate cells at 37°C in a humidified atmosphere with 5 % CO_2
Related products:	HDMVEC/TERT164-B, microvascular endothelial cells (Evercyte, Cat# CHT-013-0164-B) HUVEC/TERT2, umbilical vein endothelial cells (Evercyte, Cat# CHT-006-0008)

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