Protocol for passaging of HDMVEC/TERT164-B

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Evercyte Ord. No.:	CHT-013-0164-B
Designation:	HDMVEC/TERT164-B, human dermal microvascular endothelial cells
Growth medium:	EGM-2 MV Microvascular Endothelial cell Growth Medium-2 BulletKit (Lonza, Cat# CC-3202, w/o GA-1000 / FBS) supplemented with G418 and FBS (see Protocol for *preparation of HDMVEC/TERT164-B medium*) - 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 (Gibco, Cat# 14190-144, 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 (Gibco, Cat# 14190-144)
Passaging of cells:	- remove and discard the culture medium - wash the cells twice with PBS (each 160 µl/cm^2), remove PBS completely - add Trypsin-EDTA solution (20 µl/cm^2), 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/cm^2) - resuspend the cells in growth medium (about 160 µl/cm^2) and aspirate the cells by pipetting

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