Protocol for passaging of HUVEC/TERT66

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Evercyte Ord. No.:	CHT-006-0066
Designation:	HUVEC/TERT66, umbilical vein endothelial cells
Growth medium:	Endopan 300 SL Kit (PAN Biotech) supplemented with Panexin SL-S (PAN Biotech) and G418: <u>Final components:</u> Endopan 300 SL (PAN Biotech, Cat# P04-0065K without GA) Serum substitute Panexin SL-S (PAN Biotech, Cat# P04-0065S) 20 µg/ml G418 (InvivoGen, Cat# ant-gn-5, 100 mg/ml stock solution, ready-to-use) - take one bottle (500 ml) of Endopan 300 SL basal medium - add all supplements from Endopan 300 SL Kit but GA - add one additional vial of Panexin SL-S (25 mL) - add 100 µl of G418 stock solution - mix properly - store at 4°C for a maximum of 4 weeks - temper the medium to room temperature (not 37°C) before 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)
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 stock solution (2 %) at 37°C - add 38 ml PBS to 2 ml Gelatin stock solution (2 %) - 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
Passaging of cells:	- the new culture flasks have to be coated as described above - remove and discard the culture medium - wash the cells twice with PBS (each 160 µl/cm²), remove PBS completely - add 0.05 % 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 (about 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 - add appropriate aliquots of the cell suspension to Gelatin 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 90-95 % confluence) - 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)

EVERCYTE GmbH
Leberstrasse 20, 1110 Vienna, AUSTRIA
office@evercyte.com | www.evercyte.com