Protocol for passaging of HUVEC/TERT2

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Evercyte Ord. No.:	CHT-006-0008
Designation:	HUVEC/TERT2, human umbilical vein endothelial cells
Growth medium:	EGM™ Endothelial Cell Growth Medium BulletKit™ (Lonza, Cat# CC-3124) supplemented with FBS and G418:

Final components:
EBM™ basal medium (Lonza, Cat# CC-3121)
Components of EGM™ SingleQuot™ Supplements (Lonza, Cat# CC-4133: BBE, hEGF, Hydrocortisone, Ascorbic acid)
10 % FBS (PAN-Biotech, Cat# P30-3031)
20 µg/ml G418 (InvivoGen, Cat# ant-gn-5, 100 mg/ml stock solution, ready-to-use)

- take one bottle of EBM basal medium (500 ml)
- discard 50 ml of EBM basal medium
- add 500 µl of Hydrocortisone (EGM™ SingleQuot™ Supplements)
- add 500 µl of hEGF (EGM™ SingleQuot™ Supplements)
- add 2 ml of BBE (EGM™ SingleQuot™ Supplements)
- add 500 µl of Ascorbic Acid (EGM™ SingleQuot™ Supplements)
- add 50 ml of FBS
- 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

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 % - stored in 2 ml aliquots at 4°C) at 37°C
- add 38 ml of PBS to 2 ml Gelatin stock solution (2 %)
- mix carefully, store diluted Gelatin solution (0.1 %) 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)

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	- 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:	0.1 % Gelatin (Sigma-Aldrich, Cat# G1393, 2 %), diluted in PBS PBS (Sigma-Aldrich, Cat# D8537) 0.05 % Trypsin-EDTA (Gibco, Cat# 25300-054)
Passaging of cells:	- the new culture flasks have to be coated as described above - remove and discard the culture medium - wash the cells once 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 growth medium (about 160 µl/cm²) - add 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 1:8 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/TERT66, umbilical vein endothelial cells (Evercyte, Cat# CHT-006-0066) HDMVEC/TERT164-B, dermal microvascular endothelial cells, lymphatic (Evercyte, Cat# CHT-013-0164-B)

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