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Protocol for in vitro propagation of HUVEC/TERT66 cells

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Reagents

Gelatin solution (Sigma-Aldrich, Cat# G1393)
PBS
Trypsin-EDTA (Gibco, Cat# 25300054)
EndoUp4 Growth Kit (Evercyte, Cat# MHT-006-4) which contains:
- Components of Endopan 300 SL (PAN Biotech, Cat# P04-0065K, Cat# P04-90065S):
  Endopan 300 SL Basal media
  Serum substitute Panexin SL-S (2x)
  EGF
  FGF-2
  VEGF
  Ascorbic Acid Phosphate
  R3-IGF-1
  GA (Gentamycin / Amphotericin)
  Hydrocortison
  Heparin
- G418 (20 µg/ml, InvivoGen, Cat#ant-gn-5)

Practical application

The culture flasks have to be pre-coated with gelatin (Sigma Cat # G1393; diluted to 0.1 % in PBS). Therefore, the culture flasks are treated with gelatin solution (60 µl/cm²) at 37°C for at least 10 min (10 – 60 min). Before introducing cells, remove excess of gelatin solution. For detachment of cells remove and discard culture medium and wash cells twice with PBS. Remove PBS completely. Then, add 0.05 % Trypsin-EDTA (1x) solution (room-temperature; 20 µl/cm²; Gibco, Cat# 25300054), make sure that all cells have been in contact with this solution and incubate the culture flask at 37°C for approximately 3 - 4 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 Trypsin-Inhibitor (20 µl/cm²; Gibco, Cat# R007100). Thereafter, resuspend the cells in growth medium (about 160 µl/cm²) and centrifuge at 170 g for 5 min. Discard the supernatant, resuspend the cell pellet in the remaining droplet and add growth medium. Then, 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).

Related products

- HUVEC/TERT66 telomerized endothelial cells (Evercyte, Cat# CHT-006-0066)
- EndoUp4 Growth Kit (Evercyte, Cat# MHT-006-4)

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