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Protocol for in vitro propagation of HUVEC/TERT2 cells

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Reagents

Gelatin solution (Sigma-Aldrich, Cat# G1393)
PBS
Trypsin-EDTA (Gibco, Cat# 25300054)
EndoUp2 (Evercyte, Cat# MHT-006-2) which contains:

- EBM™ Basal Medium 500 mL (Lonza Cat# CC-3121) with selected supplements from EGM™ SingleQuots™ Kit (Lonza, Cat# CC-4133) – namely BBE (bovine brain extract), HEGF, hydrocortisone solution and ascorbic acid solution
- 10 % Fetal Bovine Serum (FBS) (Sigma-Aldrich, Cat# F7524)
- 20 µg/ml G418 (InvivoGen, Cat# ant-gn-5)

Practical application

The culture flasks have to be pre-coated with gelatin (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 and rinse flask once with PBS (160 µl/cm²).
For detachment of cells remove and discard culture medium and wash cells once with PBS. Remove PBS completely. Then, add 0.05 % Trypsin-EDTA (1x) solution (room-temperature; 20 µl/cm²), make sure that all cells have been in contact with this solution and incubate the culture flask at 37°C for approximately 2 – 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) and aspirate cells by pipetting. Add appropriate aliquots of the cell suspension to gelatin coated culture vessels supplemented with growth medium (final volume of 240 µl/cm²).
Cells are grown in EndoUp2 ready-to-use medium at 37°C in a humidified atmosphere with 5 % CO_2. A split ratio of 1:4 to 1:6 twice a week is recommended (after having reached about 90 – 95 % confluence).

Related products

- EndoUp2 ready-to-use medium, 500 ml (Evercyte, Cat# MHT-006-2)

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