EVERCYTE GmbH
the pharmacocellomics™ company
Muthgasse 18
1190 Wien
office@evercyte.com
www.evercyte.com

Protocol for in vitro propagation of HCEC-1CT cells

Version: April 2017

Material

Primaria™ culture flasks (Corning, Cat# 353808 and Cat# 353810)
PBS
Trypsin-EDTA solution (Gibco, Cat# 25300-054)
Defined Trypsin Inhibitor (Gibco, Cat# R007-100)
ColoUp ready-to-use medium (Evercyte, Cat# MHT-039) which contains:

- DMEM / Medium 199 Earle's, 4+1 (Biochrom, Cat# F0435 and Cat# FG0615)
- 4 mM GlutaMAX™-1 (100X), (Gibco, Cat# 35050-038)
- 2 % Cosmic Calf Serum (Hyclone, Cat# SH30087)
- 20 ng/ml EGF (Sigma-Aldrich, Cat# E9644)
- 10 µg/ml Insulin (Sigma-Aldrich, Cat# I9278)
- 2 µg/ml Apo-Transferrin (Sigma-Aldrich, Cat# T2036)
- 5 nM Sodium-Selenite (Sigma-Aldrich, Cat# S5261)
- 1 µg/ml Hydrocortisone (Sigma-Aldrich, Cat# H0396)

Practical application

HCEC-1CT cells are grown in ColoUp ready-to-use medium at 37°C in a humidified atmosphere with 5 % CO_2 and 2 - 5 % oxygen in Primaria™ culture ware. Cells are passaged after having reached about 85 - 95 % confluence. For detachment the cell culture medium is removed and the cells are washed once with PBS. Then, Trypsin-EDTA solution (0.05 %, 20 µl/cm²) is added and the culture flasks are incubated at 37°C for approx. 2 - 3 minutes. Cell detachment is observed under an inverted microscope. As soon as all cells are detached (if necessary agitate the cells by gently hitting the flask), Trypsin action is halted by addition of Defined Trypsin Inhibitor (20 µl/cm²).
Growth medium is then added to the cells and the cell suspension is centrifuged for 5 minutes at 170 g. Then, the supernatant is discarded, the cell pellet is resuspended in the remaining droplet and growth medium (about 160 µl/cm²) is added. Then, appropriate aliquots of the cell suspension are transferred to new culture vessels supplemented with growth medium (final volume of 240 µl/cm²).
A split ratio of 1:16 twice a week is recommended.

Related products

- ColoUp ready-to-use-medium, 500 ml (Evercyte, Cat# MHT-039)

ERSTE Bank Wien, Konto: 29513826400, BLZ: 20111, IBAN: AT872011129513826400, BIC: GIBAATWWXXX | FN358620h, HG Wien | UID: ATU66244913