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

Protocol for cryopreservation of HCEC-1CT cells

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Material

Primaria™ culture flasks (Corning, Cat# 353808 and Cat# 353810)

Trypsin-EDTA (Gibco, Cat# 25300054)
Trypsin-Inhibitor (Gibco, Cat# R007-100)
Freezing medium which contains:

- ColoUp ready-to-use medium (Evercyte, Cat# MHT-039)
- 10 % DMSO
- 10 % Cosmic Calf Serum (Hyclone, Cat# SH30087)

Storage temperature: liquid nitrogen

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)

Ethanol, 70%

Practical application

Freezing of cells:
Cells are detached from the culture vessel by using Trypsin-EDTA and Trypsin-Inhibitor as described in Evercyte's protocol for in vitro propagation of HCEC-1CT cells and centrifuged at 170 g for 5 min. Then, the supernatant is discarded, the cell pellet is resuspended in the remaining droplet and freezing medium (pre-cooled to 4°C) is added to reach a cell density of about 1 - 2 x 10^6 cells/ml (for thawing in a 25 cm² Primaria™ culture flask). Then, 1 ml of this cell suspension is added to each pre-cooled cryovial which are then immediately transferred to -80°C. After 24 hours the vials are transferred to liquid nitrogen for long-term storage.

Thawing of cells:
6 ml of growth medium are added to a 25 cm² culture flask, which is transferred for at least 30 min to a humidified incubator to allow the medium to reach 37°C and its normal pH. Then, a vial of frozen cells is taken, rinsed outside with ethanol and pre-warmed in the hand until one last piece of frozen cells is seen. Thereafter, the content of the vial is immediately transferred to a 15 ml centrifugation tube pre-filled with 9 ml of medium pre-cooled to 4°C and centrifuged for 5 min at 170 g. Then, the

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supernatant is discarded, the cell pellet is resuspended in the remaining droplet and 1 ml of the pre-warmed medium is added to the cells. This cell suspension is then transferred to the prepared culture flask and incubated at 37°C in a suitable incubator. After 24 h a medium change is performed. If the cells are already confluent at this point, they should be passaged (see Evercyte's protocol for in vitro propagation of HCEC-1CT cells).

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