

EVERCYTE GmbH
the pharmacocellomics™ company

Muthgasse 18

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 GlutaMAXTM-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 $1 - 2 \times 10^6$ cells/ml (for thawing in a 25 cm² PrimariaTM 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 prewarmed 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



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)