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

Protocol for cryopreservation of RPTEC/TERT1 cells

Version: January 2019

Reagents

Trypsin-EDTA (Gibco, Cat# 25300054)
Trypsin-Inhibitor (Gibco, Cat# R007100)
Freezing medium:
CryoStor® cell cryopreservation medium CS10 (Sigma-Aldrich, Cat# C2874)
Storage temperature: liquid nitrogen

ProxUp ready-to-use medium (Evercyte, Cat# MHT-003) which contains:

- DMEM/Ham's F-12 (1:1) (Biochrom, Cat# F4815)
- 10 mM HEPES-buffer (Biochrom, Cat# L1613)
- 2 mM GlutaMAX™-I (Gibco, Cat# 35050-038)
- 10 ng/ml hEGF (Sigma-Aldrich, Cat# E9644)
- 5 pM 3,3',5-Triiodo-L-thyronine sodium salt (Sigma-Aldrich, Cat# T6397)
- 3.5 µg/ml L-Ascorbic Acid (Sigma-Aldrich, Cat# A4544)
- 5 µg/ml Transferrin Holo (Merck Millipore, Cat# 616424)
- 25 ng/ml Prostaglandine E1 (Sigma-Aldrich, Cat# P8908)
- 25 ng/ml Hydrocortisone (Sigma-Aldrich, Cat# H0396)
- 8.65 ng/ml Sodium-Selenite (Sigma-Aldrich, Cat# S5261)
- 100 µg/ml G418 (InvivoGen, Cat# ant-gn-5)
- 5 µg/ml Insulin (Sigma-Aldrich, Cat# I9278)

Ethanol, 70 %

Practical application

Freezing of cells:
Detach the cells from the culture vessel by using Trypsin and Trypsin-Inhibitor as described above, resuspend the detached cells in growth medium and centrifuge at 170 g for 5 min. Then, discard the supernatant, resuspend in the remaining droplet and add freezing medium (4°C) to reach a cell density of about 1.5 - 2 x 10^6 cells/ml (for thawing in a 25 cm² culture flask). Transfer 1 ml of this cell suspension to each pre-cooled cryovial and immediately transfer the cells to -80°C. After 24 hours transfer the vials to the liquid nitrogen tank.

Thawing of cells:
Add 6 ml of growth medium to a 25 cm² culture flask and place the culture flask in the incubator for at least 30 min. Take a vial of frozen cells, rinse outside with ethanol and pre-warm in hand until one last piece of frozen cells is seen. Then, immediately transfer the content of the vial to a 15 ml centrifugation tube pre-filled with 9 ml of medium pre-cooled to 4°C and centrifuge for 5 min at 170 g. Then, discard the supernatant and resuspend the cells in the remaining droplet. Add 1 ml of pre-warmed

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

medium to the cells, transfer the cell suspension to the prepared culture flask and incubate at 37°C in a suitable incubator.
RPTEC/TERT1 cells are grown in ProxUp medium at 37°C in a humidified atmosphere with 5 % CO_2. Perform a medium change 24 hours after thawing. If the cells are already confluent at this point, they should be passaged (see Evercyte protocol for in vitro propagation of RPTEC/TERT1 cells). For the first passages after thawing we recommend splitting not higher than 1:2.

Related products
• ProxUp ready-to-use medium, 500 ml (Evercyte, Cat# MHT-003) • ProxUp basal medium, 500 ml (Evercyte, Cat# MHT-003-B) • ProxUp supplements, 9 vials (Evercyte, Cat# MHT-003-S)