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Protocol for cryopreservation of HUVEC/TERT2 cells

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Reagents

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

- EndoUp2 ready to use medium (Evercyte, Cat# MHT-006-2)
- 10 % DMSO

Storage temperature: liquid nitrogen

Gelatin solution (Sigma-Aldrich, Cat# G1393)
EndoUp2 ready-to-use medium (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-gn5)

Ethanol, 70 %

Practical application

Freezing of cells:
Detach cells from culture vessel by using Trypsin-EDTA as described in Evercyte's protocol for in vitro propagation of HUVEC/TERT2 cells, resuspend 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 5 x 10^5 cells/ml (for thawing in a 25 cm² culture flask). Add 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 liquid nitrogen.

Thawing of cells:
Pre-coat a 25 cm² culture flask with gelatine (see Evercyte's protocol for in vitro propagation of HUVEC/TERT2 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 to allow the medium to reach its normal pH. 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 supernatant and resuspend cells in the remaining droplet. Add 1 ml of pre-warmed medium to the cells, transfer them to the prepared culture flask and incubate at 37°C in a suitable incubator.
Perform a medium change 24 hours after thawing. If the cells are already confluent at

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this point, they should be passaged (see Evercyte's protocol for in vitro propagation of HUVEC/TERT2 cells).

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