

Protocol for cryopreservation of hTEC/SVTERT24-B cells

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Reagents
<p>Collagen human, Bornstein and Traub Type I (Sigma-Aldrich, Cat# C7624-5ML) 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 Ethanol, 70 % ThymoUp ready-to-use medium (Evercyte, Cat# MHT-010)</p>
Practical application
<p>Freezing of cells: Detach the cells from the culture vessel by using Trypsin-EDTA solution 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 the resulting cell pellet in the remaining droplet and add freezing medium (tempered to 4°C) to reach a cell density of about 1×10^6 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 the liquid nitrogen tank.</p> <p>Thawing of cells: Pre-coat a 25 cm² culture flask with human collagen I (see Evercyte's protocol for in vitro propagation of hTEC/SVTERT24-B 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 the supernatant and resuspend the cell pellet in the remaining droplet. Add 1 ml of the pre-warmed medium to the cells, transfer the cells 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 90 % confluent at this point, they should be passaged (see Evercyte's protocol for in vitro propagation of hTEC/SVTERT24-B cells).</p>
Related products
<ul style="list-style-type: none"> ThymoUp ready-to-use medium, 500 ml (Evercyte, Cat# MHT-010)