

## Protocol for cryopreservation of fHDF/TERT166 cells

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Reagents
<p>Trypsin-EDTA (Gibco, Cat# 25300054) Freezing medium which contains:</p> <ul style="list-style-type: none"> <li>• DMEM/Ham's F12 (1:1) (Biochrom, Cat# F4815)</li> <li>• 10 % Fetal Bovine Serum (FBS) (Sigma-Aldrich, Cat# F7524)</li> <li>• 10 % DMSO</li> </ul> <p>Storage temperature: liquid nitrogen</p> <p>FibroUp ready-to-use medium (Evercyte, Cat# MHT-008) which contains:</p> <ul style="list-style-type: none"> <li>• DMEM/Ham's F-12 (1:1) (Biochrom, Cat# F4815)</li> <li>• 10 % Fetal bovine serum (FBS) (Sigma-Aldrich, Cat# F7524)</li> <li>• 2 mM GlutaMAX™-I (Gibco, Cat# 35050-038)</li> <li>• 100 µg/ml G418 (InvivoGen, Cat# ant-gn5)</li> </ul> <p>Ethanol, 70 %</p>
Practical application
<p><b>Freezing of cells:</b> Detach cells from culture vessel by using Trypsin-EDTA as described in Evercyte's protocol for in vitro propagation of fHDF/TERT166 cells, resuspend detached cells in growth medium and centrifuge at 170 g for 5 min. Then, discard the supernatant, resuspend the cell pellet in the remaining droplet and add freezing medium (4°C) to reach a cell density of about <math>5 \times 10^5</math> cells/ml (for thawing in a 25 cm<sup>2</sup> 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.</p> <p><b>Thawing of cells:</b> Add 6 ml of cultivation medium to a 25 cm<sup>2</sup> 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 cells in the remaining droplet. Add 1 ml of medium (pre-warmed to 37°C), transfer to the prepared culture flask and incubate at 37°C in a suitable incubator.</p>
Related products
<ul style="list-style-type: none"> <li>• FibroUp ready-to-use medium, 500 ml (Evercyte, Cat# MHT-008)</li> </ul>