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Protocol for cryopreservation of HBEC3-KT cells

Version: April 2017

Reagents
Trypsin-EDTA (Gibco, Cat# R-001-100) Freezing medium containing: • Keratinocyte-SFM (1X) Kit (Gibco, Cat# 17005-042) • 10 % DMSO • 10 % Fetal Bovine Serum (FBS) (Sigma-Aldrich, Cat# F7524) Storage temperature: liquid nitrogen Gelatin from porcine skin (Sigma-Aldrich, Cat# G1890) Keratinocyte-SFM (1X) Kit (Gibco, Cat# 17005-042) Ethanol, 70 %
Practical application
Freezing of cells: Detach the cells from the culture vessel by using Trypsin-EDTA solution as described in Evercyte's protocol for in vitro propagation of HBEC3-KT cells 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 x 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. Thawing of cells: Add 6 ml of growth medium to a pre-coated 25 cm² culture flask (see Evercyte's protocol for in vitro propagation of HBEC3-KT) 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 growth 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 80 - 90 % confluent at this point, they should be passaged (see Evercyte's protocol for in vitro propagation of HBEC3-KT cells).