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Protocol for cryopreservation of hTCEpi cells

Version: April 2017

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 Ethanol, 70 % KGM™-2 BulletKit™ (Lonza, Cat# CC-3107) without GA-1000 from this kit
Practical application
Freezing of cells: Detach cells from culture vessel by using trypsin and trypsin-inhibitor as described in Evercyte's protocol for in vitro propagation of hTCEpi cells, resuspend detached cells in growth medium and centrifuge at 170 g for 5 min. Then, discard the supernatant, resuspend the cells in the remaining droplet and add freezing medium (4°C) to reach a cell density of 5 x 10^5 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 liquid nitrogen. 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 to warm the medium and 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 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 this point, they should be passaged (see Evercyte's protocol for in vitro propagation of hTCEpi cells).

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