Protocol for cryopreservation of fHDF/TERT166

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Evercyte Ord. No.:	CHT-031-0166
Designation:	fHDF/TERT166, human foreskin fibroblasts
Freezing medium:	DMEM/F12 (1:1) (PAN Biotech, Cat# P04-41150) 10 % FBS (PAN-Biotech, Cat# P30-3031, ready-to-use, stored at 4°C after thawing) 10 % DMSO (Sigma-Aldrich, Cat# D2650, ready-to-use, stored at RT) Preparation of 10 ml freezing medium, prepare just before use: - take 8 ml of DMEM/Ham´s F12 (1:1) and transfer to 15 ml centrifugation tube - add 1 ml of FBS - add 1 ml of DMSO - mix properly and store at 4°C
Additional reagents:	0.05 % Trypsin-EDTA (Gibco, Cat# 25300-054, ready-to-use, stored at 4°C after thawing) PBS (Sigma-Aldrich, Cat# D8537, ready-to-use, stored at room temperature)
Freezing cells:	- detach the cells from the culture vessel by using Trypsin-EDTA solution as described in protocol *Passaging of fHDF/TERT166* cells - resuspend the detached cells in growth medium and centrifuge at 170 g for 5 min - discard the supernatant - resuspend the resulting cell pellet in the remaining droplet - add freezing medium (tempered to 4°C) to reach a cell density of about 5 x 10^5 cells/ml (for thawing in a 25 cm^2 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 cells:	**When you start cultivating the cells, please transfer the content of the original Evercyte vial containing fHDF/TERT166 cells into a T25 roux flask as described in the following:** - add 6 ml of growth medium to a 25 cm^2 culture flask and place the culture flask in the incubator for at least 30 min to allow the medium to reach 37°C and its normal pH - take a vial of frozen cells, rinse it outside with ethanol and pre-warm in the 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 - 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

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- perform a medium change 24 hours after thawing, if the cells are already confluent at this point, they have to be passaged as described in protocol *Passaging of fHDF/TERT166 cells*

Related products:	HDF/TERT164, dermal fibroblasts (Evercyte, Cat#CHT-008-0164)

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