Protocol for cryopreservation of NHEK/SVTERT3-5

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Evercyte Ord. No.:	CLHT-011-0026-5
Designation:	NHEK/SVTERT3-5, human epidermal keratinocytes
Freezing medium:	CryoStor® cell cryopreservation medium CS10 (Sigma-Aldrich, Cat# C2874, ready-to-use)
Additional reagents:	0.05% Trypsin-EDTA (Gibco, Cat# 25300-054, ready-to-use, stored at 4°C) Defined Trypsin Inhibitor (Gibco, Cat# R007100, ready-to-use, stored at 4°C) PBS (Sigma-Aldrich, Cat# D8537, ready-to-use, stored at RT)
Freezing cells:	- detach the cells from the culture vessel by using Trypsin-EDTA solution as described in protocol *Passaging of NHEK/SVTERT3-5 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 5-7 x 10^5 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 cells:	**When you start cultivating the cells, please transfer the content of the original Evercyte vial containing NHEK/SVTERT3-5 cells into a T25 roux flask as described in the following:** - 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 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 - recovery from cryopreservation will take a few days, perform a medium change 24 hours after thawing; if the cells are already 60-70 % confluent at this point, they have to be passaged as described in protocol *Passaging of NHEK/SVTERT3-5 cells*

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