Protocol for cryopreservation of TB/SVTERT350

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Evercyte Ord. No.:	CLHT-070-0350
Designation:	TB/SVTERT350, human trophoblasts
Freezing medium:	CryoStor® cell cryopreservation medium CS10 (Sigma-Aldrich, Cat# C2874, ready-to-use, stored at 4°C)
Additional reagents:	Phosphate buffered saline (PBS) (Gibco, Cat# 14190-144, ready-to-use, stored at RT) Trypsin-Inhibitor (Gibco, Cat# R007100, ready-to-use, stored at 4°C) Trypsin-EDTA (Gibco, Cat# 25300054, ready-to-use, stored at 4°C) Trypsin 0.25% (Trypsin 2.5% Gibco, Cat# 15090046, diluted 1:10 in PBS, stored at 4°C)
Freezing cells:	- detach the cells from the culture vessel by using 1:1 Trypsin-EDTA and Trypsin 0.25% solution as described in protocol *Passaging of TB/SVTERT350* - resuspend the detached cells in growth medium and centrifuge at 180 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 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 TB/SVTERT350 into a T25 roux flask as described in the following:** - pre-coat a 25 cm² culture flask with Collagen I coating as described in protocol *Passaging of TB/SVTERT350 cells* - add 6 ml of growth medium to the pre-coated 25 cm² culture flask and place it 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 180 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 - perform a medium change 24 hours after thawing; if the cells are already 80 % confluent, they must be passaged as described in protocol *Passaging of TB/SVTERT350 cells*
Related products:	- TrophoUp medium (Evercyte, Cat# MHT-070), ready-to-use medium

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- TB/SVTERT350, human trophoblasts (Evercyte, Cat# CLHT-070-0350)

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