Protocol for cryopreservation of RA-MSC/TERT308

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Evercyte Ord. No.:	CHT-050-0308
Designation:	RA-MSC/TERT308, human reflected amnion-derived mesenchymal stem cells
Freezing medium:	CryoStor® cell cryopreservation medium CS10 (Sigma-Aldrich, Cat# C2874, ready-to-use)
Additional reagents:	PBS (Sigma-Aldrich, Cat# D8537, stored at RT) CTS™ TrypLE™ Select Enzyme (Gibco, Cat# A1285901, stored at RT)
Freezing cells:	- detach the cells from the culture vessel by using CTS™ TrypLE™ Select Enzyme solution as described in protocol *Passaging of RA-MSC/TERT308* - 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 RA-MSC/TERT308 cells into a T25 roux flask as described in the following:** - pre-coat a 25 cm² culture flask with NutriCoat™ Attachment Solution following the instructions of the manufacturer or as described in protocol *Passaging of RA-MSC/TERT308 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 - RA-MSC/TERT308 cells should be split the day after thawing as described in protocol *Passaging of RA-MSC/TERT308 cells*
Related products:	CP-MSC/TERT308, chorionic plate-derived MSCs (Evercyte, Cat# CHT-064-0308) P-MSC/TERT308, placental amnion-derived MSCs (Evercyte, Cat# CHT-051-0308) WJ-MSC/TERT273, Wharton´s jelly-derived MSCs (Evercyte, Cat# CHT-059-0273) ASC/TERT1, adipose-derived MSCs (Evercyte, Cat# CHS-001-0005)

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