Protocol for cryopreservation of EN-MSC/TERT352-B

Version: March 2025

Evercyte Ord. No.:	CHT-060-0352-B
Designation:	EN-MSC/TERT352-B, human endometrium derived mesenchymal stromal cells
Freezing medium:	CryoStor® Cell Cryopreservation Medium CS10 (Sigma-Aldrich, Cat# C2874, ready to use)
Material:	CellBIND™ tissue culture ware (Corning)
Additional reagents:	PBS (Gibco, Cat# 14190-144, ready-to-use, stored at 4°C) CTS™ TrypLE™ Select Enzyme (Gibco, Cat# A1285901, ready-to-use, stored at 4°C)
Freezing cells:	- detach the cells from the culture vessel by using CTS™ TrypLE™ Select Enzmye solution as described in protocol *Passaging of EN-MSC/TERT352-B cells* - resuspend the detached cells in growth medium and centrifuge at 200 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 1ml of this cell suspension to each pre-cooled cryovial and immediately transfer the cells to -80°C - after 24 hours transfer the vials the liquid nitrogen tank
Thawing of cells:	**When you start cultivating the cells, please transfer the content of the original Evercyte vial containing EN-MSC/TERT352-B into a T25 CellBIND™ flask as described in the following:** - add 6 ml of growth medium to the 25 cm² CellBIND™ 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 cell is seen - then, immediately transfer the content of the vials 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 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 at this point, they must be passaged as described in protocol *Passaging of EN-MSC/TERT352-B cells*

EVERCYTE GmbH
Leberstraße 20, 1110 Vienna, AUSTRIA
office@evercyte.com | www.evercyte.com