

Protocol for cryopreservation of BM-MSC/TERT292

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Evercyte Ord. No.:	CHT-063-0292
Designation:	BM-MSC/TERT292, human bone marrow-derived mesenchymal stromal cells
Freezing medium:	CryoStor [®] cell cryopreservation medium CS10 (Sigma-Aldrich, Cat# C2874, ready-to-use, stored at 4°C)
Additional reagents:	Phosphate buffered saline (PBS) (Sigma-Aldrich, Cat# D8537) CTS [™] TrypLE [™] Select Enzyme (Gibco, Cat# A1285901)
Freezing cells:	- detach the cells from the culture vessel by using CTS[™] TrypLE[™] Select Enzyme solution as described in protocol <i>Passaging of BM-MSC/TERT292 cells</i> - resuspend the detached cells in growth medium and centrifuge at 300 g for 5 min - discard the supernatant - resuspend the resulting cell pellet in the remaining droplet - add freezing medium (4°C) to reach a cell density of about 3-5 x 10⁵ 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 BM-MSC/TERT292 cells into a T25 roux flask as described in the following: - pre-coat a 25 cm² culture flask with ACF Cell Attachment Substrate as described in protocol <i>Passaging of BM-MSC/TERT292 cells</i> - add 6 ml of growth medium to the pre-coated 25 cm² culture flask and place it in the incubator for 1 hour to allow the medium to reach 37°C and pH adjustment - 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 - BM-MSC/TERT292 cells should be split the day after thawing as described in protocol <i>Passaging of BM-MSC/TERT292 cells</i>
Related products:	WJ-MSC/TERT273, Wharton's jelly-derived MSCs (Evercyte, Cat# CHT-059-0273) ASC/TERT300, adipose-derived MSCs (Evercyte, Cat# CHT-001-0300)