

Protocol for cryopreservation of ASC/TERT300

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Evercyte Ord. No.:	CHT-001-0300
Designation:	ASC/TERT300, human adipose-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 Enzym (Gibco, Cat# A1285901)
Freezing cells:	<ul style="list-style-type: none">- detach the cells from the culture vessel by using CTS[™] TrypLE[™] Select Enzym solution as described in protocol <i>Passaging of ASC/TERT300 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:	<p>When you start cultivating the cells, please transfer the content of the original Evercyte vial containing ASC/TERT300 cells into a T25 roux flask as described in the following:</p> <ul style="list-style-type: none">- pre-coat a 25 cm² culture flask with ACF Cell Attachment Substrate as described in protocol <i>Passaging of ASC/TERT300 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- ASC/TERT300 cells should be split the day after thawing as described in protocol <i>Passaging of ASC/TERT300 cells</i>
Related products:	WJ-MSC/TERT273, Wharton's jelly-derived MSCs (Evercyte, Cat# CHT-059-0273) ASC/TERT1, adipose-derived MSCs (Evercyte, Cat# CHS-001-0005)