

Protocol for passaging of P-MSC/TERT308

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Evercyte Ord. No.:	CHT-051-0308
Designation:	P-MSC/TERT308, human placental amnion-derived mesenchymal stem cells
Growth medium:	MSC NutriStem® XF Medium (Biological Industries/SATORIUS, Cat# 05-200-1A) supplemented with G418
	<p><u>Final components:</u></p> <p>MSC NutriStem® XF Basal Medium (Biological Industries/SATORIUS, Cat# 05-200-1)</p> <p>MSC NutriStem® XF Supplement Mix (Biological Industries/SATORIUS, Cat# 05-201-1)</p> <p>200 µg/ml G418 (InvivoGen, Cat# ant-gn5, ready-to-use, 100 mg/ml)</p> <ul style="list-style-type: none"> - take one bottle of MSC NutriStem® XF Basal Medium (500 ml) - add 3 ml of MSC NutriStem® XF Supplement Mix - add 1 ml of G418 stock solution <ul style="list-style-type: none"> - mix properly - store at 4°C for a maximum of 4 weeks (if not used up in that time prepare smaller volumes accordingly) - temper the medium to room temperature (not 37°C) before use
Coating:	<p>NutriCoat™ Attachment Solution</p> <p>The coating solution is prepared by mixing the following components:</p> <p>NutriCoat™ Attachment Solution (Biological Industries/SATORIUS, Cat# 05-760-1-15, stored at RT)</p> <p>Ringer Solution (B. Braun Ecoflac® Plus, ready-to-use, Cat# 33109, stored at RT)</p> <p>For coating of a T25 roux flask proceed as follows:</p> <ul style="list-style-type: none"> - transfer 2 ml of Ringer Solution to a sterile tube, add 4 µl of NutriCoat™ Attachment Solution and gently mix - transfer the 1:500 diluted NutriCoat™ Attachment Solution (1.8 ml) to a T25 roux flask (72 µ7/cm²) - completely wet the surface of the culture flask - incubate at 37°C for at least 1 hour - remove excess of coating solution - use culture flask immediately for seeding of cells, the surface must not to dry out
Additional reagents:	<p>PBS (Sigma-Aldrich, Cat# D8537, ready-to-use, stored at RT)</p> <p>CTS™ TrypLE™ Select Enzyme (Gibco, Cat# A1285901, ready-to-use, stored at RT)</p>

- Passaging of cells:
- remove and discard the culture medium
 - wash the cells twice with PBS (each 160 $\mu\text{l}/\text{cm}^2$), remove PBS completely
 - add CTS™ TrypLE™ Select Enzyme solution (20 $\mu\text{l}/\text{cm}^2$), make sure that all cells have been in contact with this solution
 - incubate the culture flask at 37°C for approximately 2-3 min
 - observe cell detachment under an inverted microscope
 - as soon as all cells are detached (if necessary, agitate the cells by gently hitting the flask), add growth medium (about 160 $\mu\text{l}/\text{cm}^2$)
 - centrifuge at 180 g for 5 min
 - discard the supernatant, resuspend the cell pellet in the remaining droplet and add growth medium (about 160 $\mu\text{l}/\text{cm}^2$)
 - transfer appropriate aliquots of the cell suspension to pre-coated culture vessels supplemented with growth medium (final volume of 240 $\mu\text{l}/\text{cm}^2$)
 - a split ratio of 1:6 to 1:8 twice a week is recommended (after having reached about 70-80 % confluence)
 - cultivate cells at 37°C in a humidified atmosphere with 5% CO₂

Related products: CP-MSC/TERT308, chorionic plate-derived MSCs (Evercyte, Cat# CHT-064-0308)
RA-MSC/TERT308, reflected amnion-derived MSCs (Evercyte, Cat# CHT-050-0308)
WJ-MSC/TERT273, Wharton's Jelly-derived MSCs (Evercyte, Cat# CHT-059-0273)
ASC/TERT300, adipose-derived MSCs (Evercyte, Cat# CHT-001-0300)
