Protocol for passaging of CP-MSC/TERT308

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Evercyte Ord. No.:	CHT-064-0308
Designation:	CP-MSC/TERT308, human placental-derived mesenchymal stem cells (chorionic plate)
Growth medium:	MSC NutriStem® XF Medium (Biological Industries/SATORIUS, Cat# 05-200-1A) supplemented with G418 Final components: MSC NutriStem® XF Basal Medium (Biological Industries/SATORIUS, Cat# 05-200-1) MSC NutriStem® XF Supplement Mix (Biological Industries/SATORIUS, Cat# 05-201-1) 200 µg/ml G418 (InvivoGen, Cat# ant-gn5, ready-to-use, 100 mg/ml) - 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 - 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:	NutriCoat™ Attachment Solution The coating solution is prepared by mixing the following components: NutriCoat™ Attachment Solution (Biological Industries/SATORIUS, Cat# 05-760-1-15, stored at RT) Ringer Solution (B. Braun Ecoflac® Plus, ready-to-use, Cat# 33109, stored at RT) For coating of a T25 roux flask proceed as follows: - 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 µ/cm^2) - 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:	PBS (Sigma-Aldrich, Cat# D8537, ready-to-use, stored at RT) CTS™ TrypLE™ Select Enzyme (Gibco, Cat# A1285901, ready-to-use, stored at RT)

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Passaging of cells:	- remove and discard the culture medium - wash the cells twice with PBS (each 160 µl/cm²), remove PBS completely - add CTS™ TrypLE™ Select Enzyme solution (20 µl/cm²), 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 µl/cm²) - centrifuge at 180g for 5 min - discard the supernatant, resuspend the cell pellet in the remaining droplet and add growth medium (about 160 µl/cm²) - transfer appropriate aliquots of the cell suspension to pre-coated culture vessels supplemented with growth medium (final volume of 240 µl/cm²) - 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% CO_2
Related products:	P-MSC/TERT308, placental amnion-derived MSCs (Evercyte, Cat# CHT-051-0308) 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/TERT1, adipose-derived MSCs (Evercyte, Cat# CHS-001-0005) ASC/TERT300, adipose-derived MSCs (Evercyte, Cat# CHT-001-0300)

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