

Protocol for passaging of BM-MSC/TERT292

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Evercyte Ord. No.:	CHT-063-292
Designation:	BM-MSC/TERT292, human bone marrow-derived mesenchymal stromal cells
Growth medium:	Mesencult™ ACF Plus Culture Kit (STEMCELL TECHNOLOGIES, Cat# 05448) supplemented with GlutaMAX™-I and G418 <u>Final components:</u> Mesencult™ ACF Plus medium (STEMCELL TECHNOLOGIES, Cat# 05446) Mesencult™ ACF Plus 500x supplement (STEMCELL TECHNOLOGIES, Cat# 05447) Animal Component-Free Cell Attachment (STEMCELL TECHNOLOGIES, Cat# 07130) 2 mM GlutaMAX™-I (Gibco, Cat# 35050-038, ready-to-use) 200 µg/ml G418 (InvivoGen, Cat# ant-gn5, ready-to-use, 100 mg/ml) - take one bottle of Mesencult™ ACF Plus medium (500 ml) - add 1 ml of Mesencult™ ACF Plus 500x supplement - add 5 ml of GlutaMAX™-I - add 1 ml of G418 stock solution - mix properly - store at 4°C for a maximum of 1 week (if not used up in that time prepare smaller volumes accordingly) - temper the medium to room temperature (not 37°C) before use
Preparation of culture flasks / coating:	ACF Cell Attachment Substrate The coating solution is prepared by mixing the following components: Animal Component-Free Cell Attachment (Cat# 07130, stored at 4°C) PBS (Sigma-Aldrich, Cat# D8537, ready-to-use, stored at RT) For coating of a T25 roux flask proceed as follows: - transfer 1.8 ml of PBS to a sterile tube, add 6 µl of ACF Cell Attachment Substrate and gently mix - transfer the 1:300 diluted ACF substrate (1.8 ml) to a T25 roux flask (72 µl/cm²) - completely wet the surface of the culture flask - incubate at room temperature for at least 2 hours - NOTE: coated flasks can be stored for up to 3 days at 4°C – the flasks must be sealed with Parafilm to prevent evaporation – before use let the flask warm up at room temperature for 30 min - remove excess of coating solution

	- wash the flask once with PBS (160 $\mu\text{l}/\text{cm}^2$) - remove PBS completely - add 6 ml of cell culture medium and incubate at 37°C for 1 hour for the medium to reach 37°C and pH adjustment - use culture flask immediately for seeding of cells
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)
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 300 g for 5 min - discard the supernatant, resuspend the cell pellet in the remaining droplet and add growth medium (about 80 $\mu\text{l}/\text{cm}^2$, 2 ml for cells from a T25 roux flask) - transfer appropriate aliquots of the cell suspension to pre-coated culture vessels supplemented with growth medium (240 $\mu\text{l}/\text{cm}^2$, 6 ml for T25 roux flask) - a split ratio of 1:4 to 1:6 twice a week is recommended (after having reached about 80-90 % confluence). Carefully monitor the outer parts of the roux flask - cells get confluent there first - cultivate cells at 37°C in a humidified atmosphere with 5% CO₂
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) ASC/TERT300, adipose-derived MSCs (Evercyte, Cat# CHT-001-0300)