

## Protocol for passaging of BM-MSC/TERT292

Version: September 2021

Evercyte Ord. No.:	CHT-063-292
Designation:	BM-MSC/TERT292, human bone marrow-derived mesenchymal stem cells
Growth medium:	Mesencult™ ACF Plus Culture Kit (STEMCELL TECHNOLOGIES, Cat# 05448) supplemented with GlutaMAX™-I and G418

Final components:

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 2 weeks (if not used up in that time prepare smaller volumes accordingly)
- temper the medium to room temperature (not 37°C) before use

Coating:	ACF Cell Attachment Substrate
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The coating solution is prepared by mixing the following components:  
 Animal Component-Free Cell Attachment (Cat# 05448, 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/cm<sup>2</sup>)
- 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
- use culture flask immediately for seeding of cells, the surface must not to dry out

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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  $\mu\text{l}/\text{cm}^2$ ), 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 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 1:4 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% CO<sub>2</sub>

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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)

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