

Protocol for passaging of ASC/TERT300

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Evercyte Ord. No.:	CHT-001-300
Designation:	ASC/TERT300, human adipose-derived mesenchymal stromal 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 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
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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 Enzym (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 1:6 to 1:8 twice a week is recommended (after having reached about 70-80 % 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 mesenchymal stromal cells (Evercyte, Cat# CHS-001-0005)
