

## Protocol for passing of ASC/TERT300

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Version: September 2025

Evercyte Ord. No.:	CHT-001-300
Designation:	ASC/TERT300, human adipose-derived mesenchymal stromal cells
Growth medium:	<p>Mesencult™ ACF Plus Culture Kit (STEMCELL TECHNOLOGIES, Cat# 05448) supplemented with GlutaMAX™-I and G418:</p> <p><u>Final components:</u></p> <p>Mesencult™ ACF Plus medium (STEMCELL TECHNOLOGIES, Cat# 05446)</p> <p>Mesencult™ ACF Plus 500x supplement (STEMCELL TECHNOLOGIES, Cat# 05447)</p> <p>Animal Component-Free Cell Attachment (STEMCELL TECHNOLOGIES, Cat# 07130)</p> <p>2 mM GlutaMAX™-I (Gibco, Cat# 35050-038, ready-to-use)</p> <p>200 µg/ml G418 (InvivoGen, Cat# ant-gn5, ready-to-use, 100 mg/ml)</p> <ul style="list-style-type: none"><li>- take one bottle of Mesencult™ ACF Plus medium (500 ml)</li><li>- add 1 ml of Mesencult™ ACF Plus 500x supplement</li><li>- add 5 ml of GlutaMAX™-I</li><li>- add 1 ml of G418 stock solution</li><li>- mix properly</li><li>- store at 4°C for a maximum of 1 week (if not used up in that time prepare smaller volumes accordingly)</li><li>- temper the medium to room temperature (not 37°C) before use</li></ul>
Preparation of culture flasks / coating:	<p>ACF Cell Attachment Substrate</p> <p>The coating solution is prepared by mixing the following components:</p> <p>Animal Component-Free Cell Attachment (Cat# 07130, stored at 4°C)</p> <p>PBS (Sigma-Aldrich, Cat# D8537, ready-to-use, stored at RT)</p> <p>For coating of a T25 roux flask proceed as follows:</p> <ul style="list-style-type: none"><li>- transfer 1.8 ml of PBS to a sterile tube, add 6 µl of ACF Cell Attachment Substrate and gently mix</li><li>- transfer the 1:300 diluted ACF substrate (1.8 ml) to a T25 roux flask (72 µl/cm<sup>2</sup>)</li><li>- completely wet the surface of the culture flask</li><li>- incubate at room temperature for at least 2 hours</li><li>- 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</li><li>- remove excess of coating solution</li></ul>

	<ul style="list-style-type: none"><li>- wash the flask once with PBS (160 <math>\mu\text{l}/\text{cm}^2</math>)</li><li>- remove PBS completely</li><li>- 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</li><li>- use culture flask immediately for seeding of cells</li></ul>
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:	<ul style="list-style-type: none"><li>- remove and discard the culture medium</li><li>- wash the cells twice with PBS (each 160 <math>\mu\text{l}/\text{cm}^2</math>), remove PBS completely</li><li>- add CTS™ TrypLE™ Select Enzyme solution (20 <math>\mu\text{l}/\text{cm}^2</math>), make sure that all cells have been in contact with this solution</li><li>- incubate the culture flask at 37°C for approximately 2-3 min</li><li>- observe cell detachment under an inverted microscope</li><li>- as soon as all cells are detached (if necessary, agitate the cells by gently hitting the flask), add growth medium (about 160 <math>\mu\text{l}/\text{cm}^2</math>)</li><li>- centrifuge at 300 g for 5 min</li><li>- discard the supernatant, resuspend the cell pellet in the remaining droplet and add growth medium (about 80 <math>\mu\text{l}/\text{cm}^2</math>, 2 ml for cells from a T25 roux flask)</li><li>- transfer appropriate aliquots of the cell suspension to pre-coated culture vessels supplemented with growth medium (240 <math>\mu\text{l}/\text{cm}^2</math>, 6 ml for T25 roux flask)</li><li>- 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</li><li>- cultivate cells at 37°C in a humidified atmosphere with 5% CO<sub>2</sub></li></ul>
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)