

## Protocol for passaging of EN-MSC/TERT352-B

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Evercyte Ord. No.:	CHT-060-0352-B
Designation:	EN-MSC/TERT352-B, human endometrium derived mesenchymal stromal cells
Growth medium:	RoosterNourish™-MSC-XF (RoosterBio, Cat# K82016) supplemented with G418:  <u>Final components:</u> RoosterBasal™2.0-CC (RoosterBio, Cat# M22520) RoosterBooster™-MSC-XF (RoosterBio, Cat# SU-016) 200 µg/ml G418 (InvivoGen, Cat# ant-gn5, ready-to-use, 100 mg/ml)  <ul style="list-style-type: none"><li>- take one bottle of RoosterBasal™2.0-CC medium (500 ml)</li><li>- add 10 mL RoosterBooster™-MSC-XF</li><li>- add 1 ml of G418 stock solution</li> <li>- mix properly</li><li>- store at 4°C for a maximum of 2 weeks</li><li>- temper the medium to room temperature (not 37°C) before use</li></ul>
Material:	<ul style="list-style-type: none"><li>- CellBIND™ tissue culture ware (Corning)</li></ul>
Additional reagents:	PBS (Gibco, Cat#14190-144, ready-to-use, stored at 4°C) CTS™ TrypLE™ Select Enzyme (Gibco, Cat# A1285901, ready-to-use, stored at 4°C)
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 µl/cm<sup>2</sup>), remove PBS completely</li><li>- add CTS™ TrypLE™ Select Enzyme solution (20 µl/cm<sup>2</sup>), 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 µl/cm<sup>2</sup>)</li><li>- centrifuge at 200 g for 5 min</li><li>- discard the supernatant, resuspend the cell pellet in the remaining droplet and add growth medium (about 160 µl/cm<sup>2</sup>)</li><li>- transfer appropriate aliquots of the cell suspension to CellBIND™ culture ware supplemented with growth medium (final volume of 240 µl/cm<sup>2</sup>)</li><li>- a split ratio of 1:9 to 1:12 twice a week is recommended (after having reached about 70-80 % confluence)</li><li>- cultivate cells at 37°C in a humidified atmosphere with 5% CO<sub>2</sub></li></ul>