

## Protocol for passaging of LHCN-M2

Version: May 2021

Evercyte Ord. No.:	CkHT-040-231-2
Designation:	LHCN-M2, human skeletal muscle cells
Growth medium:	<p>The MyoUp medium for cultivation of LHCN-M2 cells can either be ordered from Evercyte as ready-to-use medium (Cat# MHT-040) or can be prepared by mixing the following components:</p> <p>DMEM (Gibco, Cat # 10566016) / M199 (Gibco, Cat# 31150022) (4+1)                      15 % FBS (Sigma Aldrich, Cat# F7524)                      20 mM Hepes (Sigma Aldrich, Cat# H0887)                      0.03 µg/ml Zinc Sulfate (Sigma Aldrich, Cat# Z0251)                      1.4 µg/ml Vitamin B12 (Sigma Aldrich, Cat# V2876)                      0.055 µg/ml Dexamethasone (Sigma Aldrich, Cat# D4902)                      2.5 ng/ml HGF (Merck Millipore, Cat# GF116ml bFGF (Peprotech, Cat# 100-18B)                      10 ng/ml bFGF (Peprotech, Cat# 100-18B)</p> <ul style="list-style-type: none"> <li>- take one bottle (500 ml) of DMEM and discard 100 ml</li> <li>- add 100 ml of M199 and mix properly</li> <li>- discard 90 ml DMEM/M199 mixture</li> <li>- add 75 ml FBS (ready-to-use)</li> <li>- add 10 ml Hepes (1M stock, ready-to-use)</li> <li>- add 50 µl Zink Sulfate stock (30 mg/ml, prepared in cell culture grade water)</li> <li>- add 50 µl Vitamin B12 stock (14 mg/ml, prepared in cell culture grade water)</li> <li>- add 70 µl Dexamethasone stock (1 mM, prepared in absolute EtOH, basal medium)</li> <li>- add 25 µl HGF stock (50 µg/ml, prepared in cell culture grade water)</li> <li>- add 50 µl bFGF stock solution (100 µg/ml, prepared in PBS/Tris/BSA buffer)</li> <li>- mix properly and store at 4°C for up to 1 month</li> <li>- temper the medium to room temperature (not 37°C) before use</li> </ul>
Coating:	<p>0.1 % Gelatin solution</p> <p>The coating solution is prepared by mixing the following components:</p> <p>Gelatin (Sigma Aldrich, Cat# G1890)                      Cell culture grade water (Hyclone, Cat# SH30529.03)</p> <ul style="list-style-type: none"> <li>- weigh 2 g of Gelatin in glass bottle</li> <li>- add 200 ml cell culture grade water</li> <li>- transfer bottle to water bath to dissolve Gelatin</li> </ul>

- autoclave resulting 1% Gelatin solution
- aliquot (5 ml) and store at 4°C until use

For coating of cell culture flasks, liquefy the 1 % Gelatin solution at 37°C  
Add 45 ml cell culture grade water to 5 ml 1 % Gelatin solution (final concentration 0.1 %) and mix carefully  
Store at 37°C until use (stable for 4 weeks)

For coating of a T75 roux flask proceed as follows:

- transfer 6 ml of Gelatin solution (0.1 %) to a T75 roux flask (final 80 µl/cm<sup>2</sup>)
- completely wet the surface of the culture flask
- incubate at 37°C for at least 4 hours (up to one week)
- remove excess of Gelatin solution
- use culture flasks immediately for seeding of cells, the surface must not dry out

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Additional reagents	PBS (Sigma Aldrich, Cat# D8537, ready-to-use, stored at RT) 0.05 % Trypsin-EDTA (Gibco, Cat#25300-054, ready-to-use, stored at 4°C after thawing) 0.1 % Gelatin (Sigma Aldrich, Cat# G1890), dissolved in cell culture grade water
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Passaging of cells:	<ul style="list-style-type: none"><li>- remove and discard the culture medium</li><li>- wash the cells once with PBS, remove PBS completely</li><li>- add Trypsin-EDTA solution (20 µl/cm<sup>2</sup>), make sure that all cells have been in contact with Trypsin-EDTA and 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, add growth medium (about 160 µl/cm<sup>2</sup>) and aspirate cells by pipetting</li><li>- determine the viable cell number and add appropriate aliquots of the cell suspension to new Gelatin coated culture vessels filled with growth medium (final volume of 240 µl/cm<sup>2</sup>)</li><li>- a seeding density of 1.200 cells/cm<sup>2</sup> is recommended</li><li>- cells should be split twice a week when having reached about 30-40 % confluence, never allow the culture to become confluent!</li><li>- cultivate cells at 37°C in a humidified atmosphere with 5 % CO<sub>2</sub></li></ul>
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Related products:	MyoUp ready-to-use medium, 500 ml (Evercyte, Cat# MHT-040)
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