

# Protocol for cryopreservation of LHCN-M2

Version: August 2024

Evercyte Ord. No.:	CkHT-040-231-2
Designation:	LHCN-M2, human skeletal muscle cells
Freezing medium:	<p>The freezing medium is prepared by mixing the following components:</p> <p>DMEM (Gibco, Cat # 61965-026) / M199 (Gibco, Cat# 31150022) (4+1)                      15 % FBS (PAN Biotech, Cat# P30-3031)                      10 % DMSO (Sigma-Aldrich, Cat# D2650)</p> <p>Preparation of 10 ml freezing medium prepare just before use:</p> <ul style="list-style-type: none"> <li>- take 6 ml DMEM and transfer to 15 ml centrifugation tube</li> <li>- add 1.5 ml M199 and mix properly</li> <li>- add 1.5 ml FBS and mix carefully</li> <li>- add 1 ml DMSO and mix carefully</li> <li>- store at 4°C until use</li> </ul>
Additional reagents	<p>0.1 % Gelatin (Sigma-Aldrich, Cat# G1890, diluted in cell culture grad water)                      PBS (Sigma-Aldrich, Cat# D8537, ready-to-use, stored at room temperature)                      0.05 % Trypsin-EDTA (Gibco, Cat#25300-054)</p>
Freezing of cells:	<ul style="list-style-type: none"> <li>- detach the cells from the culture flask using Trypsin-EDTA solution as described in protocol <i>Passaging of LHCN-M2 cells</i></li> <li>- resuspend the detached cells in growth medium MyoUp and centrifuge at 170 g for 5 min</li> <li>- discard the supernatant, resuspend the cell pellet in the remaining droplet</li> <li>- add freezing medium (tempered to 4°C) to reach a cell density of 5.000-6.000 cells/cm<sup>2</sup> (3.75-4.5 x 10<sup>5</sup> cells for thawing in a 75 cm<sup>2</sup> culture flask)</li> <li>- add 1 ml of this cell suspension to each pre-cooled cryovial and immediately transfer the cells to -80°C</li> <li>- after 24 hours transfer the vials to the liquid nitrogen tank</li> </ul>
Thawing of cells:	<p><b>When you start cultivating the cells, please transfer the content of the original Evercyte vial containing LHCN-M2 cells into a T75 roux flask as described in the following:</b></p> <ul style="list-style-type: none"> <li>- pre-coat a 75 cm<sup>2</sup> culture flask with Gelatin as described in protocol <i>Passaging of LHCN-M2 cells</i></li> <li>- add 15 ml of growth medium to the 75 cm<sup>2</sup> culture flask and place the culture flask in the incubator for at least 30 min to allow the medium to reach 37°C and its normal pH</li> <li>- take a vial of frozen cells, rinse it outside with ethanol and pre-warm in hand until one last piece of frozen cells is seen</li> </ul>

- immediately transfer the content of the vial to a 15 ml centrifugation tube pre-filled with 9 ml of MyoUp medium pre-cooled to 4°C
- centrifuge for 5 min at 170 g
- discard the supernatant and resuspend the cell pellet in the remaining droplet
- add 8 ml of pre-warmed MyoUp medium to the cell suspension, transfer the cells to the prepared culture flask and incubate at 37°C in a humidified incubator with 5 % CO<sub>2</sub>
- perform a medium change 24 hours after thawing, if the cells are already 30-40 % confluent at this point, they have to be passaged as described in protocol *Passaging of LHCN-M2*

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Related products: MyoUp ready-to-use medium, 500 ml (Evercyte, Cat# MHT-040)

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