

## Protocol for differentiation of LHCN-M2 cells towards myofibers

Version: June 2025

Evercyte Protocol No.:	Diff-Myoblasts-V3
Cells:	LHCN-M2, telomerized muscle cells (Evercyte, Cat# CkHT-040-231-2)
Reagents, material:	<p>PBS (Gibco, Cat #14190-144, ready-to-use, stored at RT)</p> <p>MyoUp medium (Evercyte, Cat# MHT-040) or <i>Protocol for preparation of MyoUp medium</i></p> <p>0.05 % Trypsin-EDTA (1x) solution (Gibco, Cat# 25300054)</p> <p>1 % gelatin from porcine skin, type A (Sigma Aldrich, Cat# G1890, stock solution from powder: 1% in cell culture grade water, stored in aliquots at 4°C)</p> <p>2 x 8-well chamber slides (IBIDI, Cat# 80826)</p> <p>0.1 % gelatin solution (= coating solution)</p> <ul style="list-style-type: none"> <li>- pipette 5 ml of 1 % gelatin stock solution into a 50 ml sterile tube</li> <li>- add 45 ml sterile cell culture grade water</li> <li>- mix properly and store at 37°C for up to 4 weeks</li> </ul> <p>DMEM/M199 (4:1) with 2 % horse serum (= differentiation medium)</p> <ul style="list-style-type: none"> <li>- pipette 8 ml DMEM (Gibco, Cat# 10566-016) into a 15 ml sterile tube</li> <li>- add 2ml M199 (Gibco, Cat# 31150-022)</li> <li>- mix properly and discard 200 µl of the DMEM/M199 mixture</li> <li>- add 200 µl of horse serum (Sigma Aldrich, Cat# H1270)</li> <li>- mix carefully</li> </ul>
Seeding of cells into chamber slides:	<p>prepare 2 chamber slides:</p> <p>1x with cells seeded at higher cell density (12.000 cells / cm<sup>2</sup>) for induction of differentiation and 1x with cells seeded at lower cell density (1.200 cells / cm<sup>2</sup>) as undifferentiated control</p> <ul style="list-style-type: none"> <li>- transfer 200 µl of coating solution (0.1 % gelatin solution) to each well of the chamber slides and wet the surface of each well completely</li> <li>- incubate the slides at 37°C for at least 4 hours</li> <li>- remove excess of gelatin solution from the chamber slides just before using the slides and seed cells immediately; the surface must not dry out</li> <li>- harvest LHCN-M2 cells in growth medium as described in <i>Protocol passaging of LHCN-M2 cells</i> and determine the cell number using a hemocytometer</li> <li>- prepare 2 ml of a cell suspension containing 6 x 10<sup>4</sup> cells / ml</li> <li>- transfer 200 µl of the cell suspension into each well of the slide (final cell density / well: 12.000 cells / cm<sup>2</sup>)</li> <li>- take 200 µl of the prepared cell suspension and add 1.8 ml LHCN-M2 growth medium (1:10 dilution)</li> </ul>

- add 200 µl of the 1:10 dilution to each well of the second chamber slide (final cell density / well: 1.200 cells / cm<sup>2</sup>)
- make sure that the cell suspensions are distributed evenly in the individual chambers
- incubate the slides at 37°C for 24 hours

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Induction of  
differentiation:

- discard the growth medium and add 200 µl of differentiation medium to each well in the high cell density slides (12.000 cells / cm<sup>2</sup>)(day 1)
- perform a medium change at day 3, 6 and 9 (differentiation medium in the high cell density slides)
- monitor and document morphological changes of the cells towards differentiated cells (multinucleated myofibers) and analyse for expression of typical markers of differentiated myoblasts (e.g. MF-20, desmin, Pax-7) by immunofluorescence staining