Protocol for differentiation of LHCN-M2 cells towards myofibers

Version: September 2021

Evercyte Protocol No.:	Diff-Myoblasts-V3
Cells:	LHCN-M2, telomerized muscle cells (Evercyte, Cat# CkHT-040-231-2)
Reagents, material:	PBS (Sigma Aldrich, Cat# D8537, ready-to-use, stored at RT) MyoUp medium (Evercyte, Cat# MHT-040) or *Protocol for preparation of MyoUp medium* 0.05 % Trypsin-EDTA (1x) solution (Gibco, Cat# 25300054) 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) 2 x 8-well chamber slides (IBIDI, Cat# 80826) 0.1 % gelatin solution (= coating solution) - pipette 5 ml of 1 % gelatin stock solution into a 50 ml sterile tube - add 45 ml sterile cell culture grade water - mix properly and store at 37°C for up to 4 weeks DMEM/M199 (4:1) with 2 % horse serum (= differentiation medium) - pipette 8 ml DMEM (Gibco, Cat# 10566-016) into a 15 ml sterile tube - add 2ml M199 (Gibco, Cat# 31150-022) - mix properly and discard 200 µl of the DMEM/M199 mixture - add 200 µl of horse serum (Sigma Aldrich, Cat# H1270) - mix carefully
Seeding of cells into chamber slides:	prepare 2 chamber slides: 1x with cells seeded at higher cell density (12.000 cells / cm^2) for induction of differentiation and 1x with cells seeded at lower cell density (1.200 cells / cm^2) as undifferentiated control - 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 - incubate the slides at 37°C for at least 4 hours - remove excess of gelatin solution from the chamber slides just before using the slides and seed cells immediately; the surface must not dry out - harvest LHCN-M2 cells in growth medium as described in *Protocol passaging of LHCN-M2 cells* and determine the cell number using a hemocytometer - prepare 2 ml of a cell suspension containing 6 x 10^4 cells / ml - transfer 200 µl of the cell suspension into each well of the slide (final cell density / well: 12.000 cells / cm^2) - take 200 µl of the prepared cell suspension and add 1.8 ml LHCN-M2 growth medium (1:10 dilution)

EVERCYTE GmbH
Leberstraße 20, 1110 Vienna, AUSTRIA
office@evercyte.com | www.evercyte.com

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