Protocol for passaging of LHCN-M2

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Evercyte Ord. No.:	CkHT-040-231-2
Designation:	LHCN-M2, human skeletal muscle cells
Growth medium:	MyoUp medium contains the herein described components and is prepared as described in the following: DMEM (Gibco, Cat # 61965-026) / M199 (Gibco, Cat# 31150022) (4+1) 15 % FBS (e.g. PAN Biotech, Cat# P30-3031) 20 mM Hepes (Sigma-Aldrich, Cat# H0887) 3 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# GF116) 10 ng/ml bFGF (R&D Systems, Cat# 3718-FB-100) - take one bottle (500 ml) of DMEM and discard 100 ml - add 100 ml of M199 and mix properly - discard 90 ml DMEM/M199 mixture - add 75 ml FBS (ready-to-use) - add 10 ml Hepes (1M stock, ready-to-use) - add 50 µl Zink Sulfate stock (30 mg/ml, prepared in cell culture grade water) - add 50 µl Vitamin B12 stock (14 mg/ml, prepared in cell culture grade water) - add 70 µl Dexamethasone stock (1 mM, prepared in absolute EtOH, basal medium) - add 25 µl HGF stock (50 µg/ml, prepared in cell culture grade water) - add 50 µl bFGF stock solution (100 µg/ml, prepared in PBS) - mix properly and store at 4°C for up to 1 month - temper the medium to room temperature (not 37°C) before use
Coating:	0.1 % Gelatin solution The coating solution is prepared by mixing the following components: Gelatin (Sigma-Aldrich, Cat# G1890) Cell culture grade water (Hyclone, Cat# SH30529.03) - weigh 2 g of Gelatin in glass bottle - add 200 ml cell culture grade water - transfer bottle to water bath to dissolve Gelatin - 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^2) - 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
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
Passaging of cells:	- remove and discard the culture medium - wash the cells once with PBS, remove PBS completely - add Trypsin-EDTA solution (20 µl/cm^2), 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 - observe cell detachment under an inverted microscope - as soon as all cells are detached, add growth medium (about 160 µl/cm^2) and aspirate cells by pipetting - 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^2) - a seeding density of 1.200 cells/cm^2 is recommended - cells should be split twice a week when having reached about 30-40 % confluence, never allow the culture to become confluent! - cultivate cells at 37°C in a humidified atmosphere with 5 % CO_2
Related products:	- MyoUp (Cat# MHT-040), ready-to-use medium - MyoUp2 (Cat# MHT-040-2, for US customers), this medium contains all components of MyoUp but FBS; **before use, 75 ml FBS must be added to give rise to ready-to-use MyoUp medium**

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