Protocol for cyropreservation of LHCN-M2

Version: February 2022

Evercyte Ord. No.:	CkHT-040-231-2
Designation:	LHCN-M2, human skeletal muscle cells
Freezing medium:	The freezing medium is prepared by mixing the following components: DMEM (Gibco, Cat # 10566016) / M199 (Gibco, Cat# 31150022) (4+1) 15 % FBS (PAN Biotech, Cat# P30-3031) 10 % DMSO (Sigma-Aldrich, Cat# D2650) Preparation of 10 ml freezing medium prepare just before use: - take 6 ml DMEM and transfer to 15 ml centrifugation tube - add 1.5 ml M199 and mix properly - add 1.5 ml FBS and mix carefully - add 1 ml DMSO and mix carefully - store at 4°C until use
Additional reagents	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)
Freezing of cells:	- detach the cells from the culture flask using Trypsin-EDTA solution as described in protocol *Passaging of LHCN-M2 cells* - resuspend the detached cells in growth medium MyoUp and centrifuge at 170 g for 5 min - discard the supernatant, resuspend the cell pellet in the remaining droplet - add freezing medium (tempered to 4°C) to reach a cell density of 5.000-6.000 cells/cm^2 (3.75-4.5 x 10^5 cells for thawing in a 75 cm^2 culture flask) - add 1 ml of this cell suspension to each pre-cooled cryovial and immediately transfer the cells to -80°C - after 24 hours transfer the vials to the liquid nitrogen tank
Thawing of cells:	**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**: - pre-coat a 75 cm^2 culture flask with Gelatin as described in protocol *Passaging of LHCN-M2 cells* - add 15 ml of growth medium to the 75 cm^2 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 - 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

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

- 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_2
- 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*

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

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