Product-Data-Sheet for LHCN-M2

Version: August 2024

Evercyte Ord. No.:	CkHT-040-231-2
Designation:	LHCN-M2
Biosafety Level:	1
Shipped:	Frozen on dry ice
Medium:	MyoUp (Evercyte, Cat#MHT-040): DMEM (Gibco, Cat # 61965-026) / M199 (Gibco, Cat# 31150022) (4+1) 15 % FBS (PAN-Biotech, Cat# P30-3031, ready-to-use, stored at 4°C after thawing) 20 mM Hepes (Sigma-Aldrich, Cat# H0887, ready-to-use, stored at 4°C) 3 µg/ml Zinc Sulfate (Sigma-Aldrich, Cat# Z0251, stored at 4°C or -20°C) 1.4 µg/ml Vitamin B12 (Sigma-Aldrich, Cat# V2876, stored at 4°C) 0.055 µg/ml Dexamethasone (Sigma-Aldrich, Cat# D4902, stored at -20°C) 2.5 ng/ml HGF (Merck Millipore, Cat# GF116, stored at -20°C) 10 ng/ml bFGF (R&D Systems, Cat# 3718-FB-100)
Growth:	Adherent
Organism:	Homo sapiens (human)
Morphology:	Myoblast morphology
Source:	Pectoralis major muscle tissue, male donor, 41 years
Cell Type:	Satellite cells
Antigen Expression:	Positive for PAX7, Desmin, MF20 upon induced differentiation
Ethical statement:	Approved by Institutional Review Board (IRB) in accordance with the Declaration of Helsinki
Comments:	LHCN-M2 was developed from human satellite cells (from the pectoralis major muscle) by transduction with retroviral vectors containing the cdk-4 and hTERT gene. The cell line was continuously cultured for more than 200 population doublings without showing signs of growth retardation or replicative senescence and shows the typical myoblast morphology (Zhu, Ch.-H. et al. 2007, Aging Cell, 6(4):515-23, [PubMed UID: 17559502]).
Propagation:	Cells are grown in MyoUp medium (see above) at 37°C in a humidified atmosphere with 5 % CO_2.
Subculturing:	The new culture flasks must be pre-coated with Gelatin. Therefore, the culture flasks are treated with Gelatin solution (80 µl/cm²; Sigma-Aldrich, Cat# G1890, 0.1 % in cell culture

	grade water) at 37°C for at least 4 hours (up to one week). Before introducing cells, remove excess of Gelatin solution. For detachment of the cells remove and discard the culture medium and wash the cells twice with PBS. Remove PBS completely. Then, add 0.05 % Trypsin-EDTA solution (room-temperature; 20 µl/cm²; Gibco, Cat# 25300054), make sure that all cells have been in contact with this solution 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/cm²) 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/cm²). A seeding density of 1.200 cells/cm² is recommended. Cells should be split twice a week when having reached about 30-40 % confluence. Never allow the culture to become confluent!
Preservation:	Freezing medium: DMEM (Gibco, Cat # 61965-026) / M199 (Gibco, Cat# 31150022) (4+1) 15 % FBS (PAN Biotech, Cat# P30-3031) 10 % DMSO (Sigma-Aldrich, Cat# D2650) Storage temperature: liquid nitrogen
Freezing and thawing procedure:	Freezing of cells: Detach the cells from the culture vessel by using Trypsin-EDTA solution as described above, resuspend the detached cells in growth medium and centrifuge at 170 g for 5 min. Then, discard the supernatant, resuspend the cell pellet in the remaining droplet and add freezing medium (tempered to 4°C) to reach a cell density of 5.000-6.000 cells/cm² (3.75-4.5 x 10^5 cells for thawing in a 75 cm² 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 cm² culture flask with Gelatin (see above or protocol *Passaging of LHCN-M2 cells*). Add 15 ml of growth medium to a 75 cm² 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. Then, immediately transfer the content of the vial to a 15 ml centrifugation tube pre-filled with 9 ml of medium pre-cooled to 4°C and centrifuge for 5 min at 170 g. Then, discard the supernatant and resuspend the cell pellet in the remaining droplet. Add 8 ml of pre-warmed medium to the cell suspension, transfer the cells to the prepared culture flask and incubate at 37°C in a suitable incubator.

	Perform a medium change 24 hours after thawing. If the cells are already 30-40 % confluent at this point, they must be passaged (see above or protocol *Passaging of LHCN-M2 cells*).
Doubling Time:	35-40 hours
Virus Testing	Cells have been tested negative for HAV and Parvo B19 with Roche DPX-PCR (cobas® TaqScreen DPX-Test), for HBV, HCV, HIV nucleic acids with Roche-Multiplex-PCR (cobas® TaqScreen MPX Test, v2.0).
Other Analytical Data:	Cells are negative for Mycoplasma contaminations as tested using MycoAlert™ Mycoplasma Detection Kit from Lonza. Cells are negative for bacterial and fungal contaminations as tested according to Ph. Eur. 2.6.1. / USP <71>. STR profile has been analyzed and is as expected.

Please Note:

The classification of biosafety level is based on Austrian Legislation (Gentechnikbuch; Systemverordnung) and on recommendations of the Central Committee on Biological Safety (ZKBS). While Evercyte undertakes all reasonable measures to test for absence of a selected panel of known human pathogenic viruses, there is currently no test procedure available that guarantees for complete absence of infectious pathogens. The use of state-of-the art infectious virus assays or viral antigen assays may leave open the possible existence of a latent viral genome, even if a negative test result is obtained. Therefore, we recommend that all human cell lines should be handled with caution such as an organism of ACDP Hazard Group 2.
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