iXCells BIOTECHNOLOGIES

Human Motor Neurons (iPSC-derived, TDP-43 mutation, M337V)

SKU: 40HU-105-HET; 40HU-105-HOM; 40HU-105-ISO

PRODUCT SHEET

Product Description

Amyotrophic lateral sclerosis (ALS) is a late-onset neurodegenerative disease of the motor system, characterized by selective and progressive loss of motor neurons, eventually leading to paralysis and death within 2–5 years [1]. iPSC-derived motor neurons are valuable tools for biochemical analysis, disease modelling and clinical application of this disease. Cytoplasmic accumulation and nuclear loss of the RNA binding protein transactive response DNA-binding protein 43 (TDP-43) from affected neurons in most instances of ALS [2-3]. Over 40 dominantly inherited mutations in the gene encoding TDP-43 have subsequently been identified in familial ALS patients [4], implicating TDP-43 dysfunction in the vast majority of ALS cases.

Product Details

Catalog Number: 40HU-105-HET; 40HU-105-HOM; 40HU-105-ISO

Organism: *Homo Sapiens,* Human

Cell Type: Brain Cell

Tissue: N/A

Disease: ALS

Package Size: 1 x 10^6 cells/vial and 2 x 10^6 cells/vial

Passage Number: P0

Growth Properties: Adherent

Associated Media: Motor Neuron Culture Medium Kit (MD-0022)

Storage Conditions & Shipment

Product Format/Shipped: Cryopreserved

Storage: Liquid Nitrogen

For Research Use Only

iXCells Biotechnologies USA, Inc.

United States

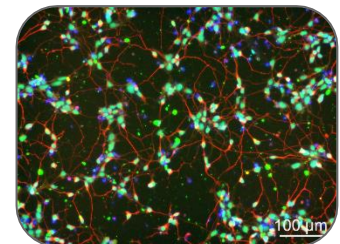
www.ixcellsbiotech.com

Customer Support

E. supports@ixcellsbiotech.com
E. orders@ixcellsbiotech.com
P. +1 (858) 412 5988

RELATED PRODUCTS

Human Motor Neurons (iPSC-derived, TDP43 mutation, N352S, HET)
SKU: 40HU-102-HET



Human Motor Neurons (iPSC-derived, TDP43 mutation, N352S, HET)
SKU: 40HU-102-HET

Overview of Human Motor Neurons (iPSC-derived, TDP-43 mutation, M337V)

Human Motor Neurons (iPSC-derived, TDP-43 mutation, M337V) is derived from a genetically modified normal iPSC line carrying the heterozygous or homozygous M337V mutation in the TDP-43 gene (Figure 1). iXCells™ hiPSC-derived motor neurons express typical markers of motor neurons, e.g. HB9 (MNX1), ISL1, CHAT, (Figure 2), with the purity higher than 85%. iXCells™ motor neurons are available in both cryopreserved vials and fresh plate formats (12, 24, 48, and 96-well plate). Most of the cells will express high level of HB9 and ISL-1 after thawing in the Motor Neuron Culture Medium Kit (Cat# MD-0022-100ML). After culturing in the medium for 5-7 days, these cells will express high levels of CHAT and MAP2.

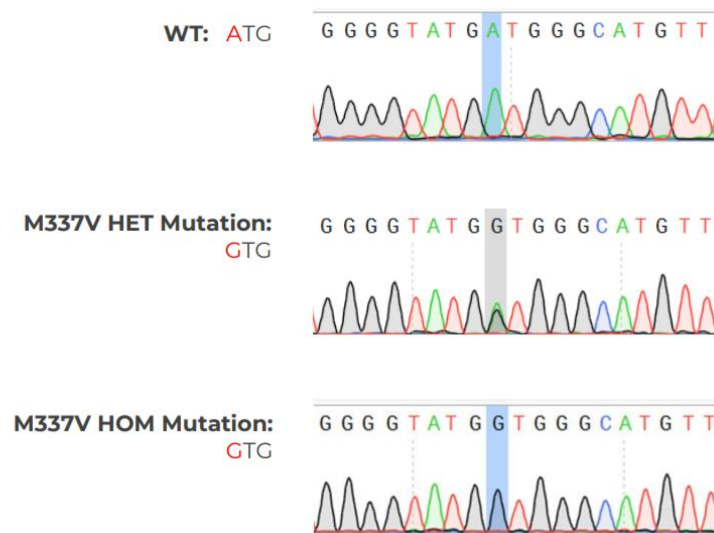


Figure 1. Heterozygous and homozygous M337V mutation (highlighted) has been introduced to TDP-43 gene using CRISPR/Cas9 based genome editing technology. The targeted site is verified by genomic PCR/Sanger .sequencing.

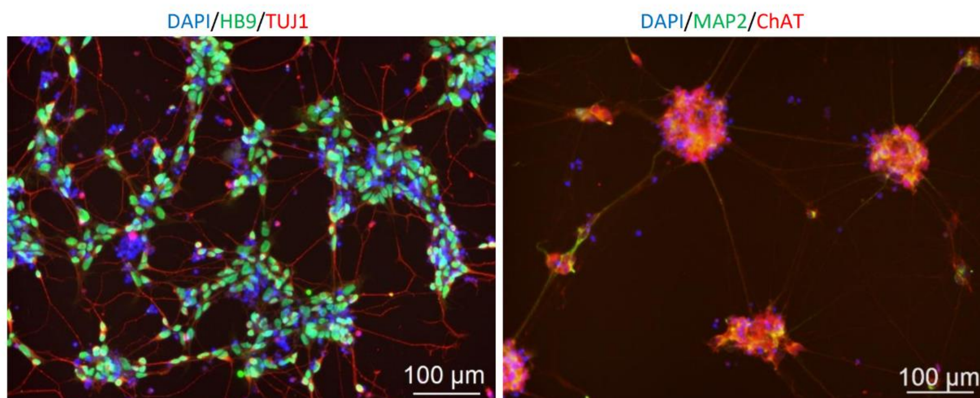


Figure 2. Immunofluorescence staining showing HB9 and ChAT positive cells on day 2 and 7 in culture respectively.

Protocols

This procedure describes the steps required for thawing and plating hiPSC-Derived Motor Neurons using iXCells™ Motor Neuron Culture Medium Kit (Cat# MD-0022-100ML).

REQUIRED REAGENTS AND MATERIALS

IXCELLS™ MOTOR NEURON CULTURE MEDIUM KIT (CAT# MD-0022-100ML) INCLUDES:

		VOLUME
MOTOR NEURON CULTURE MEDIUM A	MD-0022A-100ML	100 ML
MOTOR NEURON CULTURE MEDIUM B	MD-0022B-20UL	20 µL

The motor neuron culture medium a and b can be stored at 2-8°C for 1 month and at -20°C for 12 months.

MONO-CULTURE OF HIPSC-DERIVED MOTOR NEURONS

The following protocol is based on 48-well plate format.

1. Prepare the coating vessel before thawing the cells. Please refer to the iXCell's coating protocol.

   Note: *Upon receipt of the frozen cells, it is recommended to thaw the cells and initiate the culture immediately in order to retain the highest cell viability.*

2. To thaw the cells, put the vial in 37°C water bath with gentle agitation for ~1-2 minutes. Keep the cap out of water to minimize the risk of contamination.

3. Pipette the cells into a 15 mL conical tube with 5 mL **Motor Neuron Culture Medium A (Cat# MD-0022A-100ML).**

4. Centrifuge at 600*g* for 5 minutes at room temperature. In the meantime, prepare a mix of Motor Neuron Culture Medium A with **Motor Neuron Culture Medium B (Cat# MD-0022B-20UL; 1 in 1000)** for plating. e.g. for 5 mL Motor Neuron Culture Medium A add 5 µL Motor Neuron Culture Medium B.

   Note: *Please spin down the vial of Motor Neuron Culture Medium B before opening it.*

5. Remove the supernatant and re-suspend the cells in previously made mix of Motor Neuron Culture Medium A and B.

6. Seed the cells on precoated plate at the desired density. Incubate in 37°C CO_2 incubator overnight. Perform ***complete media change*** to remove the Motor Neuron Culture Medium B the next day.

   Note: *We recommend using a pipette when performing complete media change instead of aspirating.*

Note: *We recommend seeding 100-300K cells per cm^2 depending on the application. Cell debris may be observed after cell recovery because the cryopreserved neurons are fragile. Refer to the CoA of each lot to determine the seeding density for your experiment.*

7. Perform **half medium** change every 2-3 days with Motor Neuron Culture Medium A only. Most of the cells should express high levels of HB9 and ISL1 1-2 days after thaw, and express high levels of ChAT and MAP2 7-10 days after thaw.

Note: *Pure motor neurons tend to aggregate and detach from the plates. Change 50% of the medium with extra care to avoid cell loss.*

Co-culture of hiPSC-Derived Motor Neurons with Astrocytes

The following protocol is based on 48-well plate format.

1. Thaw a vial of iPSC-derived motor neuron as described previously, in Motor Neuron Culture Medium A (Cat# MD-0022A-100ML) with Motor Neuron Culture Medium B (Cat# MD-0022B-20UL; 1 in 1000). Seed the cells on precoated plate at the desired density. Incubate in 37ºC CO_2 incubator overnight.
2. Perform ***complete media change*** to remove the Motor Neuron Culture Medium B the next day.

Note: *We recommend using a pipette when performing complete media change instead of aspirating.*

3. Thaw a vial of Astrocyte (Cat# 40HU-008, check iPSC-derived Astrocyte product page for recovery details) and seed the cells on top of the motor neurons at $2x10^4$ cells per well (48-well plate format), in **Motor Neuron Culture Medium A** with 1% FBS. We recommend adding astrocytes 1 to 7 days post plating the iPSC derived motor neurons.
4. Incubate in 37ºC CO_2 incubator overnight.
5. Perform half medium change every 2-3 days (keeping the 1% FBS to support the astrocytes).

iXCells BIOTECHNOLOGIES

References

[1] Taylor, J. P., Brown, R. H. Jr & Cleveland, D. W. Decoding ALS: from genes to mechanism. Nature 539, 197–206 (2016).

[2] Neumann, M. et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 314, 130–133 (2006).

[3] Ling, S. C., Polymenidou, M. & Cleveland, D. W. Converging mechanisms in ALS and FTD: disrupted RNA and protein homeostasis. Neuron 79, 416–438 (2013).

[4] Lagier-Tourenne, C., Polymenidou, M. & Cleveland, D. W. TDP-43 and FUS/TLS: emerging roles in RNA processing and neurodegeneration. Hum. Mol. Genet. 19, R46–R64 (2010).

Disclaimers

This product is for laboratory research purposes only and should not be used in humans. While iXCells Biotechnologies strives to provide accurate and current information on this product sheet, we do not guarantee its accuracy. Citations from scientific literature and patents are for informational purposes and may not be confirmed as accurate.

You are responsible for the safe storage, handling, and usage of this product, and iXCells Biotechnologies is not liable for any damages or injuries resulting from its receipt or use. While we make reasonable efforts to ensure the authenticity and reliability of deposited strains, we are not liable for damages arising from misidentification or misrepresentation of cultures.

Copyright

© iXCells Biotechnologies USA, Inc. 2023. All rights reserved.

Contact Information & Customer Support

iXCells Biotechnologies USA, Inc.

United States
www.ixcellsbiotech.com

Technical Support. supports@ixcellsbiotech.com
Product Sales. orders@ixcellsbiotech.com or contact your local distributor
US Phone. +1 (858) 412 5988