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Product Information

Human Motor Neurons (iPSC-derived, SOD1 mutant, A4V, HOM)

Catalog Number	40HU-101	Cell Number	1.0 million cells/vial (Cryopreserved) 2.0 million cells/vial (Cryopreserved)
Species	*Homo sapiens*	Storage Temperature	Liquid nitrogen

Product Description

Spinal motor neurons (MNs) are a highly specialized type of neurons that reside in the ventral horns and project axons to muscles to control their movement. Degeneration of MNs is implicated in a number of devastating diseases, including spinal muscular atrophy (SMA), amyotrophic lateral sclerosis (ALS), Charcot-Marie-Tooth and poliomyelitis disease. iPSC-derived motor neurons are valuable tools for biochemical analysis, disease modelling and clinical application of these diseases. Mutations (over 150 identified to date) in the SOD1 gene have been linked to familial ALS [1-3]. The most frequent mutations are A4V and H46R. A4V (alanine at codon 4 changed to valine) is the most common ALS-causing mutation in the U.S. population, with approximately 50% of SOD1-ALS patients carrying the A4V mutation [4-6]. Approximately 10 percent of all U.S. familial ALS cases are caused by heterozygous A4V mutations in SOD1.

Human Motor Neurons (iPSC-derived, SOD1 mutant, A4V, HOM) is derived from a genetically modified normal iPSC line carrying the A4V mutation (Figure 1). iXCells™ hiPSC-derived motor neurons express typical markers of motor neurons, e.g. HB9 (MNX1), ISL1, CHAT, with the purity higher than 85%. iXCells™ motor neurons are available in both cryopreserved vials (2 million cells/vial) and fresh plate formats (12-well plate or 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). And after cultured in the medium for 5-7 days, these cells will express high levels of CHAT and MAP2.

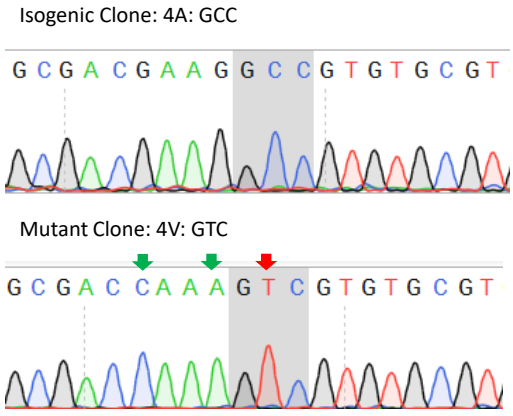

Figure 1. A4V mutation (red arrow) and two silent mutations (green arrows) have been introduced to SOD1 gene using CRISPR/Cas9 based genome editing technology. The targeted site is verified by genomic PCR/Sanger sequencing.

Product Details

Tissue Origin	Human iPSC-derived motor neurons (SOD1, A4V)
Package Size	1.0 million cells/vial; 2.0 million cells/vial
Shipped	Cryopreserved
Media	Motor Neuron Culture Medium Kit (Cat# MD-0022-100ML)

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 37°C 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 37°C CO_2 incubator overnight.
5. Perform half medium change every 2-3 days (keeping the 1% FBS to support the astrocytes).

References

[1] Conwit RA (December 2006). "Preventing familial ALS: a clinical trial may be feasible but is an efficacy trial warranted?". Journal of the Neurological Sciences. 251 (1–2): 1–2.

[2] Al-Chalabi A, Leigh PN (August 2000). "Recent advances in amyotrophic lateral sclerosis". Current Opinion in Neurology. 13 (4): 397–405.

[3] Redler RL, Dokholyan NV (2012-01-01). "The complex molecular biology of amyotrophic lateral sclerosis (ALS)". Progress in Molecular Biology and Translational Science. Progress in Molecular Biology and Translational Science. 107: 215–62.

[4] Rosen DR, Bowling AC, Patterson D, Usdin TB, Sapp P, Mezey E, McKenna-Yasek D, O'Regan J, Rahmani Z, Ferrante RJ (June 1994). "A frequent ala 4 to val superoxide dismutase-1 mutation is associated with a rapidly progressive familial amyotrophic lateral sclerosis". Human Molecular Genetics. 3 (6): 981–7.

[5] Cudkowicz ME, McKenna-Yasek D, Sapp PE, Chin W, Geller B, Hayden DL, Schoenfeld DA, Hosler BA, Horvitz HR, Brown RH (February 1997). "Epidemiology of mutations in superoxide dismutase in amyotrophic lateral sclerosis". Annals of Neurology. 41 (2): 210–21.

[6] Valentine JS, Hart PJ (April 2003). "Misfolded CuZnSOD and amyotrophic lateral sclerosis". Proceedings of the National Academy of Sciences of the United States of America. 100 (7): 3617–22.

Disclaimers

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