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Human Motor Neurons (iPSC-derived)

Catalog Number	40HU-005 40HU-006	**Cell Number**	1.0 million cells/vial (Cryopreserved) 2.0 million cells/vial (Cryopreserved) 4.0 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. Neurodegenerative diseases, such as spinal muscular atrophy (SMA), amyotrophic lateral sclerosis (ALS), Charcot-Marie-Tooth and poliomyelitis disease are a result of the progressive degeneration of motor neurons [1]. Furthermore, motor neurons derived from normal, or patient induced pluripotent stem cells (iPSCs) enable the generation of cell models with features relevant to human physiology, thus making it a valuable tool for biochemical analysis, disease modelling and other broad range of clinical applications [2,3].

iXCells Biotechnologies is proud to provide the world's first fully differentiated and functional human iPSC-derived motor neurons that display typical neuronal morphology and express all key markers of motor neurons, e.g., HB9 (MNX1), ISL1, ChAT (Figure 1) when cultured in the Motor Neuron Culture Medium Kit (Cat# MD-0022-100ML). Moreover, whole cell patch clamp revealed that when cultured in Motor Neuron Activity Medium Kit (Cat# MD-0118-100ML) over 65% of the neurons exhibited mature spiking, and over 35% of the neurons had spontaneous activity at a holding potential of -60 mV (Figure 2) indicating the presence of a highly mature population of neurons.

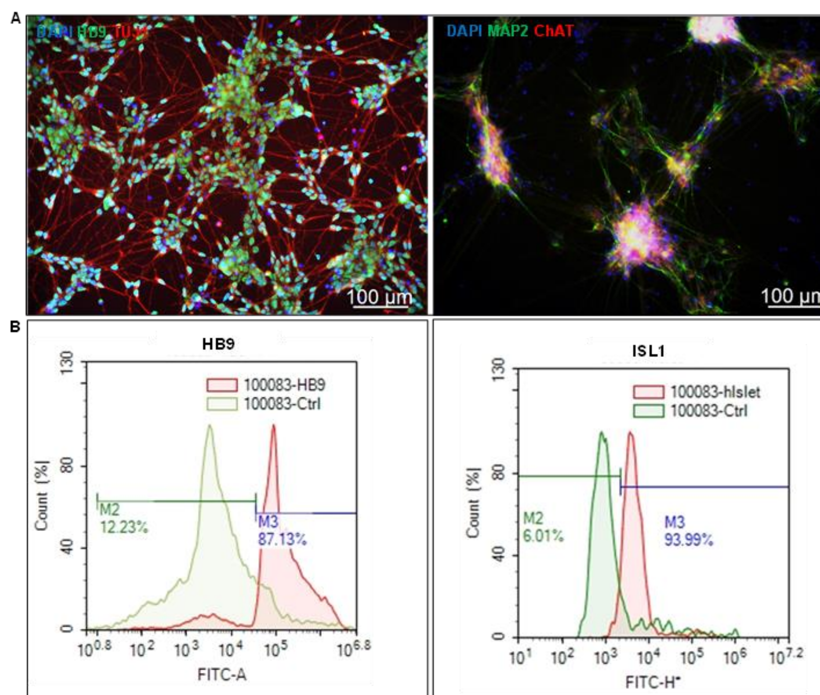

Figure 1 A) Immunofluorescence staining showing HB9 and ChAT positive cells on day 2 and 7 in culture respectively. (**B**) Flow cytometry measurements demonstrate >85% HB9 and >90% ISL1 positive cells on day 1-2.

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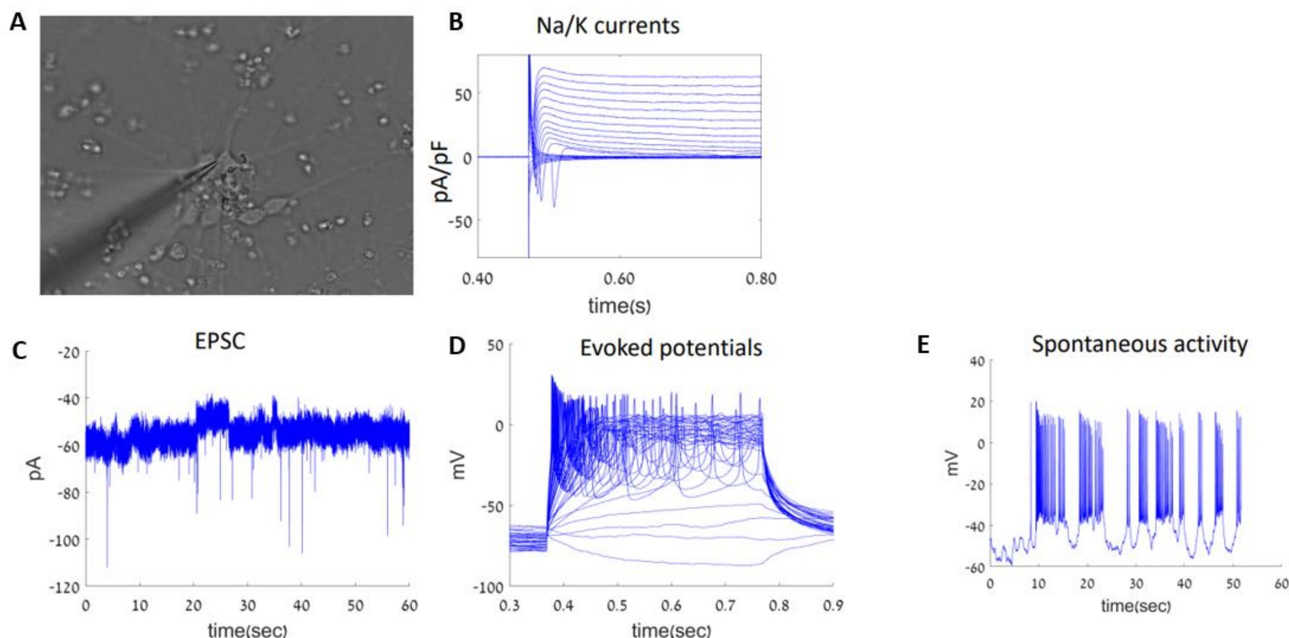

Figure 2 A) An image of the cells during the experiment. The patch pipette is visible on the left. **B)** The Sodium/potassium currents were recorded in voltage clamp mode with test potentials of -100 mV to 90 mV. **C)** Excitatory postsynaptic currents (EPSCs) were recorded in voltage clamp mode while clamping the cell at -60 mV. **D)** Evoked action potentials were recorded in current clamp mode starting with a current injection 12 pA below what is needed to hold the neuron at - 60 mV and with 3pA current steps. **E)** Spontaneous activity was recorded in current clamp mode with a current injection needed for a membrane potential of -45 mV.

Product Details

Tissue Origin	Human iPSC-derived motor neurons (Normal, ALS)
Package Size	1.0 million cells/vial; 2.0 million cells/vial; 4.0 million cells/vial (frozen)
Shipped	Cryopreserved
Storage	Liquid Nitrogen
Media	Motor Neuron Culture Medium Kit (Cat# MD-0022-100ML) Motor Neuron Activity Medium Kit (Cat# MD-0118-100ML)

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). The same procedure applies in the case of Motor Neuron Activity Medium Kit (Cat# MD-0118-100ML).

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 2-8ºC for 1 month and at -20ºC for 12 months.

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iXCells™ Motor Neuron Activity Medium Kit (Cat# MD-0118-100ML) includes:

		Volume
Motor Neuron Activity Medium A	MD-0118A-100ML	100 mL
Motor Neuron Activity Medium B	MD-0118B-20UL	20 μL

The Motor Neuron Activity Medium A and B can be stored at 2-8ºC for 1 month and at -20ºC 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 37ºC 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.*

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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] Brady ST. (1993). “Motor neurons and neurofilaments in sickness and in health. *Cell.* 9;73(1):1-3.

[2] Dolmetsch R, Geschwind DH. (2011) “The human brain in a dish: the promise of iPSC-derived neurons”. *Cell.* 145(6):831-4.

[3] Payne NL, Sylvain A, O'Brien C, Herszfeld D, Sun G, Bernard CC. (2015) “Application of human induced pluripotent stem cells for modeling and treating neurodegenerative diseases.” *Nat Biotechnology.* 25;32(1):212-28.

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