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Product Information

iXCells™ Human iPSC-Derived Motor Neurons

Catalog Number	40HU-005 40HU-006	Cell Number	2.0 million cells/vial (Cryopreserved) >5 million viable cells/plate (fresh, 96-well plate) 8-10 million viable cells/plate (fresh, 12-well plate)
Species	*Homo sapiens*	Storage Temperature	37°C or 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 [1]. iPSC-derived motor neurons are valuable tools for biochemical analysis, disease modelling and clinical application of these diseases [2,3].

iXCells Biotechnologies is proud to provide the world's first commercial human iPSC-derived motor neurons. iXCells™ hiPSC-derived motor neurons express typical markers of motor neurons, e.g. HB9 (MNX1), ISL1, CHAT (Figure 1 and Figure 2), with the purity higher than 85%. Functional validation of iPSC-derived motor neurons has been done with neuromuscular junction formation (Video 1-3).

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 (Figure 1) after thawing in the Motor Neuron Maintenance Medium (Cat# MD-0022). And after cultured in the medium for 5-7 days, these cells will express high levels of CHAT and MAP2 (Figure 2).

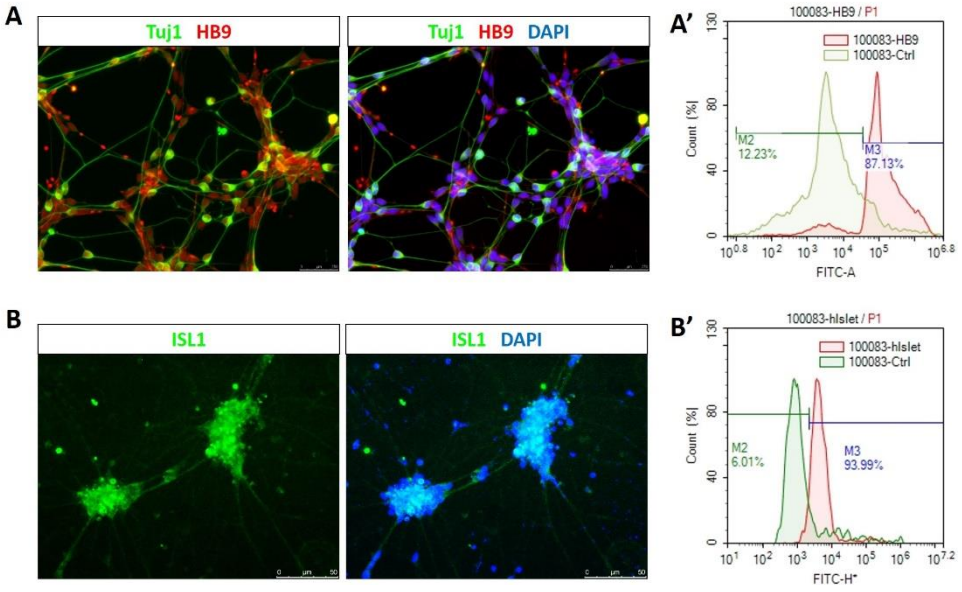

Figure 1. After cultured in Motor Neuron Maintenance Medium on the Matrigel-coated plates for 2 days, more than 85% of the iPSC-derived motor neurons express HB9 (Figure A and A'), and more than 90% of the cells express ISL1 (Figure B and B').

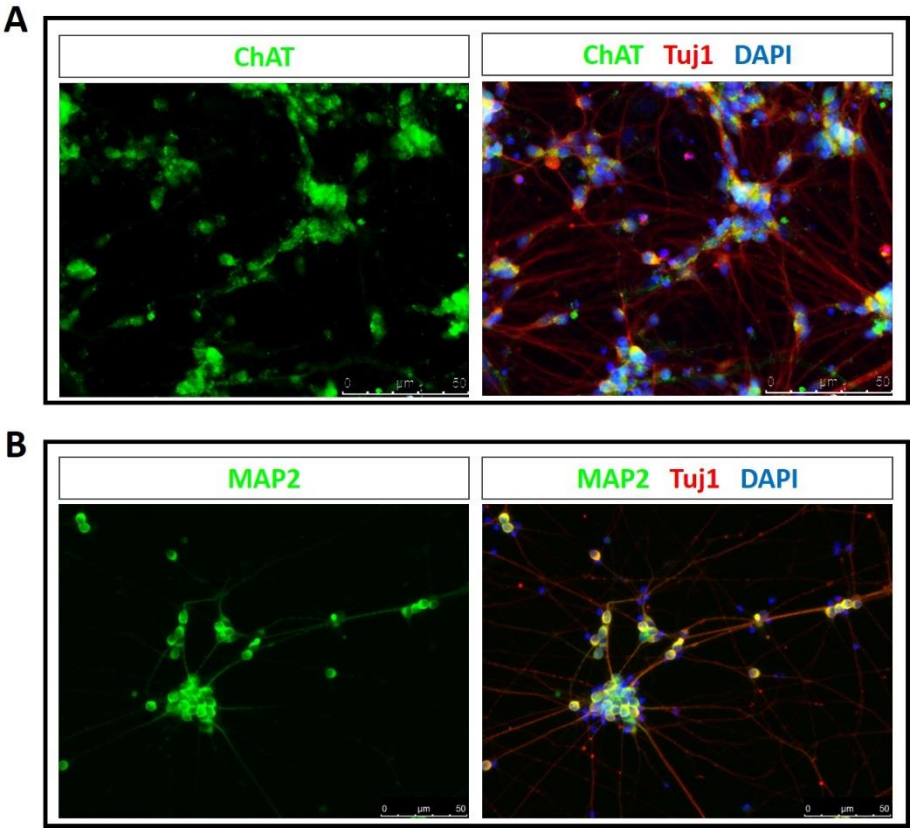

Figure 2. After cultured in Motor Neuron Maintenance Medium on the Matrigel-coated plates for 5-7 days, more than 85% of the iPSC-derived motor neurons express ChAT (Figure A) and MAP2 (Figure B).

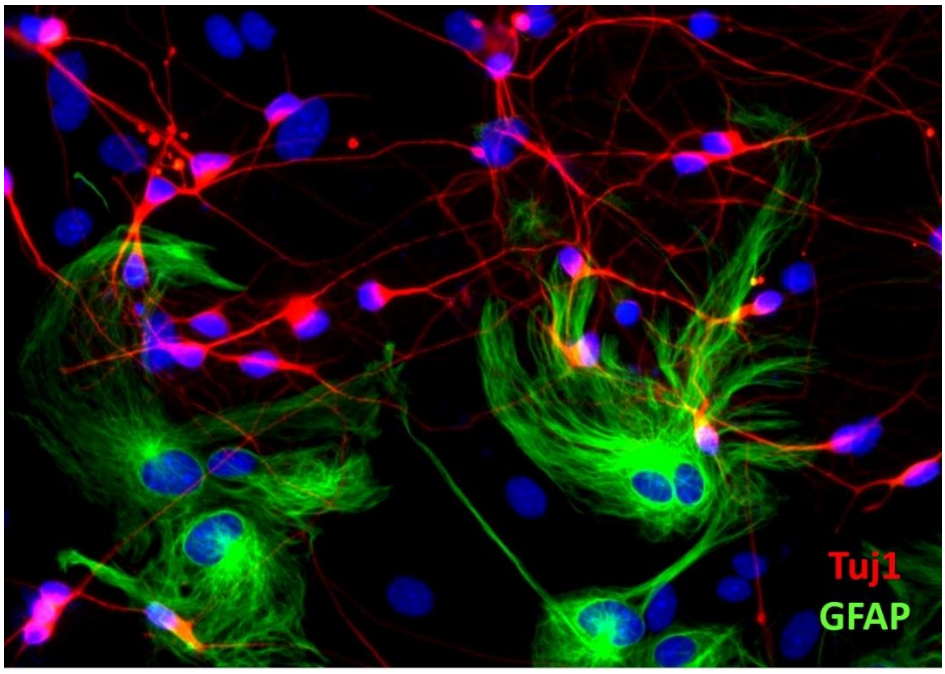

Figure 3. Co-culture of iXCells™ hiPSC-derived motor neurons with mouse astrocytes for 7 days. Motor neurons are stained with Tuj1, and mouse astrocytes are stained with GFAP.

Product Details

Tissue Origin	Human iPSC-derived motor neurons (Normal, ALS)
Package Size	2.0 million cells/vial (frozen); >5 million viable cells/plate (fresh, 96-well plate) 8-10 million viable cells/plate (fresh, 12-well plate)
Shipped	Cryopreserved or Freshly plated (12-well or 96-well plate)
Media	Human Motor Neuron Maintenance Medium (Cat # MD-0022)

Protocols

Mono-culture of hiPSC-Derived Motor Neurons

The following protocol is based on 12-well plate format

1. 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. Prepare Matrigel-coated plates the day before.

   Note: Dilute Matrigel with DMEM/F12 medium into 80 µg/ml. Add 0.5ml diluted Matrigel into each well of a 12-well plates to cover the surface. Coat the plates at room temperature for at least 2 hours before use. The coated plates can be stored at 4°C for a week.
3. To thaw the cells, put the vial in 37°C water bath with gentle agitation for ~1 minute. Keep the cap out of water to minimize the risk of contamination.
4. Pipette the cells into a 15ml conical tube with 5ml **Motor Neuron Maintenance Medium (Cat# MD-0022)**.
5. Centrifuge at 200g for 5 minutes at room temperature.
6. Remove the supernatant and re-suspend the cells in **Motor Neuron Maintenance Medium**.
7. Seed the cells on Matrigel-coated plates at the desired density.

   Note: We recommend to seed 200-500K cells/well (30-70% confluence).
8. Incubate in 37°C CO_2 incubator overnight.
9. Perform half medium change every 2-3 days. 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 12-well plate format.

1. Thaw a vial of Astrocyte and seed the cells on Matrigel coated plates at $1x10^5$ cells/well (12 well plate), in **Astrocyte Growth Medium (Cat# MD-0060)** or DMEM with 10% FBS.
2. The next day, thaw a vial of iPSC-derived motor neuron.
3. Remove the Astrocyte Growth Medium.
4. Seed Motor neuron on top of astrocytes in **Motor Neuron Maintenance Medium (Cat# MD-0022)** at the desired density (We recommend to seed 200-500k cells to one well of a 12-well plate).
5. Incubate in 37°C CO_2 incubator overnight.
6. Perform half medium change every 2-3 days. No significant cell death should be observed within 2-3 months.

Co-culture of hiPSC-Derived Motor Neurons with Myotubes

The following protocol is based on 12-well plate format.

1. Maintain C2C12 mouse myoblasts in **Myoblast Growth Medium (Cat# MD-0064)** or other myoblast culture media.
2. When the cells reach 80-90% confluency, switch the media to **Myoblast Differentiation Medium (Cat# MD-0065)**.
3. Maintain the cells in Myoblast Differentiation Medium. Most of the myoblast cells fuse and form myotubes in 3-4 days.
4. Add 0.5ml 0.05% Trypsin EDTA to one well for 3 minutes at 37°C. Myotubes always come off earlier than myoblasts. Then add 1ml Myoblast Differentiation Medium to the well. Transfer the detached myotubes to a 50ml conical tube.
5. Remove any remaining myoblasts by centrifuge at lower speed (eg, 50g, 1 minute).
6. Seed the myotubes to Matrigel coated plates in Myoblast Differentiation Medium (split ratio 1:1 to 1:2). Incubate the cells for 2-3 days.
7. Thaw a vial of iPSC-derived motor neuron.
8. Seed the motor neurons on top of myotubes in **Motor Neuron Maintenance Medium (Cat# MD-0022)**. It is recommended to seed 200-500K motor neurons in each well of a 12-well plate.
9. Muscle contractions can be observed as early as 5 days after co-culturing with motor neurons.

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.

Disclaimers

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