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# Product Information

## Human Motor Neurons (iPSC-derived, TDP-43 mutation, Q331K, HOM)

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|---|---|---|---|
| Catalog Number | 40HU-104 | Cell Number | 1.0 million cells/vial (Cryopreserved)<br>2.0 million cells/vial (Cryopreserved) |
| Species | *Homo sapiens* | Storage Temperature | Liquid nitrogen |

## 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.

**Human Motor Neurons (iPSC-derived, TDP-43 mutation, Q331K, HOM)** is derived from a genetically modified normal iPSC line carrying the homozygous Q331K mutation in the TDP43 gene (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 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.

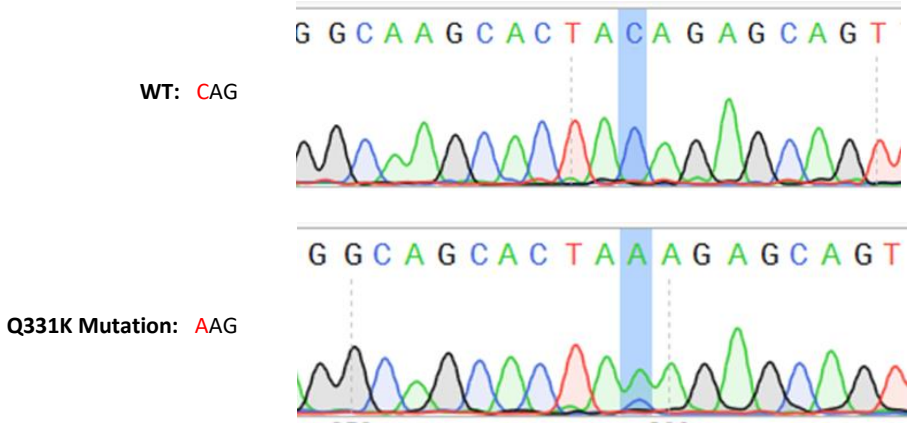


**Figure 1**. Homozygous Q331K mutation (highlighted in grey) has been introduced to TDP-43 gene using CRISPR/Cas9 based genome editing technology. The targeted site is verified by genomic PCR/Sanger sequencing.

## Product Details

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|---|---|
| **Tissue Origin** | Human iPSC-derived motor neurons (TDP-43 mutation, Q331K, homozygous) |
| **Package Size** | 1.0 million cells/vial; 2.0 million cells/vial |
| **Shipped** | Cryopreserved |
| **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 seeding 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 seeding 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] 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