

## Product Information

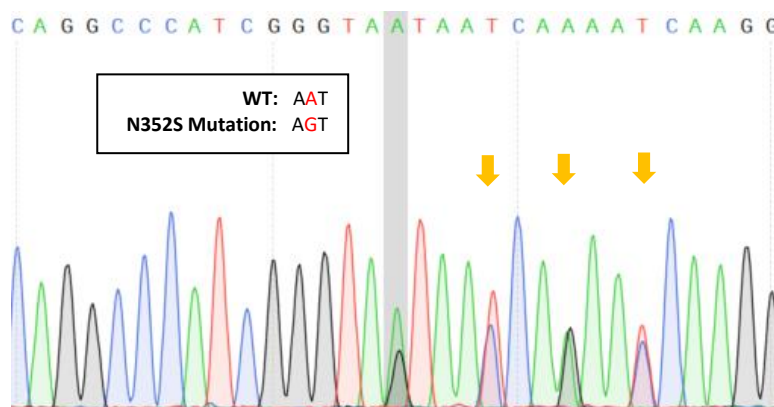
### Human Motor Neurons (iPSC-derived, TDP-43 mutation, N352S, HET)

Catalog Number	40HU-102	Cell Number	1.0 million cells/vial (Cryopreserved) 2.0 million cells/vial (Cryopreserved)
Species	<i>Homo sapiens</i>	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, N352S, HET)** is derived from a genetically modified normal iPSC line carrying the heterozygous N352S 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.** Heterozygous N352S mutation (highlighted in grey) and two silent mutations (yellow arrows) have 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

<b>Tissue Origin</b>	Human iPSC-derived motor neurons (TDP-43 mutation, N352S, heterozygous)
<b>Package Size</b>	1.0 million cells/vial; 2.0 million cells/vial
<b>Shipped</b>	Cryopreserved
<b>Media</b>	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<sub>2</sub> 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  $1 \times 10^5$  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<sub>2</sub> 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

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