



Human Pluripotent Stem Cell Derived-Motor Neurons (HPSC-MN) Catalog #1780

Cell Specification

Motor neurons are specialized efferent neurons of the central nervous system that transmit signals from the brainstem and spinal cord to skeletal muscles, thereby controlling voluntary movement, muscle tone, and motor coordination [1]. During development, motor neuron specification is regulated by defined morphogen gradients and transcriptional programs that establish ventral spinal cord identity and motor neuron fate [2]. Human pluripotent stem cell-derived motor neurons provide a valuable *in vitro* model for studying motor neuron development, neuromuscular connectivity, electrophysiological function, and neurodegenerative diseases such as amyotrophic lateral sclerosis and spinal muscular atrophy [3]. Human Pluripotent Stem Cell-derived Motor Neurons (HPSC-MN) from ScienCell Research Laboratories provide a robust, reliable, and consistent off-the-shelf option for human motor neuron research.

HPSC-MN are cryopreserved and delivered frozen. Each vial contains $> 1 \times 10^6$ cells in 1 ml volume. HPSC-MN are characterized by immunofluorescence with antibodies specific to pan neuronal marker beta tubulin III (TubbIII) and motor neuron markers MNX1. HPSC-MN are negative for mycoplasma, bacteria, yeast, and fungi. HPSC-MN can be cultured under the conditions provided by ScienCell Research Laboratories. *HPSC-MN are not recommended for expansion since the cells do not proliferate in culture.*

Product Content

Cat. #	# of vials	Product	Quantity	Storage
1780	1	HPSC-MN	1 mL	Liquid Nitrogen
5951	1	Neuronal Maintenance Medium (NMM)	100mL	4°C
5932	1	Motor Neuron Supplement (MNS 50X)	2 mL	-20°C

Recommended Medium

It is recommended to use Neuronal Maintenance Medium (NMM, Cat. #5951) for culturing HPSC-MN *in vitro*.

Additional Materials Recommended (Not provided)

Cat. #	Product	Vendor
1720/1860	HPSC-A/HiPSC-A	ScienCell Research Laboratories
3432-005-01	Cultrex Basement Membrane Extract (BME)	R&D Systems
1254	ROCK Inhibitor Y-27632	Tocris Bioscience

Product Use

HPSC-MN are for research use only. They are not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage

Upon receiving, directly and immediately transfer the cells from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are needed for experiments.

Shipping

Dry ice.

References

- [1] Jessell, T. M. (2000). Neuronal specification in the spinal cord: inductive signals and transcriptional codes. *Nature Reviews Genetics*, 1(1), 20–29.
- [2] Shirasaki, R., & Pfaff, S. L. (2002). Transcriptional codes and the control of neuronal identity. *Annual Review of Neuroscience*, 25, 251–281.
- [3] Amoroso, M. W., et al. (2013). Accelerated high-yield generation of limb-innervating motor neurons from human stem cells. *Journal of Neuroscience*, 33(2), 574–586.
- [4] Sances, S., et al. (2016). Modeling ALS with motor neurons derived from human induced pluripotent stem cells. *Nature Neuroscience*, 19(4), 542–553.

Instructions for culturing cells

Caution: Cryopreserved neurons are very delicate. Thaw the vial in a 37°C water bath and return the cells to culture as quickly as possible with minimal handling! Do not centrifuge the cells after thawing as this can damage the cells.

*Note: Experiments should be well organized before thawing HPSC-MN. **HPSC-MN cannot be subcultured or passaged, as the cells do not proliferate.***

Initiating the culture:

Note: ScienCell primary cells must be cultured in a 37°, 5% CO₂ incubator. Cells are only warranted if ScienCell media and reagents are used and the recommended protocols are followed.

1. For functional study of neurons, we recommend plating HPSC-MN on top of HPSC/HiPSC-derived astrocytes (Cat.# 1720, #1860). Please refer to the corresponding product sheet for procedures of plating and culturing astrocytes. Alternatively, HPSC-MN could also be cultured on Matrigel/Cultrex BME coated culture vessels. Prepare Matrigel/Cultrex BME-coated culture vessel according to the manufacturer's instructions, and warm to room temperature before using.
2. Prepare complete Neuronal Maintenance Medium (NMM, Cat.# 5951). Decontaminate the external surfaces of medium bottle and medium supplement tubes with 70% ethanol and transfer them to a sterile field. Aseptically transfer Motor Neuron Supplement (MNS 50X, Cat.# 5932) to the basal medium with a pipette. Rinse the supplement tube with medium to recover the entire volume.

Note: We recommend pre-warming the complete medium to room temperature (Not 37°C), prior to use.

3. Prepare a 50 ml conical tube with complete medium. We recommend adding at least 9 mL of the complete medium to the conical tube. Add ROCK inhibitor Y-27632 to a final concentration of 10 µM.

Note: Applying ROCK inhibitor Y-27632 in the first 24 hours improves the cell viability.

4. Leave the tube in the sterile field and proceed to thaw the cryopreserved cells.
5. Place the frozen vial in a 37°C water bath. Hold and rotate the vial gently until the contents completely thaw. Promptly remove the vial from the water bath, wipe it down with 70% ethanol, and transfer it to the sterile field. Carefully remove the cap without touching the interior threads.
6. Gently dispense the contents of the vial into the conical tube containing the complete medium.

Note: Centrifugation of cells after thawing is not recommended since these actions are more harmful to the cells than the effect of residual DMSO in the culture.

7. **Gently invert** the tube **1-2 times** to obtain a homogenous cell suspension in the medium. Resuspending neurons by pipetting up and down is NOT recommended.

8. Aspirate medium/coating solution in the prepared culture vessel. Gently aliquot the cell suspension onto prepared culture vessels. We recommend plating the cells at 50,000 cell/cm² if plated on astrocyte supporting layer, or 100,000 cell/cm² if plated on Matrigel/Cultrex coated surfaces.
9. Replace the lid of the culture plate and gently rock the plate to distribute the cells evenly.
10. Return the culture vessel to the incubator.
11. For best results, do not disturb the culture for at least 16 hours after the culture has been initiated. Refresh culture medium the next day to remove residual DMSO and unattached cells, then every other day thereafter.
12. Cells may take a few days to grow neurites in culture.

It is not recommended that neurons be subcultured beyond their initial plating as these cells do not proliferate.