Rev. 0

Human Pluripotent Stem Cell Derived-Sensory Neurons (HPSC-SN)

Catalog #1790

Cell Specification

Sensory neurons are specialized neurons of the peripheral nervous system that detect and transmit sensory information, including pain, temperature, touch, proprioception, and chemical stimuli, from peripheral tissues to the central nervous system [1]. Peripheral sensory neurons are primarily located in dorsal root ganglia and cranial sensory ganglia and include diverse subtypes such as nociceptors, mechanoreceptors, and proprioceptors [2]. Human pluripotent stem cell-derived sensory neurons provide a valuable *in vitro* model for studying peripheral nervous system development, pain signaling, neurotoxicity, peripheral neuropathy, and sensory neuron-related disease mechanisms [3]. Human Pluripotent Stem Cell-derived Sensory Neurons (HPSC-SN) from ScienCell Research Laboratories provide a robust, reliable, and consistent off-the-shelf option for human sensory neuron research.

HPSC-SN are cryopreserved and delivered frozen. Each vial contains > 1×10^6 cells in 1 ml volume. HPSC-SN are characterized by immunofluorescence with antibodies specific to pan neuronal marker beta tubulin III (TubbIII) and sensory neuron markers BRN3A. HPSC-SN are negative for mycoplasma, bacteria, yeast, and fungi. HPSC-SN can be cultured under the conditions provided by ScienCell Research Laboratories. *HPSC-SN are not recommended for expansion since the cells do not proliferate in culture.*

Product Content

Cat. #	# of vials	Product	Quantity	Storage
1790	1	HPSC-SN	1mL	Liquid Nitrogen
5951	1	Neuronal Maintenance Medium (NMM)	100mL	4°C
5942	1	Sensory Neuron Supplement (SNS 50X)	2mL	-20°C

Recommended Medium

It is recommended to use Neuronal Maintenance Medium (NMM, Cat. #5951) for culturing HPSC-SN i*n 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-SN 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] Basbaum, A. I., Bautista, D. M., Scherrer, G., & Julius, D. (2009). Cellular and molecular mechanisms of pain. Cell, 139(2), 267–284.

[2] Marmigère, F., & Ernfors, P. (2007). Specification and connectivity of neuronal subtypes in the sensory lineage. Nature Reviews Neuroscience, 8(2), 114–127.

[3] Chambers, S. M., et al. (2012). Combined small-molecule inhibition accelerates developmental timing and converts human pluripotent stem cells into nociceptors. Nature Biotechnology, 30(7), 715–720.

[4] Young, G. T., et al. (2014). Characterizing human stem cell-derived sensory neurons at the single-cell level reveals their ion channel expression and utility in pain research. Molecular Therapy, 22(8), 1530–1543.

Instructions for culturing cells

Caution: Cryopreserved neurons are very delicate. Thaw the vial in a 37ºC 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-SN.* ***HPSC-SN 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% CO_2 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-SN 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-SN 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 Sensory Neuron Supplement (SNS 50X, Cat.# 5942) 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 37ºC 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 20,000 cell/cm^2 if plated on astrocyte supporting layer, or 50,000 cell/cm^2 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.