Rev. 0

Human Pluripotent Stem Cell Derived-Cortical Neurons (HPSC-CN)

Catalog #1750

Cell Specification

Cortical neurons are the principal excitatory projection neurons of the cerebral cortex and are central to information processing across sensory perception, cognition, and voluntary motor control through organized laminar microcircuits and long-range connectivity [1]. During development and early life, cortical circuits undergo experience-dependent refinement ("critical period" plasticity), a process that strongly shapes synaptic connectivity and functional network maturation [2]. Disruption of cortical development or circuit function has been implicated in numerous neurological and neuropsychiatric disorders, and *in vitro* human cortical neuron models provide high value for mechanistic discovery and drug screening [3]. Human PSC-based corticogenesis platforms have demonstrated robust generation of cortical projection neuron subtypes in a temporal sequence and the formation of functional excitatory synapses and networks, supporting their use as scalable, standardized human model systems [3,4]. Human Pluripotent Stem Cell-derived Cortical Neurons (HPSC-CN) from ScienCell Research Laboratories provide a robust, reliable, and consistent off-the-shelf option for human cortical neurons.

HPSC-CN are cryopreserved and delivered frozen. Each vial contains > 1×10^6 cells in 1 ml volume. HPSC-CN are characterized by immunofluorescence with antibodies specific to pan neuronal marker beta tubulin III (Tubb3) and cortical identity marker TBR1. HPSC-CN are negative for mycoplasma, bacteria, yeast, and fungi. HPSC-CN can be further cultured under the conditions provided by ScienCell Research Laboratories. *HPSC-CN are not recommended for expansion since the cells do not proliferate in culture*.

Product Content

Cat. #	# of vials	Product	Quantity	Storage
1750	1	HPSC-CN	1mL	Liquid Nitrogen
5951	1	Neuronal Maintenance Medium (NMM)	100mL	4°C
5902	1	Neuron Maintenance Supplement (NMS 50X)	2mL	-20°C

Recommended Medium

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

Additional Materials Recommended (Not provided)

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

Product Use
HPSC-CN 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] Douglas, R. J., & Martin, K. A. C. (2004). Neuronal circuits of the neocortex. Annual Review of Neuroscience, 27, 419–451.
[2] Hensch, T. K. (2005). Critical period plasticity in local cortical circuits. Nature Reviews Neuroscience, 6(11), 877–888.
[3] Shi, Y., Kirwan, P., Smith, J., Robinson, H. P. C., & Livesey, F. J. (2012). Human cerebral cortex development from pluripotent stem cells to functional excitatory synapses. Nature Neuroscience, 15(3), 477–486.
[4] Espuny-Camacho, I., Michelsen, K. A., Gall, D., Linaro, D., Hasche, A., Bonnefont, J., … Vanderhaeghen, P. (2013). Pyramidal neurons derived from human pluripotent stem cells integrate efficiently into mouse brain circuits in vivo. Neuron, 77(3), 440–456.

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-CN.* ***HPSC-CN cannot be subcultured or passaged, as the cells do not proliferate.***

Initiating the culture:

Note: ScienCell primary cells must be cultured in a 37°C, 5% CO_2 incubator. Cells are only warranted if ScienCell media and reagents are used and the recommended protocols are followed.

1. HPSC-CN could 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. Alternatively, for functional studies of neurons, we recommend plating HPSC-CN on top of HPSC/HiPSC-derived astrocytes (Cat# 1720, and #1860). Please refer to the corresponding product sheet for procedures of plating and culturing astrocytes.
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 Neuron Maintenance Supplement (NMS 50X) 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 are completely thawed. 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 from the prepared culture vessel. Gently aliquot the cell suspension onto prepared culture vessels. We recommend plating the cells at 20,000 cells/cm^2 if plated on astrocyte supporting layer, or 50,000 cells/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.

Caution: Handling human-derived products is potentially biohazardous. Always wear gloves and safety glasses when working with these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].

[1] Grizzle WE, Polt S. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." *J Tissue Cult Methods.* 11: 191-9.