



## Human Pluripotent Stem Cell Derived-Glutamatergic Neurons (HPSC-GlutN) Catalog #1760

### Cell Specification

Glutamatergic neurons form the major excitatory neuronal population in the mammalian central nervous system and are foundational to synaptic transmission and circuit computation in the cortex and hippocampus [1]. Vesicular glutamate transporters (VGLUTs) define glutamatergic identity at excitatory synapses and distinguish functionally and anatomically distinct excitatory synapse classes and release properties [1,2]. Dysregulated glutamatergic signaling and glutamate-mediated excitotoxicity are strongly implicated in acute CNS injury (e.g., ischemia) and multiple neurodegenerative and neurological disease processes, making human glutamatergic neuron models valuable for both mechanistic studies and therapeutic screening [3]. Human pluripotent stem cell systems can robustly generate functional excitatory synapses and network activity, supporting scalable *in vitro* modeling with human excitatory neurons [4]. Human Pluripotent Stem Cell-derived Glutamatergic Neurons (HPSC-GlutN) from ScienCell Research Laboratories provide a robust, reliable, and consistent off-the-shelf option for human glutamatergic neurons.

HPSC-GlutN are cryopreserved and delivered frozen. Each vial contains  $> 1 \times 10^6$  cells in 1 mL volume. HPSC-GlutN are characterized by immunofluorescence with antibodies specific to pan neuronal marker beta tubulin III (Tubb3) and glutamatergic marker vGLUT1. HPSC-GlutN are negative for mycoplasma, bacteria, yeast, and fungi. HPSC-GlutN can be further cultured under the conditions provided by ScienCell Research Laboratories. *HPSC-GlutN are not recommended for expansion since the cells do not proliferate in culture.*

### Product Content

Cat. #	# of vials	Product	Quantity	Storage
1760	1	HPSC-GlutN	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-GlutN *in 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

Rev. 0

### **Product Use**

HPSC-GlutN 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] Fremeau, R. T., Jr., Troyer, M. D., Pahner, I., Nygaard, G. O., Tran, C. H., Reimer, R. J., ... Edwards, R. H. (2001). The expression of vesicular glutamate transporters defines two classes of excitatory synapse. *Neuron*, 31(2), 247–260.
- [2] Fremeau, R. T., Jr., Kam, K., Qureshi, T., Johnson, J., Copenhagen, D. R., Storm-Mathisen, J., ... Edwards, R. H. (2004). Vesicular glutamate transporters 1 and 2 target to functionally distinct synaptic release sites. *Science*, 304(5678), 1815–1819.
- [3] Choi, D. W. (1988). Glutamate neurotoxicity and diseases of the nervous system. *Neuron*, 1(8), 623–634.
- [4] 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.

## Instructions for culturing cells

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**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-GlutN. **HPSC-GlutN 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<sub>2</sub> incubator. Cells are only warranted if ScienCell media and reagents are used and the recommended protocols are followed.

1. HPSC-GlutN 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-GlutN 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 37°C 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<sup>2</sup> if plated on astrocyte supporting layer, or 50,000 cells/cm<sup>2</sup> 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.