Rev. 0

Human Pluripotent Stem Cell-derived Cortical Neuron Modeling Kit (HPSC-CNMK)

Catalog #1758

Cell Specification

Cortical neurons are the principal excitatory neurons of the cerebral cortex and play central roles in sensory processing, cognition, and motor control through organized cortical circuits [1,2]. Disruption of cortical neuron development or function is implicated in many neurological and neuropsychiatric disorders, making human cortical neuron models valuable tools for disease modeling and drug screening. Human PSC-based corticogenesis platforms have demonstrated robust generation of cortical projection neuron subtypes in a temporal sequence and formation of functional excitatory synapses and networks, supporting their use as scalable, standardized human model systems [3,4]. Astrocytes provide essential trophic, metabolic, and synaptic support to neurons and actively regulate neuronal maturation and network activity. In cortical culture systems, neuron–astrocyte co-culture is particularly useful for modeling disease mechanisms involving synaptic dysfunction, altered excitability, and neuroinflammatory responses [5,6].

Human Pluripotent Stem Cell-derived Cortical Neuron Modeling Kit (HPSC-CNMK) from ScienCell Research Laboratories is designed as an off-the-shelf co-culture system for establishing human cortical neuron/astrocyte assays *in vitro*. The kit combines Human Pluripotent Stem Cell-derived Cortical Neurons (HPSC-CN), Human Pluripotent Stem Cell-derived Astrocytes (HPSC-A), and the corresponding medium components required to establish an astrocyte supporting layer and initiate cortical neuron co-culture.

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 not recommended for expansion since the cells do not proliferate in culture. HPSC-A are cryopreserved and delivered frozen. Each vial contains > 1×10^6 cells in 1 mL volume. HPSC-A are characterized by immunofluorescence with antibodies specific to GFAP, S100β, and Vimentin. HPSC-A can be further cultured under the conditions provided by ScienCell Research Laboratories. Both cell types are negative for mycoplasma, bacteria, yeast, and fungi.

Product Content

Cat. #	# of vials	Product	Quantity	Storage
1720	1	HPSC-A (H9-A)	1mL	Liquid Nitrogen
1750	1	HPSC-CN	1mL	Liquid Nitrogen
5951	1	Neuronal Maintenance Medium (NMM)	100 mL	4°C
5902	1	Neuron Maintenance Supplement (NMS 50X)	2 mL	-20°C
1801-100	1	Astrocyte Medium (AM)	100mL	4°C
1852-STEM	1	Astrocyte Growth Supplement (100X)	1mL	-20°C
0002-STEM	1	Fetal Bovine Serum	2mL	-20°C

Recommended Medium

For establishment and expansion of the astrocyte supporting layer, it is recommended to use Astrocyte Medium (AM, Cat. #1801-100) supplemented with Astrocyte Growth Supplement (Cat. #1852-STEM) and Fetal Bovine Serum (Cat. #0002-STEM).

For initiation and maintenance of cortical neuron/astrocyte co-culture, it is recommended to use Neuronal Maintenance Medium (NMM, Cat. #5951) supplemented with Neuron Maintenance Supplement (NMS 50X, Cat. #5902).

Additional Materials Recommended (Not provided)

Cat. #	Product	Vendor
0413	Poly-L-lysine (10 mg/mL)	ScienCell Research Laboratories
5813	CellEase	ScienCell Research Laboratories
0303	DPBS without Ca^{2+} and Mg^{2+}	ScienCell Research Laboratories
1254	ROCK Inhibitor Y-27632 (optional)	Tocris Bioscience

Product Use

HPSC-CNMK is for research use only. It is not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage

Upon receiving, directly and immediately transfer the cryopreserved cell vials from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are needed for experiments. Store basal media at 4°C and supplements/serum at -20°C.

Shipping

Dry ice.

References

[1] Douglas, RJ., & Martin, KA. (2004). Neuronal circuits of the neocortex. *Annual Review of Neuroscience, 27*, 419–451.
[2] Hensch, TK. (2005). Critical period plasticity in local cortical circuits. *Nature Reviews Neuroscience, 6*(11), 877–888.
[3] Shi, Y., Kirwan, P., Smith, J., Robinson, HP., & Livesey, FJ. (2012). Human cerebral cortex development from pluripotent stem cells to functional excitatory synapses. *Nature Neuroscience, 15*(3), 477–486.
[4] Espuny-Camacho, I., Michelsen, KA., 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.
[5] Chung, WS., Allen, NJ., & Eroglu, C. (2015). Astrocytes control synapse formation, function, and elimination. *Cold Spring Harbor Perspectives in Biology, 7*(9), a020370.
[6] Chen, Y., & Swanson, RA. (2003). Astrocytes and brain injury. *Journal of Cerebral Blood Flow & Metabolism, 23*(2), 137–149.

Instructions for culturing cells

Caution: Cryopreserved cells 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.

Initiating the astrocyte supporting layer:

Note: ScienCell human pluripotent stem cell-derived cells must be cultured in a 37ºC, 5% CO_2 incubator. Cells are only warranted if ScienCell media and reagents are used and the recommended protocols are followed.

1. Prepare a poly-L-lysine-coated culture vessel (2 µg/cm^2, T-25 flask is recommended). To obtain a 2 µg/cm^2 poly-L-lysine-coated culture vessel, add 5 ml of sterile water to a T-25 flask and then add 5 µl of poly-L-lysine stock solution (10 mg/ml, Cat. #0413). Leave the vessel in a 37ºC incubator overnight (or for a minimum of one hour).
2. Prepare complete Astrocyte Medium. Decontaminate the external surfaces of medium bottle and medium supplement tubes with 70% ethanol and transfer them to a sterile field. Aseptically transfer supplement to the basal medium with a pipette. Rinse the supplement tube with medium to recover the entire volume.

   Note*: Applying ROCK inhibitor Y-27632 in the first 24 hours can improve the cell viability.*
3. Rinse the poly-L-lysine-coated vessel twice with sterile water and then add 5 ml of sterile water. Leave the vessel in the sterile field and proceed to thaw the cryopreserved cells.
4. Take one vial of HPSC-A out of the liquid nitrogen. Immediately transfer the vial into a 37ºC water bath and gently swirl it or until most of contents are thawed and only a small piece of ice remains.

   Note: *The viability of the cells will decrease if the vial contents are completely thawed.*
5. Immediately remove the vial from the water bath, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads. Using a 5 mL pipette, gently resuspend the contents of the vial homogenously. Transfer cell suspension to 15 mL tube containing 10 mL of Astrocyte Medium. Wash the emptied vial with 1 mL medium and combine with the cell suspension in the tube.

   Note: *Centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of residual DMSO in the culture. It is also important to minimize the time for step 3-4.*
6. Bring the poly-L-lysine-coated culture vessel to the hood and aspirate the water. Gently mix cells to get a homogenous suspension with 5 mL pipette and seed the cells into T-25 flask or equivalent.
7. Return the culture vessel to the incubator at 37ºC 5% CO_2.
8. For best results, do not disturb the culture for 16 hours after the culture has been initiated. Change the medium the next day to remove unattached cells and residual DMSO.

Maintaining the astrocyte culture:

1. Refresh supplemented culture medium the next morning after establishing a culture from cryopreserved cells.
2. Change the medium every three days, until the culture is approximately 70% confluent.
3. Once the culture reaches 70% confluency, change medium every other day until the culture is approximately 90% confluent.

Subculturing:

1. Subculture when the culture reaches 90-95% confluency.
2. Prepare poly-L-lysine-coated culture vessels at least 1 hour before.
3. Warm Astrocyte Medium, CellEase (Cat. #5813) and DPBS (Ca^{++}- and Mg^{++}-free, Cat. #0303) to **room temperature**. We do not recommend warming reagents and medium in a 37ºC water bath prior to use.
4. Rinse the cells with DPBS.
5. Dissociate the astrocytes with CellEase protocol.

   Note: *We recommend using CellEase which is optimized to minimize cell damage due to over digestion.*
6. Gently resuspend cells in Astrocyte Medium.
7. Count and plate cells in a new culture vessel at desired cell density for assay. A seeding density of 10,000-20,000 cells/cm^2 is recommended for expansion.

   Note: *We do not recommend cryopreservation of HPSC-A cells by the end user. Refreezing cells may damage them and affect cell performance. ScienCell does not guarantee HPSC-A cells cryopreserved by the end user.*

Initiating cortical neuron / astrocyte co-culture

Note: HPSC-CN are not recommended for expansion and should not be subcultured after initial plating.

1. For co-culture applications, establish a healthy astrocyte supporting layer at 70% confluence prior to plating HPSC-CN.
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 Dopaminergic Neuron 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 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. Plate HPSC-CN onto the astrocyte supporting layer at a recommended seeding density of 20,000 cells/cm².
9. Return the culture vessel to the incubator and do not disturb the culture for at least 16 hours after plating.
10. Refresh the culture medium the next day to remove residual DMSO and unattached cells, then change medium every other day thereafter.
11. Cells may take a few days to extend neurites in culture.
12. Culture the cells to desired maturity for downstream analysis.