Human iPSC-Derived Forebrain Neuron Precursor Cells

Catalog #200-0770 1 x 10^6 cells

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Product Description

Human iPSC-Derived Forebrain Neuron Precursor Cells were manufactured from human induced pluripotent stem cell (iPSC) line, Healthy Control Human iPSC Line, Female, SCTi003-A (Catalog #200-0511), using STEMdiff™ SMADi Neural Induction Kit (Catalog #08581) and STEMdiff™ Forebrain Neuron Differentiation Kit (Catalog #08600). Human iPSC-Derived Forebrain Neuron Precursor Cells should be thawed and matured using STEMdiff™ Forebrain Neuron Maturation Kit (Catalog #08605), which will result in a mixed population of highly pure forebrain-type neurons (≥ 80% class III β-tubulin-positive neurons; < 15% S100B-positive astrocytes). These neurons are functional and can be maintained long-term in culture. They are versatile tools for modeling human neurological development and disease, drug screening, toxicity testing, and cell therapy validation.

Cells were obtained using Institutional Review Board (IRB)-approved consent forms and protocols.

Stability and Storage

Cells are frozen in a cryopreservation medium containing dimethyl sulfoxide (DMSO). Product stable at -135°C or colder for 12 months from date of receipt. Short-term storage of cells (< 1 month) at -80°C is acceptable, but should be minimized to ensure maximum stability. Thawed samples must be used immediately.

Precautions

Cell Screening: iPSC master cell banks are screened for AAV2, BK virus, Epstein-Barr Virus, Hepatitis A, Hepatitis B, Hepatitis C, Herpes Simplex 1 and 2, Herpes Virus Type 6, Herpes Virus Type 7, Herpes Virus Type 8, HIV-1, HIV-2, HPV-16, HPV-18, Human Adenovirus, Human Cytomegalovirus, Human Foamy Virus, Human T-Lymphotropic Virus, John Cunningham Virus, LCMV, Parvovirus B19, Sarbecovirus (SARS Virus), Seoul Virus, Corynebacterium Bovis, and Mycoplasma (Human Comprehensive CLEAR Panel) by PCR. As testing cannot completely guarantee that the donor was virus-free, THIS PRODUCT SHOULD BE TREATED AS POTENTIALLY INFECTIOUS and only used following appropriate handling precautions such as those described in biological safety level 2.

Storage of frozen cell products in the vapor phase of a liquid nitrogen storage tank is recommended. Storage in the liquid phase can result in cross-contamination if the vial breaks or is not sealed properly. Storage in the liquid phase also increases the potential for liquid nitrogen to penetrate the vial and cause it to explode when removed from storage. Use of a face shield is required as a safety precaution when transferring cells from one container to another. When handling this product, do not use sharps such as needles and syringes.

STEMCELL cannot guarantee the biological function or any other properties associated with performance of cells in a researcher's individual assay or culture systems. STEMCELL assures the cells will meet the specifications only when assessed immediately after thawing by our test methods.

FOR IN VITRO RESEARCH USE ONLY. NOT APPROVED FOR DIAGNOSTIC, THERAPEUTIC, OR CLINICAL APPLICATIONS.

NOT APPROVED FOR HUMAN OR VETERINARY USE IN VIVO.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
D-PBS (Without Ca++ and Mg++)	37350
DMEM/F-12 with 15 mM HEPES	36254
Laminin	Sigma L2020
Poly-L-ornithine (PLO) Solution	Sigma P4957
Serological pipettes, 2 mL	e.g. 38002
STEMdiff™ Forebrain Neuron Maturation Kit	08605
Trypan Blue	07050
Y-27632 (Dihydrochloride; optional)	72302

Preparation of Reagents and Materials

A. COATING CULTUREWARE WITH PLO AND LAMININ

Using sterile technique, coat cultureware with poly-L-ornithine (PLO) and laminin prior to initial plating and expansion of Human iPSC-Derived Forebrain Neuron Precursor Cells.

1. Dilute PLO solution in sterile Dulbecco's phosphate-buffered saline (D-PBS) to reach a final concentration of 15 µg/mL PLO working solution.

   For example, add 15 mL of PLO solution to 85 mL of D-PBS.
2. Gently mix PLO working solution. Do not vortex.
3. Add PLO working solution to the cultureware to cover the entire growth surface. Refer to Table 1 for recommended coating volumes.
4. Gently tilt the cultureware to spread PLO working solution evenly across the surface and incubate at 37°C and 5% CO_2 for 2 hours or seal the cultureware (e.g. with Parafilm®) and incubate overnight at 2 - 8°C. Do not let PLO working solution evaporate. Allow stored coated cultureware to come to room temperature (15 - 25°C) before proceeding to step 5.
5. Prepare a 5 µg/mL laminin working solution in DMEM/F-12 with 15 mM HEPES. Refer to Table 1 for recommended coating volumes.
6. Rinse PLO-coated cultureware twice using sterile D-PBS by pipetting D-PBS gently toward the corner of the cultureware to avoid removing PLO coating.
7. Aspirate PBS from the cultureware and add laminin working solution (prepared in step 5) to cover the entire growth surface. Refer to Table 1 for recommended coating volumes.
8. Incubate at 37°C and 5% CO_2 for 2 hours or seal the cultureware (e.g. with Parafilm®) and incubate overnight at 2 - 8°C. Do not let laminin working solution evaporate. Warm the coated cultureware to 37°C before use.

   NOTE: Using freshly coated cultureware is recommended. However, if not used immediately, coated cultureware can be stored at 2 - 8°C in laminin working solution for up to 4 days.
9. Aspirate laminin working solution immediately prior to seeding cells. Do not let the surface dry.

   NOTE: It is not necessary to wash cultureware after removing laminin working solution.

Table 1: Recommended Volumes of PLO and Laminin Working Solutions for Coating Cultureware

CULTUREWARE	APPROXIMATE SURFACE AREA	VOLUME OF PLO WORKING SOLUTION	VOLUME OF LAMININ WORKING SOLUTION
96-well plate	0.33 cm^2/well	50 µL/well	50 µL/well
4- or 24-well plate	2 cm^2/well	250 µL/well	250 µL/well
6-well plate	10 cm^2/well	1.5 mL/well	1.5 mL/well
35 mm dish	10 cm^2	1.5 mL	1.5 mL
60 mm dish	20 cm^2	2.5 mL	2.5 mL

B. PREPARATION OF STEMDIFF™ FOREBRAIN NEURON MATURATION MEDIUM

Use sterile technique to prepare STEMdiff™ Forebrain Neuron Maturation Medium. The following example is for preparing 125 mL of medium. If preparing other volumes, adjust accordingly.

1. Thaw STEMdiff™ Forebrain Neuron Maturation Supplement at room temperature (15 - 25°C) or at 2 - 8°C overnight. Mix thoroughly.
   NOTE: If not used immediately, aliquot supplement and store at -20°C. Do not exceed the shelf life of the supplement. After thawing aliquots, use immediately. Do not re-freeze.
2. Add 25 mL of STEMdiff™ Forebrain Neuron Maturation Supplement to 100 mL of BrainPhys™ Neuronal Medium. Mix thoroughly.
   NOTE: If not used immediately, store STEMdiff™ Forebrain Neuron Maturation Medium at 2 - 8°C for up to 4 weeks. Warm medium to 37°C before use. Protect from light.

Directions for Use

IMPORTANT: To confirm the number of cells provided, a viable cell count must be done immediately after thawing (before washing). Work quickly once the cells have been thawed to ensure high viability and recovery. Use sterile technique when processing thawed cells.

NOTE: Use tissue culture-treated cultureware. Using sterile technique, coat cultureware with PLO and laminin prior to initial plating of Human iPSC-Derived Forebrain Neuron Precursor Cells.

THAWING AND PLATING HUMAN iPSC-DERIVED FOREBRAIN NEURON PRECURSOR CELLS

Generally, 1 x 10^6 of Human iPSC-Derived Forebrain Neuron Precursor Cells is enough to seed eight wells of a 24-well plate at the higher range of recommended seeding densities or twelve wells of a 24-well plate at the lower range of recommended seeding densities.

NOTE: The following instructions are for seeding cells into coated 24-well plates. If using other cultureware, adjust volumes accordingly.

1. Coat the desired number of wells of a 24-well tissue culture-treated plate with PLO and laminin (see Preparation section A).
2. Warm DMEM/F-12 with 15 mM HEPES and STEMdiff™ Forebrain Neuron Maturation Medium (see Preparation section B) to 37°C before starting the protocol to ensure that the thawing procedure is done as quickly as possible.
3. Add 10 mL of warm DMEM/F-12 with 15 mM HEPES to a 15 mL conical tube.
4. Wipe the outside of the vial of cells with 70% ethanol or isopropanol.
5. In a biosafety hood, twist the cap a quarter-turn to relieve internal pressure and then retighten.
6. Quickly thaw cells in a 37°C water bath by gently shaking the cryovial continuously until only a small frozen cell pellet remains. Do not vortex cells.
   NOTE: ThawSTAR® CFT2 Automated Thawing System (Catalog #100-0650) may be used to quickly and efficiently thaw cells. For complete instructions, refer to the Product Information sheet (Document #10000010334), available at www.stemcell.com, or contact us to request a copy.
7. Remove the cryovial from the water bath and wipe it with 70% ethanol or isopropanol.
   NOTE: It is important to work quickly in the following steps to ensure high cell viability and recovery.
8. In a biosafety hood, measure and record the total volume of the cell suspension using a 2 mL serological pipette.
9. Remove a 20 µL aliquot of cells for pre-wash counting. If using Trypan Blue to assess viability, we suggest adding a minimum of 20 µL of medium and recording the volume of medium added.
10. Transfer cells from the cryovial to the tube containing 10 mL of warm DMEM/F-12 with 15 mM HEPES. Mix gently.
11. Rinse the vial with 1 mL of warm DMEM/F-12 with 15 mM HEPES and add it dropwise to the cells, while gently swirling the 15 mL tube.
12. Centrifuge cells at 300 x g for 5 minutes at room temperature (15 - 25°C).
13. Aspirate medium, leaving the cell pellet intact.
14. Gently resuspend the cell pellet in 1 mL of STEMdiff™ Forebrain Neuron Maturation Medium.
15. Remove a 20 µL aliquot of cells for post-wash counting. If using Trypan Blue to assess viability, we suggest adding a minimum of 20 µL of medium and recording the volume of medium added. Count cells using a hemocytometer (e.g. Catalog #100-1181). Use the final post-wash cell concentration to calculate the volume of cell suspension to plate per well in step 17.
16. Using a serological pipette or by aspiration, gently remove the matrix solution from the coated cultureware (prepared in step 1). Ensure that the coated surface is not scratched.
17. Seed cells onto a warm (37°C) coated cultureware at a density of 4 - 8 x 10^4 cells/cm^2 per well in STEMdiff™ Forebrain Neuron Maturation Medium. Refer to Table 2 for recommended volumes.

NOTE: The seeding density of Human iPSC-Derived Forebrain Neuron Precursor Cells should be optimized for the application. For long-term cultures (> 30 days of maturation) and for immunocytochemistry, seed cells at 1.5 - 3 x 10^4 cells/cm^2 per well. For short-term cultures (< 30 days of maturation), seed cells at 4 - 8 x 10^4 cells/cm^2 per well.

NOTE: Post-thaw viability is typically 70 - 90%. If poor cell recovery is observed after plating, 10 µM Y-27632 (Dihydrochloride) may be added to the medium during the plating step. Ensure that the cells are plated into their final desired cultureware. Neurons are generally not amenable to passaging after the maturation period has started.

Table 2: Recommended Volume of STEMdiff™ Forebrain Neuron Maturation Medium for Various Cultureware

CULTUREWARE	VOLUME OF STEMDIFF™ NEURON MATURATION MEDIUM
96-well plate	100 µL/well
4- or 24-well plate	500 µL/well
6-well plate	2 mL/well
35 mm dish	2 mL
60 mm dish	5 mL

18. Place the plate in a 37°C incubator. Move the plate in several quick, short, back-and-forth and side-to-side motions to distribute the cells across the surface of the wells.
19. Perform full-medium change every 2 - 3 days.

    NOTE: To avoid cell detachment, perform medium changes slowly (dropwise), pointing the pipette toward the wall of the cell culture vessel. If detachment is observed, switch to half-medium changes every other day.
20. Continue maturation of neurons for a minimum of 14 days. Neurons can be cultured for up to 12 weeks if prolonged maturation time is required.

Assessment of Neuronal Differentiation

Neuronal differentiation may be assessed by immunocytochemistry using Anti-Beta-Tubulin III Antibody, Clone TUJ1. The presence of GABA-ergic neurons can be assessed using anti-GABA antibodies. The presence of synapses can be assessed by evaluating the expression and localization of synapsin.

For evaluating neuronal maturation efficiency, marker expression may be assessed after 14 days of maturation by immunocytochemistry using the following antibodies:

- Anti-FOXG1 Antibody, Polyclonal (Abcam Catalog #ab18259)
- Anti-Beta-Tubulin III Antibody, Clone TUJ1 (Catalog #60052)
- Anti-Human MAP2 Antibody, Polyclonal (Catalog #100-1342)
- Anti-Human Synaptophysin Antibody, Clone 249 (Catalog #100-1345)
- *Anti-Glial Fibrillary Acidic Protein (GFAP) Antibody, Polyclonal (Aves Labs Catalog #GFAP)

* GFAP is an astrocyte marker with low expression in the culture.

Related Products

For related products, including specialized cell culture and storage media, supplements, antibodies, cytokines, and small molecules, visit www.stemcell.com/hPSCNCworkflow, or contact us at techsupport@stemcell.com.