Human iPSC-Derived Neural Progenitor Cells

Catalog #200-0620	1 x 10^6 cells
#200-0621	5 x 10^6 cells

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Product Description

Human iPSC-Derived Neural Progenitor Cells (NPCs) are highly pure central nervous system (CNS)-type progenitors manufactured from the human induced pluripotent stem cell (iPSC) line, Healthy Control Human iPSC Line, Female, SCTi003-A (Catalog #200-0511), using the STEMdiff™ SMADi Neural Induction Kit (Catalog #08581). NPCs express high levels of CNS-type markers such as PAX6, SOX1, and Nestin, and express low levels of neural crest marker SOX10.

NPCs may be expanded using STEMdiff™ Neural Progenitor Medium (Catalog #05833) for several passages and cryopreserved using STEMdiff™ Neural Progenitor Freezing Medium (Catalog #05838), allowing for workflow flexibility.

NPCs can be reliably differentiated to several cell types, including forebrain neurons (STEMdiff™ Forebrain Neuron System; Catalog #08600/08605), midbrain neurons (STEMdiff™ Midbrain Neuron System; Catalog #100-0038/100-0041), and astrocytes (STEMdiff™ Astrocyte System; Catalog #100-0013/100-0016).

NPCs are also suitable as a starting point for customized downstream differentiation to various central nervous system cell types using BrainPhys™ hPSC Neuron Kit (Catalog #05795). NPCs, as well as neurons and astrocytes generated from them, can be used for studying human nervous system development, modeling neurological disorders, and developing assays.

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.

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Materials Required but Not Included

PRODUCT NAME	CATALOG #
12-well flat-bottom tissue culture-treated plates	e.g. Corning 3513
ACCUTASE™	07920
Conical tubes, 15 mL	e.g. 38009
Corning® Matrigel® hESC-Qualified Matrix OR Poly-L-ornithine and Laminin	Corning 354277 OR Sigma P4957 and Sigma L2020
D-PBS (Without Ca++ and Mg++)	37350
DMEM/F-12 with 15 mM HEPES	36254
Serological pipettes, 2 mL and 5 mL	e.g. 38002 and 38003
STEMdiff™ Neural Progenitor Medium	05833
Trypan Blue	07050
Y-27632 (Dihydrochloride) (Optional)	72302

Preparation of Reagents and Materials

Using sterile technique, coat cultureware with (A) poly-L-ornithine (PLO) and laminin or (B) Corning® Matrigel® prior to initial plating and expansion of NPCs. Refer to the instructions below for coating cultureware and preparing STEMdiff™ Neural Progenitor Medium.

NOTE: Use tissue culture-treated cultureware.

A. COATING CULTUREWARE WITH PLO AND LAMININ

1. Dilute the PLO solution in sterile Dulbecco's phosphate-buffered saline (D-PBS) to reach a final concentration of 15 µg/mL.
   For example, add 15 mL of PLO to 85 mL of D-PBS.
2. Gently mix diluted PLO solution. Do not vortex.
3. Add the PLO 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 the substrate solution evenly across the surface and incubate at 37°C and 5% CO_2 for 2 hours. Do not let the coating solution evaporate.
   NOTE: If not used immediately, cultureware must be sealed to prevent evaporation of the substrate solution (e.g. with Parafilm®). Sealed cultureware can be stored at 2 - 8°C overnight. Allow stored coated cultureware to come to room temperature (15 - 25°C) before proceeding to step 5.
5. Prepare a 10 µg/mL working solution of laminin in DMEM/F-12 with 15 mM HEPES. Refer to Table 1 for recommended coating volumes.
6. Gently tilt the PLO-coated cultureware onto one side and allow excess PLO solution to collect at the edge. Remove the excess solution using a serological pipette or by aspiration. Ensure that the coating is not scratched.
7. Wash the PLO-coated cultureware twice using D-PBS by pipetting D-PBS gently toward the corner of the cultureware to avoid removing the PLO substrate.
8. Remove the D-PBS from the cultureware and immediately add the laminin solution (prepared in step 5) to cover the entire growth surface.
9. Incubate at 37°C and 5% CO_2 for 2 hours. Do not let the coating solution evaporate.
   NOTE: Using freshly coated cultureware is recommended. However, if not used immediately, sealed cultureware can be stored at 2 - 8°C for up to 4 days after coating. Warm the coated cultureware to 37°C before use.
10. Gently remove the laminin solution immediately prior to seeding cells. Do not let the surface dry.
    NOTE: It is not necessary to wash cultureware after removing the laminin solution.

B. COATING CULTUREWARE WITH CORNING® MATRIGEL®

Corning® Matrigel® should be aliquoted and frozen. Consult the Certificate of Analysis supplied with Matrigel® for the recommended aliquot size ("Dilution Factor") to prepare 24 mL of diluted matrix. Make sure to always keep Matrigel® on ice when thawing and handling to prevent it from gelling.

1. Thaw one aliquot of Corning® Matrigel® on ice.
2. Dispense 24 mL of cold DMEM/F-12 with 15 mM HEPES into a 50 mL conical tube and keep on ice.
3. Add thawed Matrigel® to the cold DMEM/F-12 with 15 mM HEPES (in the 50 mL tube) and mix well. The vial may be washed with cold medium if desired.
4. Immediately use the diluted Matrigel® solution to coat tissue culture-treated cultureware. Refer to Table 1 for recommended coating volumes.
5. Swirl the cultureware to spread the solution evenly across the surface.

   NOTE: If the surface of the cultureware is not fully coated by the Matrigel® solution, it should not be used.
6. Incubate at room temperature (15 - 25°C) for at least 1 hour before use. Do not let the Matrigel® solution evaporate.

   NOTE: If not used immediately, the cultureware must be sealed to prevent evaporation of the Matrigel® solution (e.g. with Parafilm®) and can be stored at 2 - 8°C for up to 1 week after coating. Allow stored coated cultureware to come to room temperature before proceeding to step 7.
7. Immediately prior to seeding cells, gently tilt the cultureware onto one side and allow the excess solution to collect at the edge. Remove the excess solution using a serological pipette or by aspiration. Ensure that the coated surface is not scratched.

Table 1: Recommended Volumes for Coating Cultureware with Diluted PLO/Laminin Solution or Corning® Matrigel®

CULTUREWARE	APPROXIMATE SURFACE AREA	VOLUME OF COATING SOLUTION
96-well plate	0.32 cm^2/well	50 µL/well
24-well plate	1.9 cm^2/well	250 µL/well
12-well plate	3.8 cm^2/well	500 µL/well
6-well plate	9.5 cm^2/well	1 mL/well
35 mm dish	9 cm^2	1 mL
60 mm dish	21 cm^2	2.5 mL

C. PREPARATION OF STEMDIFF™ NEURAL PROGENITOR MEDIUM

STEMdiff™ Neural Progenitor Medium consists of STEMdiff™ Neural Progenitor Basal Medium, STEMdiff™ Neural Progenitor Supplement A (50X), and STEMdiff™ Neural Progenitor Supplement B (1000X).

Prior to thawing and plating your NPCs, prepare complete STEMdiff™ Neural Progenitor Medium. For instructions on preparing STEMdiff™ Neural Progenitor Medium, refer to the STEMdiff™ Neural Progenitor Medium Product Information Sheet (Document #10000003488), available at www.stemcell.com, or contact us to request a copy.

Directions for Use

A. THAWING AND PLATING NEURAL PROGENITOR CELLS

Generally, 1 x 10^6 of Human iPSC-Derived Neural Progenitor Cells is enough to seed one well of a 12-well plate at the higher range of recommended seeding densities or two wells of a 12-well plate at the lower range of recommended seeding densities.

NOTE: The following instructions are for seeding cells into coated 12-well plates. If using other cultureware, adjust volumes accordingly.

1. Coat the desired number of wells of a 12-well tissue culture-treated plate with either PLO/laminin or Corning® Matrigel® (see Preparation sections A and B).
2. Warm DMEM/F-12 with 15 mM HEPES and STEMdiff™ Neural Progenitor Medium (see Preparation section C) 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 NPCs.

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. 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 10mL 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 2 mL of STEMdiff™ Neural Progenitor 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. 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 12-well plate (prepared in step 1). Ensure that the coated surface is not scratched.
17. Plate cells at 1.25 - 2 x 10^5 cells/cm^2 per well of the matrix-coated 12-well plate.
    NOTE: Post-thaw viability is typically 80 - 90%. If poor NPC recovery is observed after plating, 10 µM Y-27632 may be added to the medium during the plating step.
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 NPCs across the surface of the wells.
19. Perform daily full-medium changes until the cells are ready to passage (after approximately 7 days of culture).
    NOTE: Cells may appear very dense by the time of passage; this is normal for NPCs.

B. PASSAGING NEURAL PROGENITOR CELLS FOR EXPANSION

The following instructions are for passaging NPCs from one well of a 12-well plate and plating them onto matrix-coated wells of a new 12-well plate for expansion. Indicated volumes are for a single well. If using other cultureware, adjust volumes accordingly. For complete instructions on passaging NPCs using STEMdiff™ Neural Progenitor Medium, refer to the Technical Manual: Generation and Culture of Neural Progenitor Cells Using the STEMdiff™ Neural System (Document #10000005588), available at www.stemcell.com, or contact us to request a copy.

1. Coat the desired number of wells of a 12-well tissue culture-treated plate with either PLO/laminin or Corning® Matrigel®.
2. Warm (37°C) sufficient volumes of STEMdiff™ Neural Progenitor Medium and DMEM/F-12 with 15 mM HEPES. Warm ACCUTASE™ to room temperature (15 - 25°C).
3. Aspirate medium and add 0.5 mL of ACCUTASE™ per well.
4. Incubate at 37°C for 5 - 10 minutes.
5. Using a 1 mL pipette, pipette the cell suspension up and down to dislodge remaining attached cells.
6. Add 3 mL of DMEM/F-12 with 15 mM HEPES and transfer the NPC suspension to a 15 mL conical tube.
7. Centrifuge at 300 x g for 5 minutes.
8. Carefully aspirate the supernatant and add 1 mL of STEMdiff™ Neural Progenitor Medium.
9. Count viable cells using Trypan Blue and a hemocytometer.
10. Using a serological pipette or by aspiration, gently remove the matrix solution from the new plate (prepared in step 1). Ensure that the coated surface is not scratched.
11. Plate cells at 1.25 - 2 x 10^5 cells/cm^2 in 1 mL of STEMdiff™ Neural Progenitor Medium onto the new matrix-coated plate.
    NOTE: High densities are critical to maintain NPCs in a proliferative state and may prevent spontaneous differentiation.
12. 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 NPCs across the surface of the wells.
13. Perform daily full-medium changes using STEMdiff™ Neural Progenitor Medium.
14. Visually assess cultures to monitor growth and to determine timing of the next passage (after approximately 7 days of culture).
    NOTE: Cells may appear very dense by the time of passage; this is normal for NPCs.

NPCs have been tested for capacity to expand at least 5 passages for the purposes of expansion and cell banking. Record the passage number of the NPCs and periodically assess (below). For best efficiency in downstream differentiation to forebrain neurons or midbrain neurons, it is generally recommended to keep the number of passages post-thaw to 5 or fewer.

Assessment of Neural Progenitor Cells

Refer to Table 2 below for recommended antibody clones and expected expression levels for assessing neural progenitor cells by immunocytochemistry or flow cytometry. Results may vary depending on the number of passages in which the NPCs have been expanded. Therefore, it is important to assess after expanding NPCs for several passages.

Table 2. Recommended Clones and Expected Expression Levels for Neural Progenitor Assessment

ANTIBODY TARGET	RECOMMENDED ANTIBODY FOR IMMUNOCYTOCHEMISTRY	RECOMMENDED ANTIBODY FOR FLOW CYTOMETRY	EXPECTED EXPRESSION
PAX6	Poly19013 (BioLegend Catalog #901301)	PAX6-PE O18-1330 (BD Biosciences Catalog #561552)	≥ 80 - 90%
Nestin	Anti-Human Nestin Antibody, Clone 10C2 (Catalog #60091)	Anti-Human Nestin Antibody, Clone 10C2 (Catalog #60091)	≥ 80 - 90%
SOX1	Human/Mouse/Rat SOX1 Antibody (R&D Systems Catalog #AF3369)	SOX1-APC REA698 (Miltenyi Biotec Catalog #130-111-159)	≥ 80 - 90%
SOX10	A-2 (Santa Cruz Biotechnology Catalog #sc-365692)	SOX10-FITC (Novus Biologicals Catalog #NBP2-47708AF488)	≤ 10%
OCT4	Anti-Human OCT4 (OCT3) Antibody, Clone 3A2A20 (Catalog #60093)	Anti-Human OCT4 (OCT3) Antibody, Clone 3A2A20 (Catalog #60093)	Negligible

For information about assessing neural/ectodermal marker expression by flow cytometry, refer to the Tech Tip: The STEMdiff™ Trilineage Differentiation Kit: Assessing Differentiation to Three Germ Lineages, available at www.stemcell.com.

C. CRYOPRESERVATION OF NEURAL PROGENITOR CELLS

Expanded NPCs may be cryopreserved at 1.5 - 6 x 10^6 viable cells per cryovial using STEMdiff™ Neural Progenitor Freezing Medium (Catalog #05838). For detailed instructions on freezing and thawing NPCs, refer to the Product Information Sheet (Document #10000000232) for STEMdiff™ Neural Progenitor Freezing Medium, available at www.stemcell.com.

D. PASSAGING NEURAL PROGENITOR CELLS FOR DOWNSTREAM DIFFERENTIATION

Expanded NPCs may be differentiated further to downstream cell types, including forebrain neurons, midbrain neurons, and astrocytes (Refer to Table 3 for recommended products). For best efficiency in downstream differentiation to forebrain neurons or midbrain neurons, it is generally recommended to keep the number of passages post-thaw to five or fewer. NPCs are also suitable as a starting point for customized downstream differentiation to various central nervous system cell types using BrainPhys™ hPSC Neuron Kit (Catalog #05795).

The following instructions are for passaging NPCs from one well of a 12-well plate and plating them onto matrix-coated wells of a new 12-well plate for differentiation. Indicated volumes are for a single well. If using other cultureware, adjust volumes accordingly.

1. Coat the desired number of wells of a 12-well tissue culture-treated plate with either PLO/laminin or Corning® Matrigel®.
   NOTE: Refer to Table 3 for the recommended coating matrix for the downstream cell type of interest.
2. Warm (37°C) sufficient volumes of STEMdiff™ Neural Progenitor Medium and DMEM/F-12 with 15 mM HEPES. Warm ACCUTASE™ to room temperature (15 - 25°C).
3. Aspirate medium and add 0.5 mL of ACCUTASE™.
4. Incubate at 37°C for 5 - 10 minutes.
5. Using a 1 mL pipette, pipette the cell suspension up and down to dislodge remaining attached cells.
6. Add 3 mL of DMEM/F-12 with 15 mM HEPES and transfer the NPC suspension to a 15 mL conical tube.
7. Centrifuge at 300 x g for 5 minutes.
8. Carefully aspirate the supernatant and add 1 mL of STEMdiff™ Neural Progenitor Medium.
9. Count viable cells using Trypan Blue and a hemocytometer.
10. Using a serological pipette or by aspiration, gently remove the matrix solution from the new plate (prepared in step 1). Ensure that the coated surface is not scratched.
11. Plate cells at desired density in 1 mL of STEMdiff™ Neural Progenitor Medium onto the new matrix-coated plate.
    NOTE: Refer to Table 3 for the recommended seeding density for the downstream cell type of interest.
12. 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 NPCs across the surface of the wells.
13. After 24 hours, aspirate the medium and replace with the appropriate differentiation medium.

14. Continue the differentiation as outlined in the Product Information Sheets for the relevant downstream kits (under Directions for Use: Part A, section II: Starting from the Monolayer Protocol, step 4):
    - STEMdiff™ Forebrain Neuron Differentiation Kit/STEMdiff™ Forebrain Neuron Maturation Kit (Document #10000005464)
    - STEMdiff™ Midbrain Neuron Differentiation Kit/STEMdiff™ Midbrain Neuron Maturation Kit (Document #10000007220)
    - STEMdiff™ Astrocyte Differentiation Kit/ STEMdiff™ Astrocyte Maturation Kit (Document #10000006879)

    These documents are available at www.stemcell.com.

    NOTE: If thawed NPCs will be used directly for differentiation (without an expansion step in STEMdiff™ Neural Progenitor Medium), they may be seeded directly into the appropriate differentiation medium and seeding density indicated in Table 3.

Table 3. Cell Types and Products Applicable for Downstream Differentiation of NPCs

CELL TYPE	SEEDING DENSITY	COATING MATRIX	PRODUCTS
Forebrain Neurons	1.25 x 10^5 cells/cm^2	PLO and Laminin	STEMdiff™ Forebrain Neuron Differentiation Kit (Catalog #08600) STEMdiff™ Forebrain Neuron Maturation Kit (Catalog #08605)
Midbrain Neurons	1.25 x 10^5 cells/cm^2	PLO and Laminin	STEMdiff™ Midbrain Neuron Differentiation Kit (Catalog #100-0038) STEMdiff™ Midbrain Neuron Maturation Kit (Catalog #100-0041)
Astrocytes	2 x 10^5 cells/cm^2	Corning® Matrigel® hESC-Qualified Matrix	STEMdiff™ Astrocyte Differentiation Kit (Catalog #100-0013) STEMdiff™ Astrocyte Maturation Kit (Catalog #100-0016)
Other Cell Types (Customized)*	Optimized according to protocol used	Optimized according to protocol used	BrainPhys™ hPSC Neuron Kit (Catalog #05795)

* BrainPhys™ has been used as a basal medium for the differentiation of NPCs to downstream cell types, including mixed cortical neurons (Bardy C et al.). For more details on customized NPC differentiation, contact us at techsupport@stemcell.com.

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.

References

Bardy C et al. (2015) Neuronal medium that supports basic synaptic functions and activity of human neurons in vitro. Proc Natl Acad Sci USA 112(20): E2725–34.

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