STEMdiff™ Forebrain Neuron Differentiation Kit
STEMdiff™ Forebrain Neuron Maturation Kit

Catalog #08600 1 Kit
Catalog #08605 1 Kit

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Product Description

STEMdiff™ Forebrain Neuron Differentiation Kit (Catalog #08600) is used to generate neuronal precursors from neural progenitor cells (NPCs) derived from human pluripotent stem cells using STEMdiff™ SMADi Neural Induction Kit (Catalog #08581) via either the embryoid body or monolayer protocol. The neuronal precursors generated can be matured using STEMdiff™ Forebrain Neuron Maturation Kit (Catalog #08605) to produce a mixed population of excitatory and inhibitory forebrain-type (FOXG1-positive) neurons (≥ 90% class III β-tubulin-positive neurons; < 10% GFAP-positive astrocytes). Cells derived using these products are versatile tools for modeling human neurological development and disease, drug screening, toxicity testing, and cell therapy validation.

Product Information

The following components are sold as part of a complete kit (Catalog #08600 or Catalog #08605) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMdiff™ Forebrain Neuron Differentiation Kit (Catalog #08600)				
STEMdiff™ Forebrain Neuron Differentiation Basal Medium	08601	80 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Forebrain Neuron Differentiation Supplement	08602	20 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Forebrain Neuron Maturation Kit (Catalog #08605)				
BrainPhys™ Neuronal Medium	05797	100 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Forebrain Neuron Maturation Supplement	08606	25 mL	Store at -20°C.	Stable until expiry date (EXP) on label.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
Poly-L-ornithine hydrobromide (PLO)	Sigma P3655
Laminin	Sigma L2020
DMEM/F-12 with 15 mM HEPES	36254
ACCUTASE™	07920
Trypan Blue	07050

Preparation of Reagents and Materials

A. COATING CULTUREWARE WITH POLY-L-ORNITHINE (PLO)/LAMININ

1. Dilute PLO solution in phosphate-buffered saline (PBS) to reach a final concentration of 15 µg/mL.
2. Add PLO solution to cultureware to cover the entire growth surface (see Table 1 for required volumes).
3. Distribute the solution evenly 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 the PLO solution evaporate.
4. Prepare a 5 µg/mL working solution of laminin in DMEM/F-12 (see Table 1 for required volumes).
5. Rinse PLO-coated vessel twice with sterile PBS. Pipette PBS gently toward the corner of the cultureware to avoid removing the PLO coating.
6. Aspirate PBS from the cultureware and add the laminin solution to cover the entire growth surface (see Table 1 for required volumes).

7. 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 the laminin solution evaporate.

   NOTE: Using freshly coated cultureware is recommended. However, if not used immediately, coated cultureware can be stored at 2 - 8°C in laminin solution for up to 4 days.
8. Warm coated cultureware to 37°C before use.
9. Aspirate laminin solution immediately prior to seeding cells. Do not let the surface dry. It is not necessary to wash cultureware after removal of laminin solution.

Table 1. Recommended Volumes of PLO and Laminin for Coating Cultureware

CULTUREWARE	APPROXIMATE SURFACE AREA	PLO	LAMININ
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 DIFFERENTIATION MEDIUM

Use sterile technique to prepare STEMdiff™ Forebrain Neuron Differentiation Medium (Differentiation Basal Medium + Differentiation Supplement). The following example is for preparing 100 mL of medium. If preparing other volumes, adjust accordingly.

1. Thaw Differentiation Supplement at room temperature (15 - 25°C) or at 2 - 8°C overnight. Mix thoroughly.
2. Add 20 mL of Differentiation Supplement to 80 mL of Differentiation Basal Medium. Mix thoroughly.
3. NOTE: If not used immediately, store STEMdiff™ Forebrain Neuron Differentiation Medium at 2 - 8°C for up to 4 weeks. Warm medium to 37°C before use.

C. PREPARATION OF STEMdiff™ FOREBRAIN NEURON MATURATION MEDIUM

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

1. Thaw Maturation Supplement at room temperature (15 - 25°C) or at 2 - 8°C overnight. Mix thoroughly.
2. Add 25 mL of 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.

Directions for Use

Please read the entire protocol before proceeding. Use sterile techniques when performing the following protocols. Coat cultureware with PLO/laminin as described in the Preparation section.

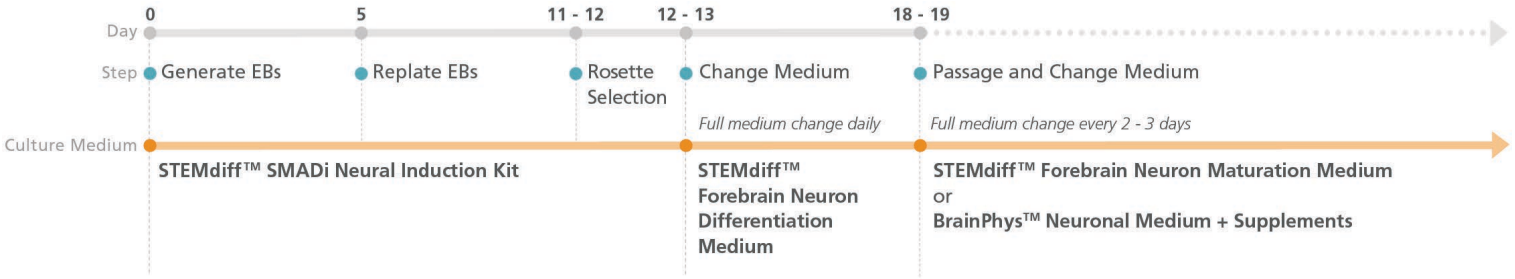
A. DIFFERENTIATION OF NPCs TO NEURONAL PRECURSORS

The following protocols integrate into the STEMdiff™ SMADi Neural Induction Kit (Catalog #08581) embryoid body protocol after rosette selection (section I), or the monolayer protocol (section II).

For complete instructions for generating central nervous system (CNS)-type NPCs using embryoid body (EB) formation with the AggreWell™800 plate (Catalog #34811) or using the monolayer protocol, refer to the Technical Manual: Generation and Culture of Neural Progenitor Cells Using the STEMdiff™ Neural System, available at www.stemcell.com or contact us to request a copy.

I. Starting from the EB Protocol

Protocol Diagram

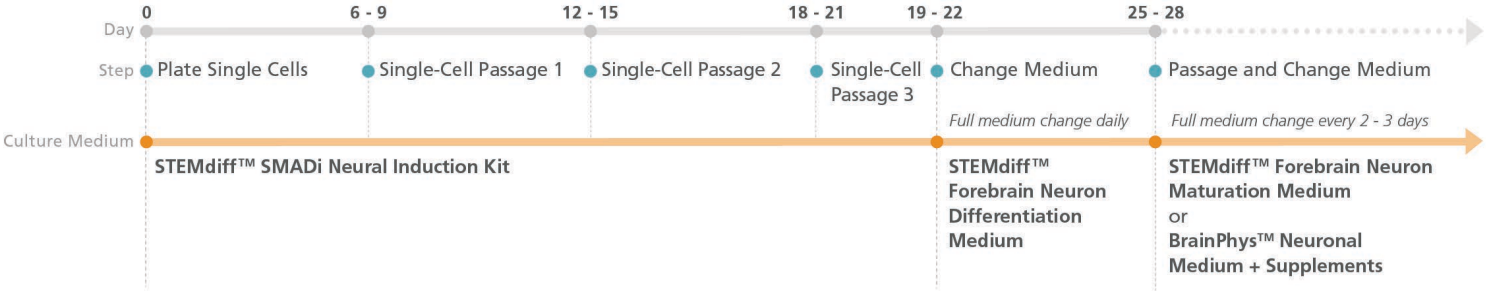


The following instructions are for a single well of a 6-well plate; if using other cultureware, adjust volumes accordingly.

1. **Day 11/12 after EB formation**: Place selected neural rosettes onto a coated well of a 6-well plate containing 2 mL of STEMdiff™ Neural Induction Medium + SMADi. Incubate at 37°C and 5% CO_2 for 24 hours.
2. **Day 12/13 after EB formation**: Aspirate medium and add 2 mL STEMdiff™ Forebrain Neuron Differentiation Medium. Incubate at 37°C and 5% CO_2.
3. Perform daily full medium changes with warm (37°C) STEMdiff™ Forebrain Neuron Differentiation Medium. Incubate at 37°C and 5% CO_2.
4. **Day 18/19 after EB formation**: Cells will reach 80 - 90% confluence and will be ready to passage. Proceed to section B.

II. Starting from the Monolayer Protocol

Protocol Diagram



The following instructions are for a single well of a 6-well plate; if using other cultureware, adjust volumes accordingly.

1. **Day 18 - 21 (Passage 3) of the monolayer protocol**: Passage the cells as single cells using ACCUTASE™ as described in the Technical Manual: Generation and Culture of Neural Progenitor Cells Using the STEMdiff™ Neural System (section 6.2).
2. Add cells to a coated well of a 6-well plate at a density of 80 - 125,000 cells/cm^2 in 2 mL STEMdiff™ Neural Induction Medium + SMADi. Incubate at 37°C and 5% CO_2 for 24 hours.

   NOTE: Cell plating density may need to be optimized for each cell line.
3. **Day 19 - 22**: Aspirate medium and add 2 mL STEMdiff™ Forebrain Neuron Differentiation Medium. Incubate at 37°C and 5% CO_2.
4. Perform daily full medium changes with warm (37°C) STEMdiff™ Forebrain Neuron Differentiation Medium. Incubate at 37°C and 5% CO_2.
5. **Day 25 - 28**: Cells will reach 80 - 90% confluence and will be ready to passage. Proceed to section B.

B. PASSAGING NEURONAL PRECURSORS INTO STEMdiff™ FOREBRAIN NEURON MATURATION MEDIUM

1. Aspirate medium and add 1 mL ACCUTASE™.
2. Incubate at 37°C and 5% CO_2 for 5 - 10 minutes.
3. Add 5 mL DMEM/F-12 and wash the cells off of the well.
4. Centrifuge cell suspension at 400 x *g* for 5 minutes. Remove and discard supernatant.

5. Resuspend cells in a suitable volume (e.g. 5 mL) of STEMdiff™ Forebrain Neuron Maturation Medium. Perform a cell count using Trypan Blue and a hemocytometer.
6. Proceed to section C for neuron maturation.

C. NEURON MATURATION

1. Seed neuronal precursors onto warm (37°C) coated cultureware at a density of 4 x 10^4 - 6 x 10^4 cells/cm^2 in STEMdiff™ Forebrain Neuron Maturation Medium. See Table 2 for recommended volumes.

   NOTE: The seeding density of neuronal precursors should be optimized for the application and cell line. For long-term cultures (> 30 days of maturation) and for immunocytochemistry, seed cells at 1.5 x 10^4 - 3 x 10^4 cells/cm^2. For short-term cultures (< 30 days of maturation), seed cells at 4 x 10^4 - 6 x 10^4 cells/cm^2.

   NOTE: Neurons can be cultured with BrainPhys™ Neuronal Medium (Catalog #05790) + desired supplements in place of STEMdiff™ Forebrain Neuron Maturation Medium.

   Table 2. Recommended Volumes 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

2. Distribute cells evenly. Incubate at 37°C and 5% CO_2.
3. Perform a full medium change every 2 - 3 days.

   NOTE: To avoid cell detachment, perform medium changes slowly (dropwise), pointing the pipette tip toward the wall of the cell culture vessel. If detachment is observed, switch to half-medium changes every other day.
4. Continue maturation of neurons for a minimum of 8 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 (Catalog #60052). 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. Results may vary depending on cell line used.

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.