STEMdiff™ Neuron Differentiation Kit
STEMdiff™ Neuron Maturation Kit

Catalog #08500	1 Kit
Catalog #08510	1 Kit

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Product Description

STEMdiff™ Neuron Differentiation Kit (Catalog #08500) is used to generate neuronal precursors from neural progenitor cells (NPCs) derived from human pluripotent stem cells (hPSCs) using the STEMdiff™ Neural Induction Medium (Catalog #05835) embryoid body protocol. The neuronal precursors generated can be matured using STEMdiff™ Neuron Maturation Kit (Catalog #08510) to produce functional neurons. STEMdiff™ Neuron Differentiation Kit and STEMdiff™ Neuron Maturation Kit are also compatible with cryopreserved NPCs (Human PSC-Derived NPCs; Catalog #70901) and cryopreserved neuronal precursor cells (Human PSC-Derived Mixed Neurons; Catalog #70905), respectively. These media will produce a mixed population of excitatory and inhibitory forebrain-type neurons (≥ 90% class III β-tubulin-positive neurons; < 1% GFAP-positive astrocytes). Neurons 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 #08500 or Catalog #08510) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMdiff™ Neuron Differentiation Kit (Catalog #08500)				
STEMdiff™ Neuron Differentiation Basal Medium	08501	100 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Neuron Differentiation Supplement A	08502	8 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Neuron Differentiation Supplement B	08503	2 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Neuron Differentiation Supplement C	08504	200 µL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Neuron Maturation Kit (Catalog #08510)				
STEMdiff™ Neuron Maturation Basal Medium	08511	100 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Neuron Maturation Supplement A	08512	100 µL	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
STEMdiff™ Neural Rosette Selection Reagent	05832
Trypan Blue	07050

Neuron Differentiation and Maturation Media

Generation of neurons from human pluripotent stem cell-derived NPCs requires both STEMdiff™ Neuron Differentiation Kit (Catalog #08500) and STEMdiff™ Neuron Maturation Kit (Catalog #08510). If cryopreserved neuronal precursors are used, only STEMdiff™ Neuron Maturation Kit (Catalog #08510) is required. Refer to Table 1 for media requirements according to starting cell type.

Table 1: Media Requirements by Starting Cell Type

STARTING CELL TYPE	STEMdiff™ NEURAL INDUCTION MEDIUM (Catalog #05835)	NEURAL PROGENITOR MEDIUM 2 (Catalog #08560)	STEMdiff™ NEURON DIFFERENTIATION KIT (Catalog #08500)	STEMdiff™ NEURON MATURATION KIT (Catalog #08510)
hPSCs	✓	✗	✓	✓
Cryopreserved NPCs (Catalog #70901 and 70902)	✗	✓	✓	✓

Preparation of Reagents and Materials

A. COATING CELL CULTURE VESSELS WITH POLY-L-ORNITHINE/LAMININ

1. Dilute poly-L-ornithine (PLO) solution in phosphate-buffered saline (PBS) to reach a final concentration of 15 µg/mL.
2. Add PLO solution into cell culture vessel to cover entire growth surface (see Table 2 for required volumes).
3. Distribute the solution evenly and incubate at 37°C and 5% CO2 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 10 µg/mL working solution of laminin in DMEM/F-12. (see Table 2 for required volumes).
5. Rinse PLO-coated vessel twice with sterile PBS. Pipette PBS gently toward the corner of the vessel to avoid removal of PLO coating.
6. Aspirate PBS from the vessel and add the laminin solution to cover entire growth surface (see Table 2 for required volumes).
7. Incubate at 37°C and 5% CO2 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 vessels is recommended. However, if not used immediately, coated vessels can be stored at 2 - 8°C in laminin solution for up to 4 days.
8. Warm coated vessel 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 the vessel after removal of laminin solution.

Table 2: 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 COMPLETE STEMdiff™ NEURON DIFFERENTIATION MEDIUM

Use sterile techniques to prepare complete STEMdiff™ Neuron Differentiation Medium (Differentiation Basal Medium + Differentiation Supplement A + Differentiation Supplement B + Differentiation Supplement C). The following example is for preparing 100 mL of complete medium. If preparing other volumes, adjust accordingly. For recommended volumes of media for various cultureware, refer to Table 3.

1. Thaw Supplement B and Supplement C at room temperature (15 - 25°C) or at 2 - 8°C overnight. Mix thoroughly.

   NOTE: If not used immediately, aliquot and store at -20°C. Do not exceed the shelf life of the supplements. Once aliquots are thawed, do not re-freeze.
2. Add 8 mL of Supplement A, 2 mL of Supplement B, and 200 µL of Supplement C to 100 mL of Basal Medium. Mix thoroughly.

   NOTE: If not used immediately, store complete STEMdiff™ Neuron Differentiation Medium at 2 - 8°C for up to 10 days. Warm complete medium to 37°C before use.

C. PREPARATION OF COMPLETE STEMdiff™ NEURON MATURATION MEDIUM

Use sterile techniques to prepare complete STEMdiff™ Neuron Maturation Medium (Maturation Basal Medium + Maturation Supplement A). The following example is for preparing 100 mL of complete medium. If preparing other volumes, adjust accordingly. For recommended volumes of medium for various cultureware, refer to Table 3.

1. Thaw Basal Medium and Supplement A at room temperature (15 - 25°C) or at 2 - 8°C overnight. Mix thoroughly.

   NOTE: If not used immediately, aliquot Supplement A and store at -20°C. Do not exceed the shelf life of the supplement. Once aliquots are thawed, do not re-freeze.

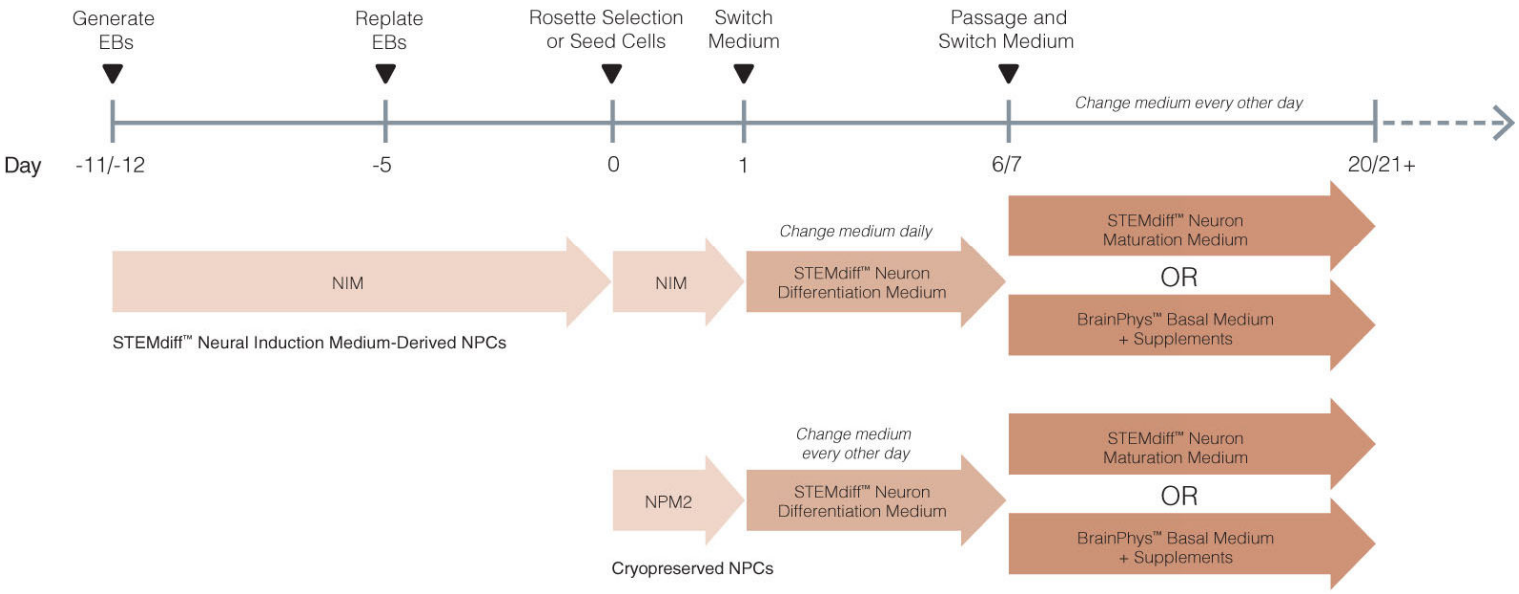
2. Add 100 µL of Supplement A to 100 mL of Basal Medium. Mix thoroughly.

   NOTE: If not used immediately, store complete STEMdiff™ Neuron Maturation Medium at 2 - 8°C for up to 3 weeks. Warm complete medium to 37°C before use.

Table 3: Recommended Volumes of Complete STEMdiff™ Neuron Differentiation or Maturation Medium for Various Cultureware

CULTUREWARE	VOLUME OF COMPLETE STEMdiff™ NEURON DIFFERENTIATION OR 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

Protocol Diagram



NIM: STEMdiff™ Neural Induction Medium; NPM2: Neural Progenitor Medium 2

Directions for Use

Please read the entire protocol before proceeding. Use sterile techniques when performing the following protocols.
For differentiation of NPCS derived from STEMdiff™ Neural Induction Medium, refer to section A. For differentiation of cryopreserved NPCs, refer to section B.

A. DIFFERENTIATION OF STEMdiff™ NEURAL INDUCTION MEDIUM-DERIVED NPCs TO NEURONAL PRECURSORS

For a detailed protocol for generating central nervous system (CNS)-type NPCs using embryoid body (EB) formation with the AggreWell™800 plate (Catalog #34811), refer to the Technical Manual: Generation and Culture of Neural Progenitor Cells Using the STEMdiff™ Neural System (Document #28782), available at www.stemcell.com or contact us to request a copy.

Neuronal Differentiation

The following instructions are for a single well of a 6-well plate; if using other cultureware, refer to Table 3 and adjust volumes accordingly.

1. On **day 0** (day 11/12 after EB formation), place selected neural rosettes onto a PLO/laminin-coated well of a 6-well plate in 2 mL of STEMdiff™ Neural Induction Medium. Incubate at 37°C and 5% CO_2 for 24 hours.
2. On **day 1** (day 12/13 after EB formation), aspirate medium and replace with 2 mL of complete STEMdiff™ Neuron Differentiation Medium.
3. Incubate at 37°C and 5% CO_2, performing daily full medium changes with warm (37°C) complete STEMdiff™ Neuron Differentiation Medium.
4. On **day 6/7** (day 18/19 after EB formation), cells will reach 90 - 95% confluence and will be ready to passage.

Passaging Neuronal Precursors

5. Aspirate medium and wash cells with 1 mL of sterile PBS to remove cell debris.
6. Add 1 mL of ACCUTASE™ and incubate at 37°C and 5% CO_2 for 5 - 10 minutes.
7. Add 5 mL of DMEM/F-12 and wash the cells off of the well.
8. Centrifuge cell suspension at 400 x *g* for 5 minutes and remove supernatant.
9. Resuspend cells in a suitable volume (e.g. 5 mL) of complete STEMdiff™ Neuron Maturation Medium. Perform a cell count using Trypan Blue and a hemocytometer.
10. Proceed to section C for neuron maturation.

B. DIFFERENTIATION OF CRYOPRESERVED NPCs TO NEURONAL PRECURSORS

For instructions on thawing, expanding, and passaging NPCs, refer to the Product Information Sheet (PIS) for Human PSC-Derived Neural Progenitor Cells (Document #DX21378) and the PIS for Neural Progenitor Medium 2 (Document #DX20712), available at www.stemcell.com or contact us to request a copy.

Cryopreserved NPCs can be passaged for up to 10 passages without loss of differentiation capacity. We recommend that cryopreserved NPCs be expanded for at least one passage prior to differentiation to allow the cells to recover and to sufficiently expand cells before seeding.

Neuronal Differentiation

Cryopreserved NPCs are ready to passage for differentiation when they reach 95 - 100% confluence.

The following instructions are for a single well of a 6-well plate; if using other cultureware, refer to Table 3 and adjust volumes accordingly.

1. On **day 0**, aspirate medium and wash cells with 1 mL of sterile PBS to remove cell debris.
2. Add 1 mL of ACCUTASE™ and incubate at 37°C and 5% CO_2 for 5 minutes.
3. Add 5 mL of 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 complete Neural Progenitor Medium 2 (Catalog #08560). Perform a cell count using Trypan Blue and a hemocytometer.
6. Seed NPCs onto a pre-warmed cell culture vessel coated with PLO/laminin at a density of 4 x 10^4 - 6 x 10^4 cells/cm^2 in complete Neural Progenitor Medium 2.
7. Distribute cells evenly and incubate at 37°C and 5% CO_2 for 24 hours.
8. On **day 1**, aspirate medium and replace with 2 mL of complete STEMdiff™ Neuron Differentiation Medium.
9. Incubate at 37°C and 5% CO_2, performing a full medium change every other day with warm (37°C) complete STEMdiff™ Neuron Differentiation Medium.
10. On **day 6/7**, cells will reach 90 - 95% confluence and will be ready for passaging.

Passaging Neuronal Precursors

11. Aspirate medium and wash cells with 1 mL of sterile PBS to remove cell debris.
12. Add 1 mL of ACCUTASE™ and incubate at 37°C and 5% CO_2 for 5 minutes.

13. Add 5 mL of DMEM/F-12 and wash the cells off of the well.
14. Centrifuge cell suspension at 400 x *g* for 5 minutes. Remove and discard supernatant.
15. Resuspend cells in a suitable volume (e.g. 1 - 2 mL) of complete STEMdiff™ Neuron Maturation Medium. Perform a cell count using Trypan Blue and a hemocytometer.
16. Proceed to section C for neuron maturation.

C. NEURON MATURATION

1. Seed neuronal precursors onto a pre-warmed (37°C) cell culture vessel coated with PLO/laminin at a density of 1.5 x 10^4 - 6 x 10^4 cells/cm^2 in complete STEMdiff™ Neuron Maturation Medium. See Table 3 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.
2. Distribute cells evenly. Incubate at 37°C and 5% CO_2. Perform a full medium change every other day.

   NOTE: To avoid cell detachment, perform medium changes slowly (dropwise), pointing the pipette tip toward the wall of the cell culture vessel.
3. Mature neurons for a minimum of 1 week in STEMdiff™ Neuron Maturation Medium. Neurons can be cultured for up to 5 weeks if prolonged maturation time is required.

   NOTE: For improved neuronal activity (see www.brainphys.com for details), neurons can be cultured with supplemented BrainPhys™ Neuronal Medium (Catalog #05790) in place of STEMdiff™ Neuron Maturation Medium. For details, including supplementation requirements, refer to section B of Directions for Use in the PIS for BrainPhys™ Neuronal Medium (Document #DX20519), available at www.stemcell.com or contact us to request a copy.

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.