# STEMdiff™ Midbrain Neuron Differentiation Kit
# STEMdiff™ Midbrain Neuron Maturation Kit

Catalog #100-0038 1 Kit
Catalog #100-0041 1 Kit

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

## Product Description

STEMdiff™ Midbrain Neuron Differentiation Kit (Catalog #100-0038) is used to generate midbrain neuronal precursors from neural progenitor cells (NPCs) derived from human pluripotent stem cells (hPSCs) using STEMdiff™ SMADi Neural Induction Kit (Catalog #08581) via either the embryoid body or monolayer protocol. The midbrain neuronal precursors are further matured into midbrain neurons using STEMdiff™ Midbrain Neuron Maturation Kit (Catalog #100-0041). These media will produce a population of midbrain neurons (≥ 15% TH-positive dopaminergic neurons; ≥ 90% class III βtubulin-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 a complete kit (Catalog #100-0038 or Catalog #100-0041) and are not available for individual sale.

| COMPONENT NAME | COMPONENT # | SIZE | STORAGE | SHELF LIFE |
|---|---|---|---|---|
| STEMdiff™ Midbrain Neuron Differentiation Kit (Catalog #100-0038) | | | | |
| STEMdiff™ Midbrain Neuron Differentiation Basal Medium | 100-0039 | 80 mL | Store at 2 - 8°C. | Stable for 12 months from date of manufacture (MFG) on label. |
| STEMdiff™ Midbrain Neuron Differentiation Supplement* | 100-0040 | 20 mL | Store at -20°C. | Stable for 12 months from date of manufacture (MFG) on label. |
| STEMdiff™ Midbrain Neuron Maturation Kit (Catalog #100-0041) | | | | |
| BrainPhys™ Neuronal Medium† | 05797 | 100 mL | Store at 2 - 8°C. | Stable for 2 years from date of manufacture (MFG) on label. |
| STEMdiff™ Midbrain Neuron Maturation Supplement* | 100-0042 | 25 mL | Store at -20°C. | Stable for 12 months from date of manufacture (MFG) on label. |

*This product contains material derived from human plasma. Donors have been tested and found negative for HIV-1 and -2, hepatitis B, and hepatitis C prior to donation. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.

†Protect from light.

## Materials Required But Not Included

| PRODUCT NAME | CATALOG # |
|---|---|
| Human Recombinant Shh (C24II) | 78065 |
| Poly-L-ornithine (PLO) solution | Sigma P4957 |
| 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/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 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 10 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™ MIDBRAIN NEURON DIFFERENTIATION MEDIUM

Use sterile technique to prepare STEMdiff™ Midbrain Neuron Differentiation Medium (Differentiation Basal Medium + Differentiation Supplement + Human Recombinant Shh). The following example is for preparing 100 mL of complete 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. Add Human Recombinant Shh at a concentration of 200 ng/mL. Mix thoroughly.

   NOTE: If not used immediately, store STEMdiff™ Midbrain Neuron Differentiation Medium at 2 - 8°C for up to 1 month. Warm medium to 37°C before use.

## C. PREPARATION OF STEMdiff™ MIDBRAIN NEURON MATURATION MEDIUM

Use sterile technique to prepare STEMdiff™ Midbrain Neuron Maturation Medium (BrainPhys™ Neuronal Medium + Maturation Supplement). The following example is for preparing 125 mL of complete 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™ Midbrain Neuron Maturation Medium at 2 - 8°C for up to 1 month. Warm medium to 37°C before use. Protect from light.

**Table 2: Recommended Volumes of STEMdiff™ Midbrain Neuron Differentiation or Maturation Medium for Various Cultureware**

| CULTUREWARE | VOLUME OF STEMdiff™ MIDBRAIN 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 or 6-well plate | 2 mL |
| 60 mm dish | 5 mL |

# Directions for Use

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

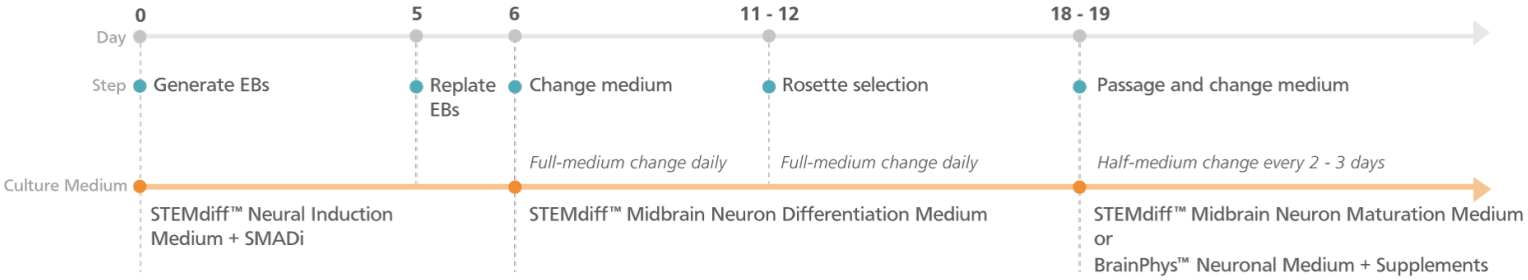
## A. DIFFERENTIATION OF NPCs TO MIDBRAIN NEURONAL PRECURSORS

Generation of midbrain neurons from NPCs requires both STEMdiff™ Midbrain Neuron Differentiation Kit and STEMdiff™ Midbrain Neuron Maturation Kit. The procedure integrates into the STEMdiff™ SMADi Neural Induction Kit (Catalog #08581) embryoid body (EB) protocol (section I), or the monolayer protocol (section II).

For complete instructions for generating central nervous system (CNS)-type NPCs using 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, available at www.stemcell.com or contact us to request a copy.

### I. Starting From the EB Protocol

#### Protocol Diagram



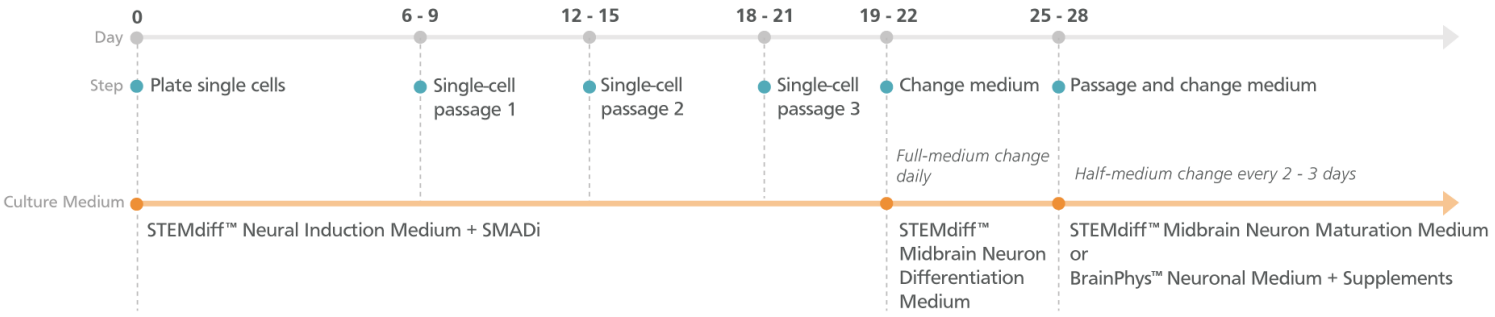

The following instructions are for a single well of a 6-well plate; if using other cultureware, refer to Table 2 and adjust volumes accordingly.

1. Day 5 after EB formation: Replate EBs onto PLO/laminin-coated plates in 2 mL of STEMdiff™ Neural Induction Medium + SMADi. Incubate at 37°C and 5% $CO_2$ for 24 hours.
2. Day 6 after EB formation: Aspirate medium and add 2 mL STEMdiff™ Midbrain Neuron Differentiation Medium. Incubate at 37°C and 5% $CO_2$.
3. Perform daily full-medium changes with warm (37°C) STEMdiff™ Midbrain Neuron Differentiation Medium. Incubate at 37°C and 5% $CO_2$.

   NOTE: The optimal timing of application of STEMdiff™ Midbrain Neuron Differentiation Medium may vary from day 1 to day 4, depending on the cell line used.
4. Day 11 - 12 after EB formation: Perform neural rosette selection using STEMdiff™ Neural Rosette Selection Reagent (Catalog #05832). Replate in 2 mL of STEMdiff™ Midbrain Neuron Differentiation Medium onto PLO/laminin-coated plates.
5. Incubate at 37°C and 5% $CO_2$ for 7 days, performing daily full-medium changes with warm (37°C) STEMdiff™ Midbrain Neuron Differentiation Medium.
6. Day 18 - 19 after EB formation: Cells will reach approximately 80 - 90% confluence and will be ready to passage.

### 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, refer to Table 2 and 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.
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™ Midbrain Neuron Differentiation Medium. Incubate at 37°C and 5% $CO_2$.
4. Perform daily full-medium changes with warm (37°C) STEMdiff™ Midbrain 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 MIDBRAIN NEURONAL PRECURSORS INTO STEMdiff™ MIDBRAIN NEURON MATURATION MEDIUM

1. Aspirate medium and wash cells with 1 mL of sterile PBS to remove cell debris.
2. Add 1 mL of ACCUTASE™. Incubate at 37°C and 5% $CO_2$ for 5 - 10 minutes.
3. Add 5 mL DMEM/F-12 and wash the cells off 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™ Midbrain Neuron Maturation Medium. Perform a cell count using Trypan Blue and a hemocytometer.
6. Proceed to section C for neuron maturation.

### C. MIDBRAIN NEURON MATURATION

1. Seed midbrain 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™ Midbrain 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$^2$.
2. Distribute cells evenly. Incubate at 37°C and 5% $CO_2$.
3. Perform a half-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.
4. Continue maturation of neurons for a minimum of 2 weeks. Neurons can be cultured for up to 5 weeks if prolonged maturation time is required.

## Assessment of Midbrain Neuronal Differentiation

Midbrain neuron differentiation may be assessed by immunocytochemistry using antibodies selective for the general neuronal marker tubulin III (e.g. Anti-Beta-Tubulin III Antibody, Clone TUJ1; Catalog #60052) and the dopaminergic neuron-specific marker tyrosine hydroxylase (e.g. Anti-Tyrosine Hydroxylase Antibody, Clone TH-2; Catalog #60058). 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.