STEMdiff™ Dopaminergic Neuron Differentiation Kit and STEMdiff™ Dopaminergic Neuron Maturation Kit

Catalog #08520 1 Kit
Catalog #08530 1 Kit

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Product Description

STEMdiff™ Dopaminergic Neuron Differentiation Kit (Catalog #08520) is used to generate dopaminergic 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 dopaminergic neuronal precursors generated can be matured using STEMdiff™ Dopaminergic Neuron Maturation Kit (Catalog #08530). STEMdiff™ Dopaminergic Neuron Differentiation Kit and STEMdiff™ Dopaminergic Neuron Maturation Kit are also compatible with cryopreserved NPCs (Human PSC-Derived NPCS; Catalog #70901) and cryopreserved dopaminergic neuron precursor cells (Human PSC-Derived Dopaminergic Neurons; Catalog #70909), respectively. These media will produce a population of midbrain dopaminergic neurons (15 - 30% TH-positive dopaminergic 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 #08520 or Catalog #08530) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMdiff™ Dopaminergic Neuron Differentiation Kit (Catalog #08520)				
STEMdiff™ Dopaminergic Neuron Differentiation Basal Medium	08521	100 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Dopaminergic Neuron Differentiation Supplement A	08522	8 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Dopaminergic Neuron Differentiation Supplement B	08523	2 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Dopaminergic Neuron Differentiation Supplement C	08524	300 µL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Dopaminergic Neuron Maturation Kit (Catalog #08530)				
STEMdiff™ Dopaminergic Neuron Maturation Basal Medium	08531	100 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Dopaminergic Neuron Maturation Supplement A	08532	160 µL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Dopaminergic Neuron Maturation Supplement B	08533	120 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
Recombinant Human Sonic Hedgehog (SHH)	Peprotech 100-45
Trypan Blue	07050

Dopaminergic Neuron Differentiation and Maturation Media

Generation of dopaminergic neurons from NPCs requires both STEMdiff™ Dopaminergic Neuron Differentiation Kit (Catalog #08520) and STEMdiff™ Dopaminergic Neuron Maturation Kit (Catalog #08530). If cryopreserved dopaminergic neuronal precursors are used, only STEMdiff™ Dopaminergic Neuron Maturation Kit 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™ DOPAMINERGIC DIFFERENTIATION KIT (Catalog #08520)	STEMdiff™ DOPAMINERGIC NEURON MATURATION KIT (Catalog #08530)
hPSCs	✓	✗	✓	✓
Cryopreserved NPCs (Catalog #70901 - 70904)	✗	✓	✓	✓

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).
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. For required volumes, see Table 2.
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).
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 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. Pre-warm coated vessel at 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™ DOPAMINERGIC NEURON DIFFERENTIATION MEDIUM

Use sterile techniques to prepare complete STEMdiff™ Dopaminergic Neuron Differentiation Medium (Differentiation Basal Medium + Differentiation Supplement A + Differentiation Supplement B + Differentiation Supplement C + SHH). 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 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 supplements 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 300 µL of Supplement C to 100 mL of Basal Medium. Mix thoroughly.

3. Add SHH at a concentration of 200 ng/mL. Mix thoroughly.

   NOTE: If not used immediately, store complete STEMdiff™ Dopaminergic 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™ DOPAMINERGIC NEURON MATURATION MEDIA

There are two formulations of complete STEMdiff™ Dopaminergic Neuron Maturation Medium:

- Medium 1 (Maturation Basal Medium + Maturation Supplement A) for day 13/14 to day 18/19 of culture
- Medium 2 (Maturation Basal Medium + Maturation Supplement B) for day 18/19+ of culture

Use sterile techniques to prepare complete STEMdiff™ Dopaminergic Neuron Maturation Media. For recommended volumes of medium for various cultureware, refer to Table 3.

MEDIUM 1 (DAY 13/14 TO DAY 18/19 OF CULTURE)

The following example is for preparing 40 mL of complete STEMdiff™ Dopaminergic Neuron Maturation Medium (**Medium 1**). If preparing other volumes, adjust accordingly.

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

2. Add 160 µL of Supplement A to 40 mL of Basal Medium. Mix thoroughly.

   NOTE: If not used immediately, store complete STEMdiff™ Dopaminergic Neuron Maturation Medium (**Medium 1**) at 2 - 8°C for up to 3 weeks. Warm complete medium to 37°C before use.

MEDIUM 2 (DAY 18/19+ OF CULTURE)

The following example is for preparing 60 mL of complete STEMdiff™ Dopaminergic Neuron Maturation Medium (**Medium 2**). If preparing other volumes, adjust accordingly.

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

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

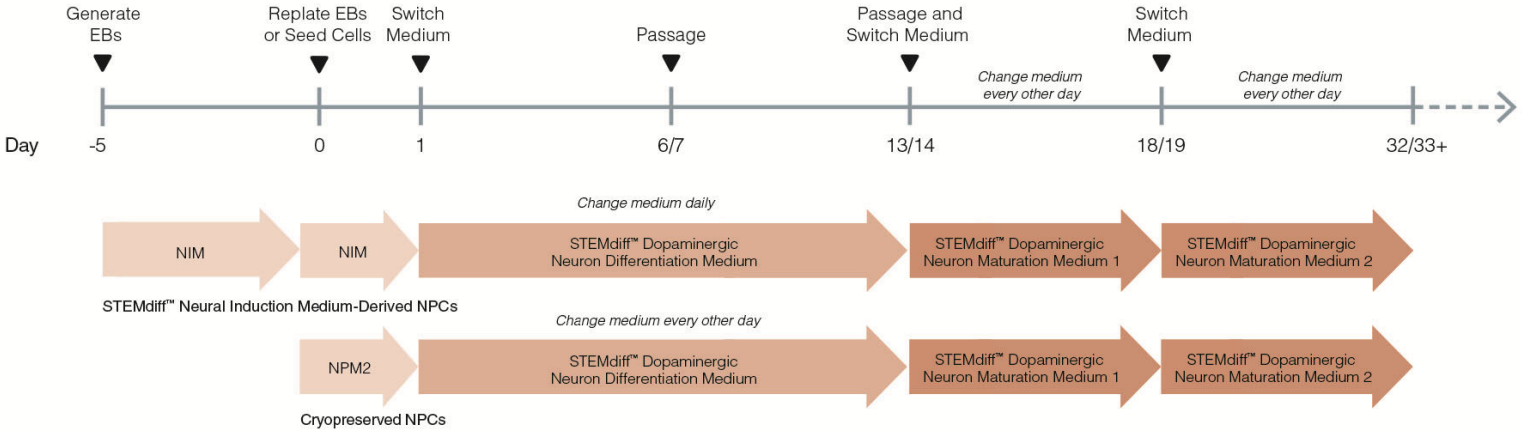
2. Add 120 µL of Supplement B to 60 mL of Basal Medium. Mix thoroughly.

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

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

CULTUREWARE	VOLUME OF COMPLETE STEMdiff™ DOPAMINERGIC 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 DOPAMINERGIC 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 #27865), refer to the Technical Manual: Generation and Culture of Neural Progenitor Cells Using the STEMdiff™ Neural System (Document #28782), available on our website at www.stemcell.com or contact us to request a copy.

Dopaminergic 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 5 after EB formation), replate EBs onto PLO/laminin-coated plates in 2 mL of STEMdiff™ Neural Induction Medium. Incubate at 37°C and 5% CO_2 for 24 hours.
2. On **day 1** (day 6 after EB formation), aspirate medium. Add 2 mL of complete STEMdiff™ Dopaminergic Neuron Differentiation Medium.
3. Incubate at 37°C and 5% CO_2 for 5 - 6 days, performing daily full medium changes with warm (37°C) complete STEMdiff™ Dopaminergic Neuron Differentiation Medium.

   NOTE: The optimal timing of application of complete STEMdiff™ Dopaminergic Neuron Differentiation Medium may vary from day 1 to day 4, depending on the cell line used.
4. On approximately **day 6/7** (day 11/12 after EB formation), perform neural rosette selection using STEMdiff™ Neural Rosette Selection Reagent. Replate in 2 mL of STEMdiff™ Dopaminergic 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) complete STEMdiff™ Dopaminergic Neuron Differentiation Medium.
6. On **day 13/14** (day 18/19 after EB formation), cells will reach approximately 80 - 90% confluence and will be ready to passage.

Passaging Neuronal Precursors

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

B. DIFFERENTIATION OF CRYOPRESERVED NPCs TO DOPAMINERGIC 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 #21378) and the PIS for Neural Progenitor Medium 2 (Document #DX20712), available on our website 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.

Dopaminergic Neuron 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; for other cultureware, refer to Table 3 and adjust volumes accordingly.

1. On **day 0**, aspirate medium and wash 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).
6. Perform a cell count using Trypan Blue and a hemocytometer.
7. Seed NPCs onto pre-warmed (37°C) PLO/laminin-coated cultureware at a density of 4 x 10^4 - 6 x 10^4 cells/cm^2 in complete Neural Progenitor Medium 2.
8. Distribute cells evenly and incubate at 37°C and 5% CO_2 for 24 hours.
9. On **day 1**, aspirate medium and replace with 2 mL of complete STEMdiff™ Dopaminergic Neuron Differentiation Medium. Incubate at 37°C and 5% CO_2 for 5 - 6 days, performing a full medium change every other day with warm (37°C) complete STEMdiff™ Dopaminergic Neuron Differentiation Medium.
10. On **day 6/7**, cells will reach 90 - 95% confluence. Passage cells according to steps 1 - 6.
11. Seed cells onto pre-warmed (37°C) PLO/laminin-coated cultureware at a density of 4 x 10^4 - 6 x 10^4 cells/cm^2 in complete STEMdiff™ Dopaminergic Neuron Differentiation Medium. Incubate at 37°C and 5% CO_2 for 7 days, performing a full medium change every other day with warm (37°C) complete STEMdiff™ Dopaminergic Neuron Differentiation Medium.
12. On **day 13/14**, cells will reach 90 - 95% confluence and will be ready for passaging.

Passaging Dopaminergic Neuronal Precursors

13. Aspirate medium and wash cells with 1 mL of sterile PBS to remove cell debris.
14. Add 1 mL of ACCUTASE™ and incubate at 37°C and 5% CO_2 for 5 minutes.
15. Add 5 mL of DMEM/F-12 and wash the cells off of the well.
16. Centrifuge cell suspension at 400 x *g* for 5 minutes. Remove and discard supernatant.
17. Resuspend cells in a suitable volume (e.g. 1 - 2 mL) of complete STEMdiff™ Dopaminergic Neuron Maturation Medium (**Medium 1**). Perform a cell count using Trypan Blue and a hemocytometer.
18. Proceed to section C for dopaminergic neuron maturation.

C. DOPAMINERGIC NEURON MATURATION

1. Seed dopaminergic 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™ Dopaminergic Neuron Maturation Medium (**Medium 1**). See **Table 3** for recommended volumes.

   NOTE: **The seeding density of dopaminergic 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, performing a full medium change every other day for 5 days with **Medium 1**.

   NOTE: To avoid cell detachment, perform medium changes slowly (dropwise), pointing the pipette tip toward the wall of the cell culture vessel.
3. On **day 18/19**, replace medium with STEMdiff™ Dopaminergic Neuron Maturation Medium (**Medium 2**). Incubate at 37°C and 5% CO_2.
4. Continue maturation of dopaminergic neurons in **Medium 2** for a minimum of 2 weeks, performing a full medium change every other day. Dopaminergic neurons can be cultured for up to 5 weeks if prolonged maturation time is required.

Assessment of Dopaminergic Neuron Differentiation

Dopaminergic neuron differentiation may be assessed by immunochemistry using antibodies selective for the general neuronal marker beta 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). 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.