

# STEMdiff™ Astrocyte Differentiation Kit STEMdiff™ Astrocyte Maturation Kit



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Catalog #100-0013 1 Kit  
Catalog #100-0016 1 Kit

**Differentiation and maturation kits for generation of astrocytes from hPSC-derived neural progenitor cells**

## Product Description

STEMdiff™ Astrocyte Differentiation Kit (Catalog #100-0013) is used to rapidly and efficiently generate astrocytic precursors from neural progenitor cells (NPCs) derived from human pluripotent stem cells (hPSCs) using STEMdiff™ SMADi Neural Induction Kit (Catalog #08581). These astrocytic precursors are then matured further into astrocytes using STEMdiff™ Astrocyte Maturation Kit (Catalog #100-0016). Using this system, a highly pure population of astrocytes (> 70% S100β-positive and > 60% GFAP-positive astrocytes; < 15% doublecortin-positive neurons) can be generated from hPSCs in as little as 7 weeks and can be maintained long-term in culture. 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 #100-0013 or 100-0016) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMdiff™ Astrocyte Differentiation Kit (Catalog #100-0013)				
STEMdiff™ Astrocyte Differentiation Basal Medium	100-0014	80 mL	Store at 2 - 8°C.	Stable for 12 months from date of manufacture (MFG) on label.
STEMdiff™ Astrocyte Differentiation Supplement	100-0015	20 mL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.
STEMdiff™ Astrocyte Maturation Kit (Catalog #100-0016)				
STEMdiff™ Astrocyte Maturation Basal Medium	100-0035	80 mL	Store at 2 - 8°C.	Stable for 12 months from date of manufacture (MFG) on label.
STEMdiff™ Astrocyte Maturation Supplement A	100-0037	20 mL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.
STEMdiff™ Astrocyte Maturation Supplement B*	100-0017	1 mL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.

\* Lot-to-lot color variations include light to dark yellow or brown. This will not affect performance.

## Materials Required But Not Included

PRODUCT NAME	CATALOG #
Corning® Matrigel® hESC-Qualified Matrix	Corning 354277
DMEM/F-12 with 15 mM HEPES	36254
STEMdiff™ SMADi Neural Induction Kit	08581
ACCUTASE™	07920
Trypan Blue	07050

## Preparation of Reagents and Materials

### A. COATING CULTUREWARE WITH CORNING® MATRIGEL®

Corning® Matrigel® hESC-Qualified Matrix should be aliquoted and frozen. Consult the Matrigel® Certificate of Analysis for the recommended aliquot size ("Dilution Factor") to prepare 25 mL of diluted matrix. Ensure to always keep Matrigel® on ice when thawing and handling to prevent it from gelling.

NOTE: Use tissue culture-treated cultureware.

1. Thaw one aliquot of Matrigel® on ice.
2. Dispense 25 mL of cold DMEM/F-12 into a 50 mL conical tube and keep on ice.
3. Add thawed Matrigel® to the cold DMEM/F-12 (in the 50 mL tube) and mix thoroughly. 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 Matrigel® solution evenly across the surface.

NOTE: If the surface of the cultureware is not fully coated by the 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 (15 - 25°C) for 30 minutes before continuing to step 7.
7. Immediately prior to seeding cells, gently tilt the cultureware onto one side and allow the excess Matrigel® 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 of Diluted Matrigel® for Coating Cultureware**

CULTUREWARE	APPROXIMATE SURFACE AREA	VOLUME OF DILUTED MATRIGEL®
96-well plate	0.33 cm <sup>2</sup> /well	50 µL/well
4- or 24-well plate	2 cm <sup>2</sup> /well	250 µL/well
6-well plate	10 cm <sup>2</sup> /well	1.5 mL/well
35 mm dish	10 cm <sup>2</sup>	1.5 mL
60 mm dish	20 cm <sup>2</sup>	2.5 mL

### B. PREPARATION OF STEMdiff™ ASTROCYTE DIFFERENTIATION MEDIUM

Use sterile technique to prepare STEMdiff™ Astrocyte Differentiation Medium (Differentiation Basal Medium + Differentiation Supplement). 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.  
NOTE: If not used immediately, aliquot the supplement and store at -20°C. Do not exceed the shelf life of the supplement. Once aliquots are thawed, do not re-freeze.
2. Add 20 mL of Differentiation Supplement to 80 mL of Differentiation Basal Medium. Mix thoroughly.  
NOTE: If not used immediately, store STEMdiff™ Astrocyte Differentiation Medium at 2 - 8°C for up to 2 weeks. Warm complete medium to 37°C before use.

### C. PREPARATION OF STEMdiff™ ASTROCYTE MATURATION MEDIUM

Use sterile technique to prepare STEMdiff™ Astrocyte Maturation Medium (Maturation Basal Medium + Maturation Supplement A + Maturation Supplement B). The following example is for preparing 100 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw Maturation Supplements A & B at room temperature (15 - 25°C) or at 2 - 8°C overnight. Mix thoroughly.  
NOTE: If not used immediately, aliquot the supplements and store at -20°C. Do not exceed the shelf life of the supplements. Once aliquots are thawed, do not re-freeze.
2. Add 20 mL of Maturation Supplement A and 1 mL of Maturation Supplement B to 80 mL of Maturation Basal Medium. Mix thoroughly.  
NOTE: If not used immediately, store STEMdiff™ Astrocyte Maturation Medium at 2 - 8°C for up to 2 weeks. Warm complete medium to 37°C before use.

## Directions for Use

Please read the entire protocol before proceeding. Use sterile technique when performing the following protocols:

### A. Differentiation of hPSCs to Astrocytic Precursors

- I. Starting from the EB Protocol
- II. Starting from the Monolayer Protocol

### B. Passaging Astrocytic Precursors

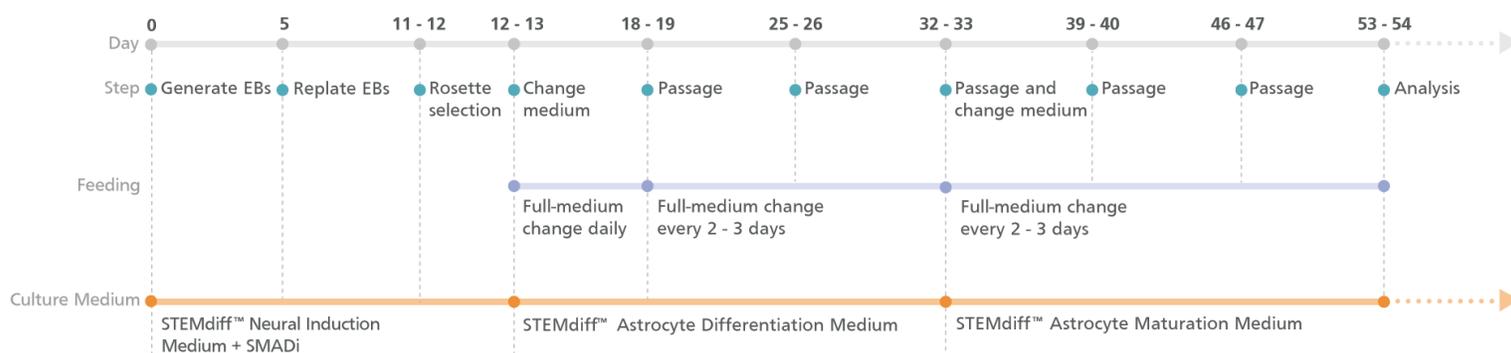
### C. Astrocyte Maturation

## A. DIFFERENTIATION OF hPSCs TO ASTROCYTIC PRECURSORS

The following protocols integrate into the STEMdiff™ SMADi Neural Induction Kit (Catalog #08581) embryoid body (EB) protocol after rosette selection (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) 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](http://www.stemcell.com) or contact us to request a copy.

### I. Starting from the EB Protocol

#### Protocol Diagram

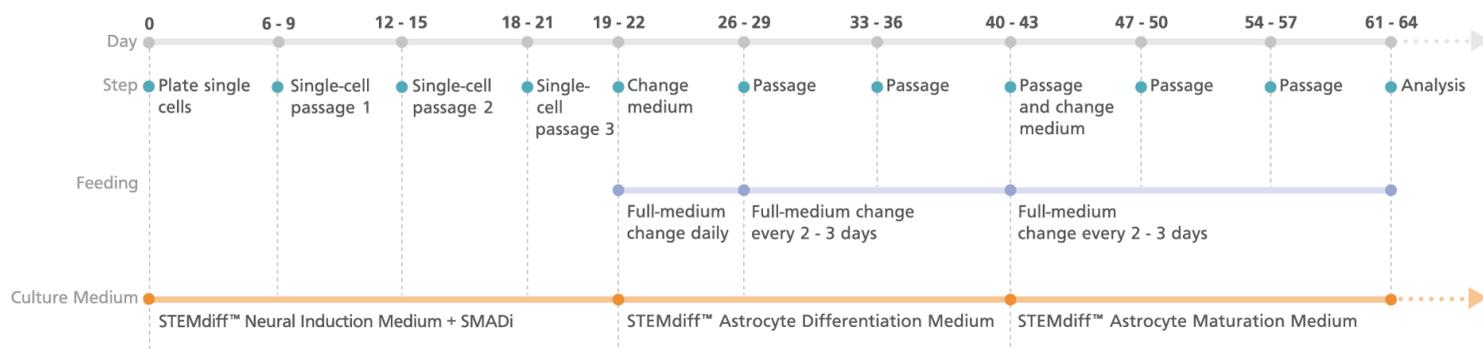


The following instructions are for a single well of a 6-well plate; if using other cultureware, adjust volumes accordingly.

1. **Day 11 - 12 after EB formation:** Place selected neural rosettes into a Matrigel®-coated well of a 6-well plate containing 2 mL STEMdiff™ Neural Induction Medium + SMADi. Incubate at 37°C and 5% CO<sub>2</sub> for 24 hours.
2. **Day 12 - 13 after EB formation:** Aspirate medium and replace with 2 mL of STEMdiff™ Astrocyte Differentiation Medium. Incubate at 37°C and 5% CO<sub>2</sub>, performing a full-medium change daily with warm (37°C) STEMdiff™ Astrocyte Differentiation Medium.
3. **Day 18 - 19 after EB formation:** Cells will reach 80 - 90% confluence and will be ready for passaging. 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.

- Day 18 - 21 (Passage 3) of the monolayer protocol:** Passage 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).
- Add cells to a Matrigel®-coated well of a 6-well plate at a density of  $1.5 - 2 \times 10^5$  cells/cm<sup>2</sup> in 2 mL STEMdiff™ Neural Induction Medium + SMADi. Incubate at 37°C and 5% CO<sub>2</sub> for 24 hours.  
NOTE: Cell plating density may need to be optimized for each cell line.
- Day 19 - 22:** Aspirate medium and add 2 mL STEMdiff™ Astrocyte Differentiation Medium. Incubate at 37°C and 5% CO<sub>2</sub>.
- Perform a full-medium change daily with warm (37°C) STEMdiff™ Astrocyte Differentiation Medium. Incubate at 37°C and 5% CO<sub>2</sub>.
- Day 26 - 29:** Cells will reach 80 - 90% confluence and will be ready to passage. Proceed to section B.

## B. PASSAGING ASTROCYTIC PRECURSORS

The following instructions are for a single well of a 6-well plate. If using other cultureware, adjust volumes accordingly. The indicated incubation times of 7 days may be adjusted to 6 - 8 days.

- Aspirate medium from the well and add 1 mL ACCUTASE™.
- Incubate at 37°C and 5% CO<sub>2</sub> for 5 - 10 minutes.
- Add 5 mL DMEM/F-12 and wash the cells off of the well. Transfer cell suspension to a 15 mL conical tube (e.g. Catalog #38009).
- Centrifuge at 400 x g for 5 minutes. Remove and discard supernatant.
- Resuspend cells in a suitable volume (e.g. 5 mL) of STEMdiff™ Astrocyte Differentiation Medium. Perform a cell count using Trypan Blue and a hemocytometer.
- Seed cells onto Matrigel®-coated cultureware at a density of  $1 - 1.5 \times 10^5$  cells/cm<sup>2</sup>.
- Incubate at 37°C and 5% CO<sub>2</sub> for 7 days, performing a full-medium change every other day with warm (37°C) STEMdiff™ Astrocyte Differentiation Medium.
- On **day 13 - 14** (25 - 26 days after EB formation), cells will be approximately 80 - 90% confluent. Passage cells according to steps 1 - 6, seeding cells onto new Matrigel®-coated cultureware at a density of  $1.5 - 2 \times 10^5$  cells/cm<sup>2</sup>.
- Incubate at 37°C and 5% CO<sub>2</sub> for 7 days, performing a full-medium change every other day with warm (37°C) STEMdiff™ Astrocyte Differentiation Medium. Proceed to section C.

## C. ASTROCYTE MATURATION

Prepare STEMdiff™ Astrocyte Maturation Medium. Passage cells as described below.

The following instructions are for a single well of a 6-well plate; if using other cultureware, adjust volumes accordingly. The indicated incubation times of 7 days may be adjusted to 6 - 8 days.

- After 20 - 21 days of astrocyte differentiation,** aspirate medium from the well and add 1 mL ACCUTASE™.
- Incubate at 37°C and 5% CO<sub>2</sub> for 5 - 10 minutes.
- Add 5 mL of DMEM/F-12 and wash cells off the well. Transfer cell suspension to a 15 mL conical tube.
- Centrifuge at 400 x g for 5 minutes. Remove and discard supernatant.
- Resuspend cells in a suitable volume of STEMdiff™ Astrocyte Maturation Medium (e.g. 5 mL). Perform a cell count using Trypan Blue and a hemocytometer.
- Seed cells onto Matrigel®-coated cultureware at a density of  $1.5 - 2 \times 10^5$  cells/cm<sup>2</sup>.
- Incubate at 37°C and 5% CO<sub>2</sub> for 7 days, performing a full-medium change every other day with warm (37°C) STEMdiff™ Astrocyte Maturation Medium.
- Passage cells according to steps 1 - 6.  
NOTE: Recommended seeding density is  $1.5 - 2 \times 10^5$  cells/cm<sup>2</sup>; if astrocytes are to be used for immunocytochemistry, use a lower seeding density of  $5 \times 10^4$  to  $1 \times 10^5$  cells/cm<sup>2</sup>.
- Incubate at 37°C and 5% CO<sub>2</sub>, performing a full-medium change every other day with warm (37°C) STEMdiff™ Astrocyte Maturation Medium.
- After two passages in STEMdiff™ Astrocyte Maturation Medium, mature astrocytes (S100β+, GFAP+) will be visible.

## Assessment of Astrocyte Differentiation

Astrocyte differentiation may be assessed by immunocytochemistry using antibodies selective for the astrocyte-specific marker S100 $\beta$ . Further assessment can be done using antibodies selective for other glial/neuron markers such as GFAP (e.g. Anti-GFAP Antibody, Polyclonal; Catalog #60128 or Anti-GFAP Antibody, Clone 2E1.E9; Catalog #60048),  $\beta$ III-Tubulin, or doublecortin (DCX). 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](http://www.stemcell.com/hPSCNCworkflow) or contact us at [techsupport@stemcell.com](mailto:techsupport@stemcell.com).

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