

# 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 2 years from date of manufacture (MFG) on label.
STEMdiff™ Astrocyte Differentiation Supplement	100-0015	20 mL	Store at -20°C.	Stable for 2 years 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 2 years from date of manufacture (MFG) on label.
STEMdiff™ Astrocyte Maturation Supplement A	100-0037	20 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Astrocyte Maturation Supplement B*	100-0017	1 mL	Store at -20°C.	Stable for 2 years 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 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

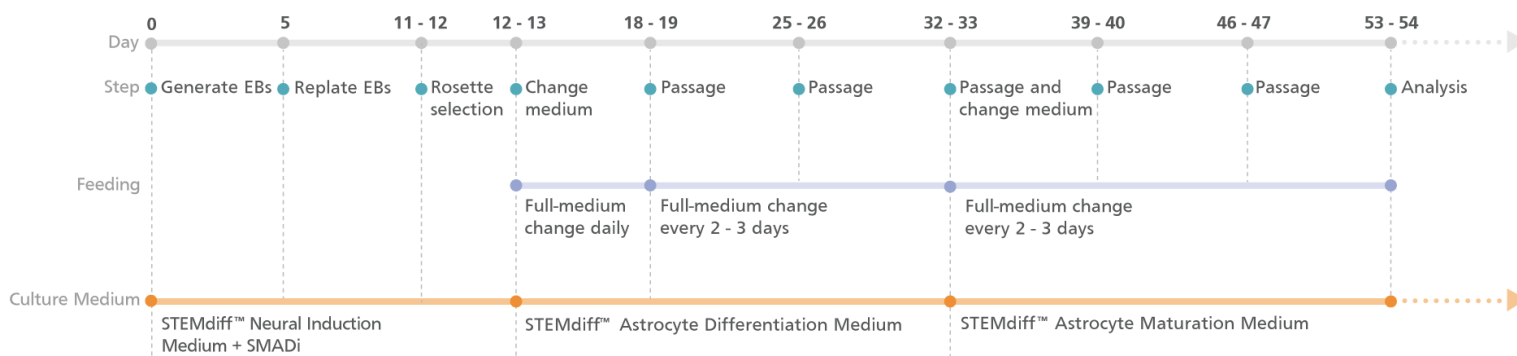
### C. Astrocyte Maturation

## 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 (Document #1000005588), available at [www.stemcell.com](http://www.stemcell.com) or contact us to request a copy.

### I. Starting from the EB Protocol

#### Protocol Diagram

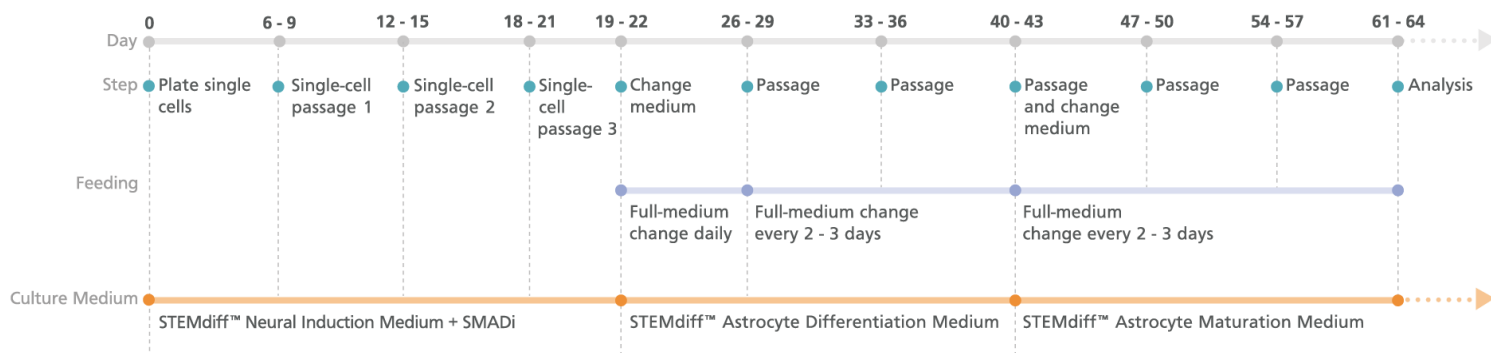


The following instructions are for a single well of a 6-well plate; if using other cultureware, adjust volumes accordingly.

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.

1. **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).
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.
3. **Day 19 - 22:** Aspirate medium and add 2 mL STEMdiff™ Astrocyte Differentiation Medium. Incubate at 37°C and 5% CO<sub>2</sub>.
4. Perform a full-medium change daily with warm (37°C) STEMdiff™ Astrocyte Differentiation Medium. Incubate at 37°C and 5% CO<sub>2</sub>.
5. **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.

1. Aspirate medium from the well and add 1 mL ACCUTASE™.
2. Incubate at 37°C and 5% CO<sub>2</sub> for 5 - 10 minutes.
3. 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).
4. Centrifuge at 400 x g for 5 minutes. Remove and discard supernatant.
5. 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.
6. Seed cells onto Matrigel®-coated cultureware at a density of  $1 - 1.5 \times 10^5$  cells/cm<sup>2</sup>.
7. 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.
8. 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>.
9. 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.

1. **After 20 - 21 days of astrocyte differentiation**, aspirate medium from the well and add 1 mL ACCUTASE™.
2. Incubate at 37°C and 5% CO<sub>2</sub> for 5 - 10 minutes.
3. Add 5 mL of DMEM/F-12 and wash cells off the well. Transfer cell suspension to a 15 mL conical tube.
4. Centrifuge at 400 x g for 5 minutes. Remove and discard supernatant.
5. 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.
6. Seed cells onto Matrigel®-coated cultureware at a density of  $1.5 - 2 \times 10^5$  cells/cm<sup>2</sup>.
7. 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.
8. 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>.
9. 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.
10. 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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