# STEMdiff™ Astrocyte Differentiation Kit
# STEMdiff™ Astrocyte Maturation Kit

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

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

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## 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 18 months from date of manufacture (MFG) on label. |
| STEMdiff™ Astrocyte Differentiation Supplement | 100-0015 | 20 mL | Store at -20°C. | Stable for 18 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 18 months from date of manufacture (MFG) on label. |
| STEMdiff™ Astrocyte Maturation Supplement A | 100-0037 | 20 mL | Store at -20°C. | Stable for 18 months from date of manufacture (MFG) on label. |
| STEMdiff™ Astrocyte Maturation Supplement B* | 100-0017 | 1 mL | Store at -20°C. | Stable for 18 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 $cm^2$/well | 50 µL/well |
| 4- or 24-well plate | 2 $cm^2$/well | 250 µL/well |
| 6-well plate | 10 $cm^2$/well | 1.5 mL/well |
| 35 mm dish | 10 $cm^2$ | 1.5 mL |
| 60 mm dish | 20 $cm^2$ | 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:

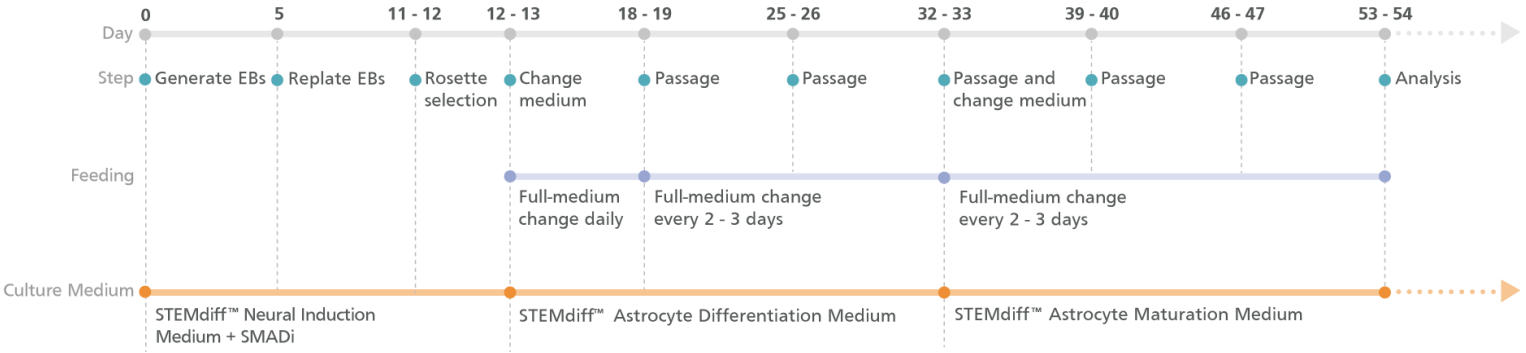
A. Differentiation of hPSCs to Astrocytic Precursors
   I. Starting from the EB Protocol
   II. Starting from the Monolayer Protocol
B. Passaging Astrocytic Precursors
C. Astrocyte Maturation

## 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 #10000005588), available at www.stemcell.com or contact us to request a copy.

### I. Starting from the EB Protocol

#### Protocol Diagram

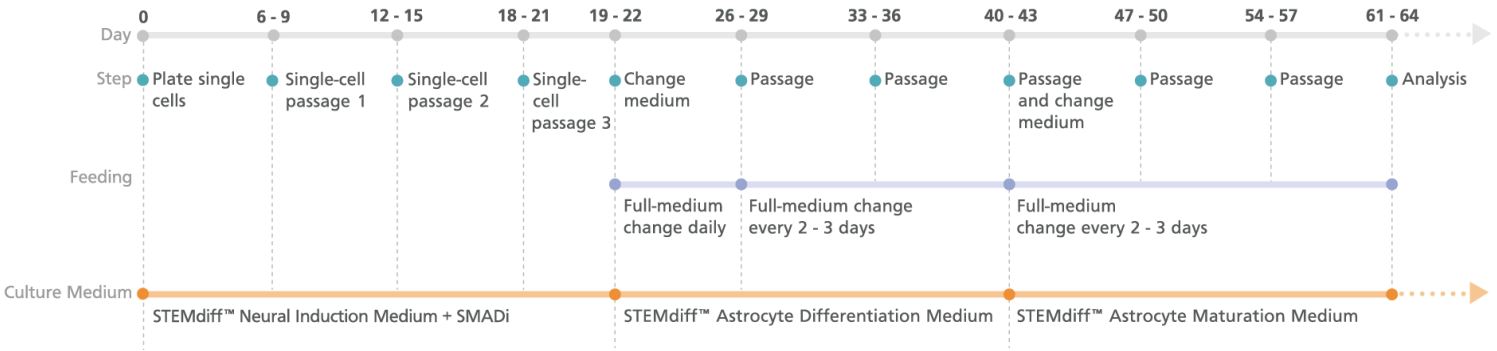



The following instructions are for a single well of a 6-well plate; if using other cultureware, adjust volumes accordingly.

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% $CO_2$ 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% $CO_2$, 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 x 10^5 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™ Astrocyte Differentiation Medium. Incubate at 37°C and 5% $CO_2$.
4. Perform a full-medium change daily with warm (37°C) STEMdiff™ Astrocyte Differentiation Medium. Incubate at 37°C and 5% $CO_2$.
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% $CO_2$ 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 x 10^5 cells/cm$^2$.
7. Incubate at 37°C and 5% $CO_2$ 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 x 10^5 cells/cm$^2$.
9. Incubate at 37°C and 5% $CO_2$ 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% $CO_2$ 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 x 10^5 cells/cm$^2$.
7. Incubate at 37°C and 5% $CO_2$ 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 x 10^5 cells/cm$^2$; if astrocytes are to be used for immunocytochemistry, use a lower seeding density of 5 x 10^4 to 1 x 10^5 cells/cm$^2$.
9. Incubate at 37°C and 5% $CO_2$, 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 immunochemistry using antibodies selective for the astrocyte-specific marker S100β. 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), βIII-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 or contact us at techsupport@stemcell.com.