STEMdiff™ Astrocyte Differentiation Kit and STEMdiff™ Astrocyte Maturation Kit

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Catalog #08540 1 Kit
Catalog #08550 1 Kit

Product Description

STEMdiff™ Astrocyte Differentiation Kit (Catalog #08540) is used to rapidly and efficiently generate astrocyte 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 astrocyte precursors generated can be matured using STEMdiff™ Astrocyte Maturation Kit (Catalog #08550). STEMdiff™ Astrocyte Differentiation Kit and STEMdiff™ Astrocyte Maturation Kit are also compatible with cryopreserved NPCs (Human PSC-Derived NPCs; Catalog #70901) and cryopreserved astrocyte precursor cells (Human PSC-Derived Astrocytes; Catalog #70913), respectively. These media will produce a highly pure population of GFAP-positive astrocytes (> 85% GFAP-positive astrocytes; < 15% class III β-tubulin-positive neurons). 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 #08540 or Catalog #08550) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMdiff™ Astrocyte Differentiation Kit (Catalog #08540)				
STEMdiff™ Astrocyte Differentiation Basal Medium	08541	100 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Astrocyte Differentiation Supplement A	08542	8 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Astrocyte Differentiation Supplement B	08543	2 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Astrocyte Differentiation Supplement C	08544	100 µL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Astrocyte Maturation Kit (Catalog #08550)				
STEMdiff™ Astrocyte Maturation Basal Medium	08551	100 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Astrocyte Maturation Supplement A	08552	9 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Astrocyte Maturation Supplement B	08553	2 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Astrocyte Maturation Supplement C	08554	100 µL	Store at -20°C.	Stable until expiry date (EXP) on label.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
Corning® Matrigel® hESC-qualified Matrix	Corning 354277
DMEM/F-12 with 15 mM HEPES	36254
ACCUTASE™	07920
STEMdiff™ Neural Rosette Selection Reagent	05832
Trypan Blue	07050

Astrocyte Differentiation and Maturation Media

Generation of astrocytes from NPCs requires both STEMdiff™ Astrocyte Differentiation Kit (Catalog #08540) and STEMdiff™ Astrocyte Maturation Kit (Catalog #08550). If cryopreserved astrocyte precursors (Catalog #70913 or 70916) are used, only STEMdiff™ Astrocyte Maturation Kit (Catalog #08550) 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™ ASTROCYTE DIFFERENTIATION KIT (Catalog #08540)	STEMdiff™ ASTROCYTE MATURATION KIT (Catalog #08550)
hPSCs	✓	×	✓	✓
Cryopreserved NPCs (Catalog #70901 - 70904)	×	✓	✓	✓

Preparation of Reagents and Materials

A. COATING CULTUREWARE WITH CORNING® MATRIGEL®

Corning® Matrigel® hESC-qualified Matrix should be aliquoted and frozen. Consult the Certificate of Analysis supplied with Corning® Matrigel® for the recommended aliquot size ("Dilution Factor") to prepare 24 mL of diluted matrix. Ensure to always keep Corning® Matrigel® on ice when thawing and handling to prevent it from gelling.

NOTE: Use tissue culture-treated cultureware.

1. Thaw one aliquot of Corning® Matrigel® on ice.
2. Dispense 24 mL of cold DMEM/F-12 into a 50 mL conical tube and keep on ice.
3. Add thawed Corning® Matrigel® to the cold DMEM/F-12 (in the 50 mL tube) and mix well. The vial may be washed with cold medium if desired.
4. Immediately use the diluted Corning® Matrigel® solution to coat tissue culture-treated cultureware. Refer to Table 2 for recommended coating volumes.
5. Swirl the cultureware to spread the Corning® Matrigel® solution evenly across the surface.

   NOTE: If the surface of the cultureware is not fully coated by the Corning® Matrigel® 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 Corning® Matrigel® solution evaporate.

   NOTE: If not used immediately, the cultureware must be sealed to prevent evaporation of the Corning® 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 the next step.
7. Immediately prior to seeding cells, gently tilt the cultureware onto one side and allow the excess Corning® Matrigel® solution to collect at the edge. Remove the excess Corning® Matrigel® solution using a serological pipette or by aspiration. Ensure that the coated surface is not scratched.

Table 2: Recommended Volumes of Corning® Matrigel® for Coating Cultureware

CULTUREWARE	APPROXIMATE SURFACE AREA	VOLUME OF CORNING® 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 COMPLETE STEMdiff™ ASTROCYTE DIFFERENTIATION MEDIUM

Use sterile techniques to prepare complete STEMdiff™ Astrocyte Differentiation Medium (Differentiation Basal Medium + Differentiation Supplement A + Differentiation Supplement B + Differentiation Supplement C). 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 Supplements B & C and store 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 100 µL of Supplement C to 100 mL of Basal Medium. Mix thoroughly.
   NOTE: If not used immediately, store complete STEMdiff™ Astrocyte Differentiation Medium at 2 - 8°C for up to 10 days. Warm complete medium to 37°C before use.

C. PREPARATION OF COMPLETE STEMdiff™ ASTROCYTE MATURATION MEDIUM

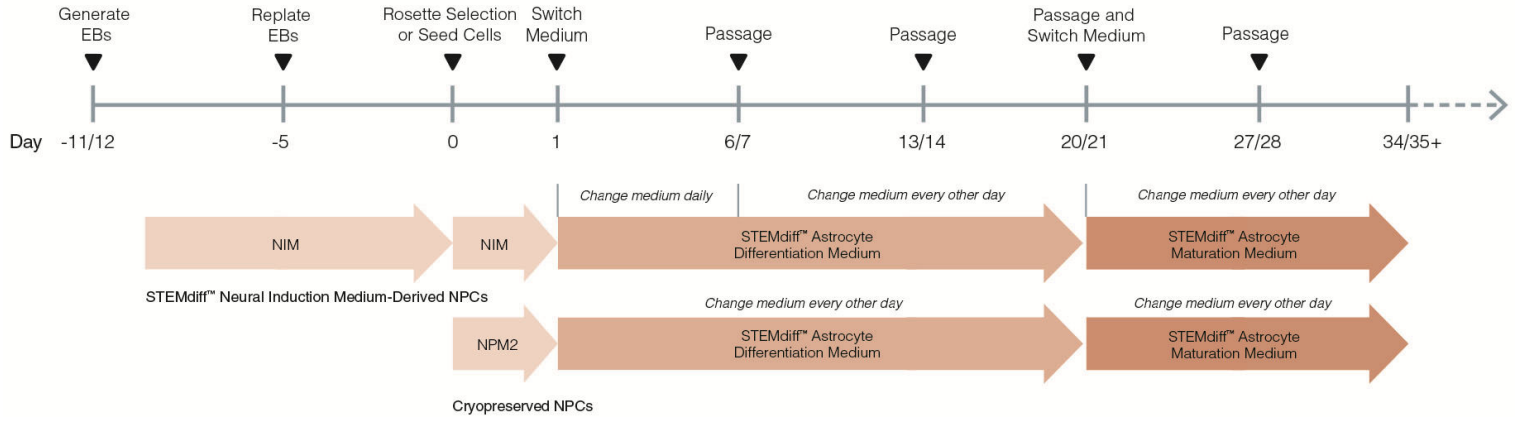
Use sterile techniques to prepare complete STEMdiff™ Astrocyte Maturation Medium (Maturation Basal Medium + Maturation Supplement A + Maturation Supplement B + Maturation Supplement C). The following example is for preparing 100 mL of complete medium. If preparing other volumes, adjust accordingly. For 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 Supplements B & C and store at -20°C. Do not exceed the shelf life of the supplements. Once aliquots are thawed, do not re-freeze.
2. Add 9 mL of Supplement A, 2 mL of Supplement B, and 100 µL of Supplement C to 100 mL of Basal Medium. Mix thoroughly.
   NOTE: If not used immediately, store complete STEMdiff™ Astrocyte Maturation Medium at 2 - 8°C for up to 1 week. Warm complete medium to 37°C before use.

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

CULTUREWARE	VOLUME OF COMPLETE STEMdiff™ ASTROCYTE 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.

A. DIFFERENTIATION OF STEMdiff™ NEURAL INDUCTION MEDIUM-DERIVED NPCs TO ASTROCYTE 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.

Astrocyte 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 11/12 after EB formation), place selected neural rosettes into a Corning® Matrigel®-coated well of a 6-well plate in 2 mL of STEMdiff™ Neural Induction Medium. Incubate at 37°C and 5% CO_2 for 24 hours.
2. On **day 1** (day 12/13 after EB formation), aspirate medium and replace with 2 mL of complete STEMdiff™ Astrocyte Differentiation Medium.
3. Incubate at 37°C and 5% CO_2, performing daily full medium changes with warm (37°C) complete STEMdiff™ Astrocyte Differentiation Medium.
4. On **day 6/7** (day 17 - 19 after EB formation), cells will reach 90 - 95% confluence and will be ready for passaging.

Passaging Astrocyte Precursors

5. Aspirate medium and wash cells with 1 mL of sterile PBS to remove cell debris.
6. Add 1 mL of ACCUTASE™ and incubate at 37°C and 5% CO_2 for 5 - 10 minutes.
7. Add 5 mL of DMEM/F-12 and wash the cells off of the well.
8. Centrifuge cell suspension at 400 x *g* for 5 minutes. Remove and discard supernatant.
9. Resuspend cells in a suitable volume (e.g. 5 mL) of complete STEMdiff™ Astrocyte Differentiation Medium. Perform a cell count using Trypan Blue and a hemocytometer.
10. Seed cells onto Corning® Matrigel®-coated cultureware at a density of 1 x 10^5 cells/cm^2.
11. 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™ Astrocyte Differentiation Medium.
12. On **day 13/14** (24 - 26 days after EB formation) cells will be approximately 90 - 95% confluent. Passage cells according to section B steps 1 - 6. Seed cells onto new Corning® Matrigel®-coated cultureware at a density of 1.5 x 10^5 cells/cm^2.
13. 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™ Astrocyte Differentiation Medium.
14. On **day 20/21** (31 - 33 days after EB formation) cells will be approximately 90 - 95% confluent. Proceed to section C for astrocyte maturation.

B. DIFFERENTIATION OF CRYOPRESERVED NPCs TO ASTROCYTE 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.

Astrocyte Differentiation

Cryopreserved NPCs are ready for passaging 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. 1 - 2 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 Corning® Matrigel®-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 STEMdiff™ Astrocyte Differentiation Medium.
10. 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™ Astrocyte Differentiation Medium.
11. On **day 6/7**, cells will be 90 - 95% confluent and will be ready for passaging.

Passaging Astrocyte Precursors

12. Aspirate medium and wash cells with 1 mL of sterile PBS to remove cell debris.
13. Add 1 mL of ACCUTASE™ and incubate at 37°C and 5% CO_2 for 5 minutes.
14. Add 5 mL DMEM/F-12 and wash the cells off the well.
15. Centrifuge cell suspension at 400 x *g* for 5 minutes. Remove and discard supernatant.
16. Resuspend cells in suitable volume (e.g. 1 - 2 mL) of complete STEMdiff™ Astrocyte Differentiation Medium.
17. Perform a cell count using Trypan Blue and a hemocytometer.
18. Seed cells onto pre-warmed Corning® Matrigel®-coated cultureware at a density of 1 x 10^5 cells/cm^2.
19. Incubate at 37°C and 5% CO_2 for 6 - 7 days, performing a full medium change every other day with warm (37°C) complete STEMdiff™ Astrocyte Differentiation Medium.
20. On **day 13/14**, cells will reach 90 - 95% confluence. Passage cells according to steps 12 - 18.
21. Incubate at 37°C and 5% CO_2 for 6 - 7 days, performing a full medium change every other day with warm (37°C) complete STEMdiff™ Astrocyte Differentiation Medium.
22. On **day 20/21**, cells will reach 90 - 95% confluence. Proceed to section C for astrocyte maturation.

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

1. Aspirate medium and add 1 mL of 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 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 of complete STEMdiff™ Astrocyte Maturation Medium (e.g. 5 mL). Perform a cell count using Trypan Blue and a hemocytometer.
6. Seed cells onto Corning® Matrigel®-coated cultureware at a density of 1.5 x 10^5 cells/cm^2.
7. Incubate at 37°C and 5% CO_2 for 6 - 7 days, performing a full medium change every other day with warm (37°C) complete STEMdiff™ Astrocyte Maturation Medium.
8. On **day 27/28**, cells will be approximately 90 - 95% confluent. Passage cells according to steps 1 - 6. Incubate at 37°C and 5% CO_2, performing a full medium change every other day with warm (37°C) complete STEMdiff™ Astrocyte Maturation Medium.
9. After two passages in STEMdiff™ Astrocyte Maturation Medium, mature astrocytes (GFAP+) will be observed.

   NOTE: Recommended seeding densities range from 1.5 x 10^5 cells/cm^2 to 2 x 10^5 cells/cm^2. If astrocytes are to be used for immunocytochemistry, a lower seeding density is recommended, ranging from 5 x 10^4 cells/cm^2 to 1 x 10^5 cells/cm^2.

   NOTE: Astrocytes can continue to be passaged up to day 100 or longer if desired.

Assessment of Astrocyte Differentiation

Astrocyte differentiation may be assessed by immunochemistry using antibodies selective for the astrocyte-specific marker GFAP (e.g. Anti-GFAP Antibody, Polyclonal; Catalog #60128 or Anti-GFAP Antibody, Clone 2E1.E9; Catalog #60048). 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.