STEMdiff™ Astrocyte Differentiation Kit
STEMdiff™ Astrocyte Serum-Free Maturation Kit

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

Catalog #100-0013 1 Kit
Catalog #100-1666 1 Kit

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Product Description

STEMdiff™ Astrocyte Differentiation Kit (Catalog #100-0013) is used to rapidly and efficiently generate astrocytic precursor cells from neural progenitor cells (NPCs) derived from human pluripotent stem cells (hPSCs) using STEMdiff™ SMADi Neural Induction Kit (Catalog #08581). These astrocytic precursor cells are then matured into astrocytes using STEMdiff™ Astrocyte Serum-Free Maturation Kit (Catalog #100-1666). This is the first completely serum-free kit available on the market for differentiation of hPSCs to astrocytes. 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. hPSC-derived astrocytes generated using this system 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 #100-1666) 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 Serum-Free Maturation Kit (Catalog #100-1666)				
STEMdiff™ Astrocyte Serum-Free Maturation Basal Medium	100-1667	70 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 Serum-Free Maturation Supplement	100-1665	10 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
ACCUTASE™	07920
Conical tubes, 15 mL and 50 mL	e.g. 38009 and 38010
Corning® Matrigel® hESC-Qualified Matrix	Corning 354277
CryoStor® CS10	07930
DMEM/F-12 with 15 mM HEPES	36254
Hausser Scientific™ Bright-Line Hemocytometer	100-1181
Trypan Blue	07050
Y-27632 (Dihydrochloride)	72302

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. If desired, wash the vial with cold medium.
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
24-well plate	2 cm^2/well	250 µL/well
12-well plate	4 cm^2/well	500 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 using immediately, aliquot the supplement and store at -20°C. Do not exceed the shelf life of the supplement. After thawing aliquots, use immediately. Do not re-freeze.
2. Add 20 mL of Differentiation Supplement to 80 mL of Differentiation Basal Medium. Mix thoroughly. Warm complete medium to room temperature before use.

   NOTE: If not using 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 SERUM-FREE MATURATION MEDIUM

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

1. Thaw Maturation Supplement A and Serum-Free Maturation Supplement at room temperature (15 - 25°C) or at 2 - 8°C overnight. Mix thoroughly.

   NOTE: If not using immediately, aliquot and store at -20°C. Do not exceed the shelf life of the supplements. After thawing aliquots, use immediately. Do not re-freeze.
2. Add 20 mL of Maturation Supplement A and 10 mL of Serum-Free Maturation Supplement to 70 mL of Serum-Free Maturation Basal Medium. Mix thoroughly. Warm complete medium to room temperature before use.

   NOTE: If not using immediately, store STEMdiff™ Astrocyte Serum-Free Maturation Medium at 2 - 8°C for up to 2 weeks. Do not freeze complete medium.

Directions for Use

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

A. Obtaining NPCs to Initiate Astrocyte Differentiation
   I. Starting from the EB Protocol
   II. Starting from the Monolayer Protocol
   III. Starting from Cryopreserved Human iPSC-Derived NPCs
   IV. Starting from Expanded NPC Monolayer Culture
B. Continuation of Astrocyte Differentiation
C. Astrocyte Maturation
D. Cryopreserving Astrocyte Precursor Cells or Astrocytes
E. Thawing Astrocyte Precursor Cells or Astrocytes

A. OBTAINING NPCs TO INITIATE ASTROCYTE DIFFERENTIATION

Astrocyte differentiation requires a high-quality starting population of NPCs. hPSC-derived NPCs can be generated with STEMdiff™ SMADi Neural Induction Kit (Catalog #08581) following a 3D embryoid body (EB; section I) or 2D monolayer (section II) neural induction workflow.

Additionally, cryopreserved NPCs (section III) or fresh NPCs maintained in STEMdiff™ Neural Progenitor Medium (section IV) may also be used for astrocyte differentiation.

I. Starting from the EB Protocol

For complete instructions on generating central nervous system (CNS)-type NPCs through EB formation with AggreWell™800 plates (Catalog #34811), refer to section 5.1 in 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. For complete instructions on preparing STEMdiff™ Neural Induction Medium + SMADi, refer to the Product Information Sheet (PIS) for STEMdiff™ SMADi Neural Induction Kit (Catalog #08581), available at www.stemcell.com.

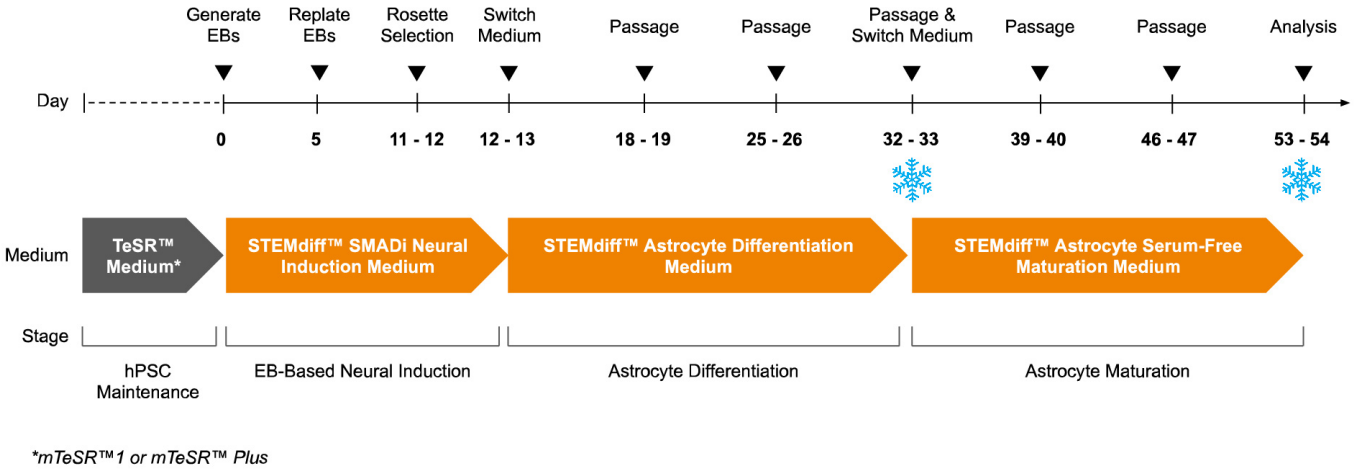


Figure 1. Protocol for Obtaining NPCs via EB-Based Neural Induction

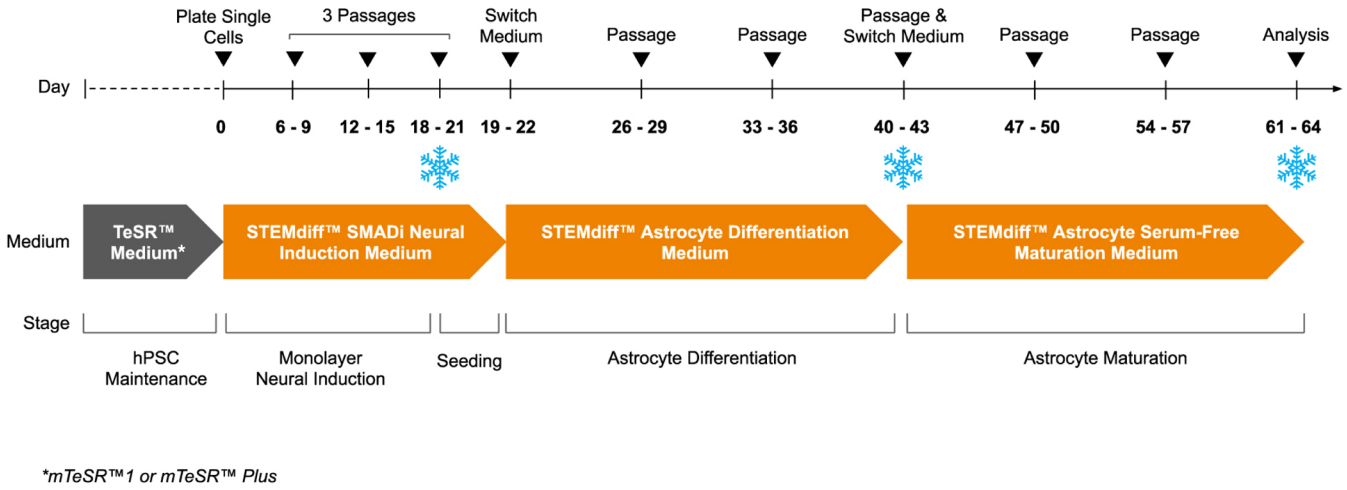
Snowflake symbols indicate optional time points for cryopreservation. For more details on cryopreservation, refer to section D.

The following instructions are for plating selected neural rosettes into one well of a 6-well plate. If using other cultureware, adjust volumes accordingly.

1. **Day 11 - 12**: After performing selection with STEMdiff™ Neural Rosette Selection Reagent (Catalog #05832), place selected neural rosettes into one well of a Matrigel®-coated 6-well plate containing 2 mL of warm (37°C) STEMdiff™ Neural Induction Medium + SMADi. Incubate at 37°C and 5% CO_2 for 24 hours.
2. **Day 12 - 13**: Aspirate medium and add 2 mL of warm STEMdiff™ Astrocyte Differentiation Medium (Preparation section B). Incubate at 37°C and 5% CO_2.
3. Perform daily full-medium changes with warm STEMdiff™ Astrocyte Differentiation Medium. Incubate at 37°C and 5% CO_2.
4. **Day 18 - 19**: Cells will reach 80 - 90% confluence and will be ready for passaging. Proceed to section B to continue astrocyte differentiation.

II. Starting from the Monolayer Protocol

For complete instructions on generating CNS-type NPCs using monolayer neural induction, refer to section 5.2 in 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. For complete instructions on preparing STEMdiff™ Neural Induction Medium + SMADi, refer to the PIS for STEMdiff™ SMADi Neural Induction Kit (Catalog #08581), available at www.stemcell.com.



**mTeSR™1 or mTeSR™ Plus*

Figure 2. Protocol for Obtaining NPCs via Monolayer Neural Induction

Snowflake symbols indicate optional time points for cryopreservation. For more details on cryopreservation, refer to section D.

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

1. Once the NPC culture is ~80 - 90% confluent (approximately **Day 18 - 21**), harvest cells with ACCUTASE™ as described in section 6.2 of the Technical Manual: Generation and Culture of Neural Progenitor Cells Using the STEMdiff™ Neural System.
2. Seed cells at a density of 1.5 - 2 x 10^5 cells/cm^2 onto one well of a Matrigel®-coated 6-well plate containing 2 mL of 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 of warm (37°C) STEMdiff™ Astrocyte Differentiation Medium (Preparation section B). Incubate at 37°C and 5% CO_2.
4. Perform daily full-medium changes with warm 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 to continue astrocyte differentiation.

III. Starting from the Cryopreserved Human iPSC-Derived NPCs

The following instructions are for seeding thawed Human iPSC-Derived Neural Progenitor Cells (Catalog #200-0620) into a coated 6-well plate; if using other cultureware, adjust volumes accordingly. For complete instructions on thawing Human iPSC-Derived NPCs, refer to the PIS (Document #10000018478), available at www.stemcell.com, or contact us to request a copy.

1. Prepare warmed (37°C) STEMdiff™ Neural Progenitor Medium (Catalog #05833), DMEM/F-12 with 15 mM HEPES, and Matrigel®-coated cultureware before starting the protocol to ensure that the thawing procedure is completed as quickly as possible.

   NOTE: For complete instructions on preparing STEMdiff™ Neural Progenitor Medium, refer to the PIS (Document #10000003488), available at www.stemcell.com, or contact us to request a copy.
2. Thaw and count Human iPSC-Derived Neural Progenitor Cells as described in steps 1 - 15 of section A: Thawing and Plating Neural Progenitor Cells of the PIS (Document #10000018478) to obtain a 2 mL single-cell suspension of NPCs in STEMdiff™ Neural Progenitor Medium.
3. Using a serological pipette or by aspiration, gently remove the excess Matrigel® solution from the 6-well plate (prepared in step 1). Ensure that the coated surface is not scratched. Immediately add 2 mL/well of STEMdiff™ Neural Progenitor Medium.
4. Seed cells at a density of 1.5 - 2 x 10^5 cells/cm^2 per well of the 6-well plate.

   NOTE: Post-thaw viability is typically 80 - 90%. If poor NPC recovery is observed after plating, STEMdiff™ Neural Progenitor Medium may be supplemented with 10 µM Y-27632 (Dihydrochloride; Catalog #72304) during the plating step.
5. Place the plate in a 37°C and 5% CO_2 incubator. Move the plate in several quick, short, back-and-forth and side-to-side motions to distribute the NPCs across the surface of the wells.

6. After 24 hours, remove the medium and add 2 mL/well of warm (37°C) STEMdiff™ Astrocyte Differentiation Medium (Preparation section B). Incubate at 37°C and 5% CO_2 for 24 hours.
7. Perform daily full-medium changes with STEMdiff™ Astrocyte Differentiation Medium until the culture is 80 - 100% confluent and is ready to passage (after ~7 days of culture). Proceed to section B to continue astrocyte differentiation.

IV. Starting from Expanded NPC Monolayer Culture

Astrocyte differentiation may be initiated using hPSC-derived NPCs generated with STEMdiff™ SMADi Neural Induction Medium (using either EB-based or 2D monolayer neural induction workflows) and expanded in STEMdiff™ Neural Progenitor Medium. For complete instructions on generating CNS-type NPCs, refer to section 5.1 or 5.2 of the Technical Manual: Generation and Culture of Neural Progenitor Cells Using the STEMdiff™ Neural System (Document #10000005588). The following instructions are for a seeding expanded NPCs onto a 6-well plate; if using other cultureware, adjust volumes accordingly.

1. Prepare STEMdiff™ Neural Progenitor Medium and coat a 6-well plate with Corning® Matrigel® (Preparation section A). Warm (37°C) sufficient volumes of STEMdiff™ Neural Progenitor Medium, DMEM/F-12 with 15 mM HEPES, and ACCUTASE™.

   NOTE: For complete instructions on preparing STEMdiff™ Neural Progenitor Medium, refer to the PIS (Document #10000003488), available at www.stemcell.com, or contact us to request a copy.
2. Passage NPCs once the culture reaches 80 - 90% confluence (after ~6 - 8 days of culture in STEMdiff™ Neural Progenitor Medium) as directed in section B step 2, using warm (37°C) STEMdiff™ Neural Progenitor Medium as the resuspension and seeding medium. Seed NPCs at a density of 1.5 - 2 x 10^5 cells/cm^2 onto a Corning® Matrigel®-coated 6-well plate containing 2 mL/well of warm (37°C) STEMdiff™ Neural Progenitor Medium.
3. After 24 hours, remove the medium and add 2 mL/well of warm (37°C) STEMdiff™ Astrocyte Differentiation Medium (Preparation section B). Incubate at 37°C and 5% CO_2 for 24 hours.
4. Perform daily full-medium changes with warm STEMdiff™ Astrocyte Differentiation Medium until the culture is 80 - 100% confluent and is ready to passage (after ~7 days of culture). Proceed to section B to continue astrocyte differentiation.

B. CONTINUATION OF ASTROCYTE DIFFERENTIATION

The following instructions are for one well of a 6-well plate; if using other cultureware, adjust volumes accordingly. Cells typically reach 80 - 100% confluence after 6 - 8 days of culture in STEMdiff™ Astrocyte Differentiation Medium.

NOTE: An 80 - 100% confluent culture will display tightly packed cells covering the majority of the well. It is normal for the spent medium to appear yellow at this stage. Refer to Figure 3A for a representative image of a 90% confluent culture of astrocyte precursor cells.

1. Prepare STEMdiff™ Astrocyte Differentiation Medium (Preparation section B) and coat a 6-well plate with Corning® Matrigel® (Preparation section A). Warm (37°C) sufficient volumes of STEMdiff™ Astrocyte Differentiation Medium, DMEM/F-12 with 15 mM HEPES, and ACCUTASE™.
2. Passage astrocyte precursor cells once the culture reaches 80 - 100% confluence as follows:
   a. Aspirate medium and add 1 mL of ACCUTASE™.
   b. Incubate at 37°C and 5% CO_2 for 5 - 10 minutes.
   c. Add 5 mL of DMEM/F-12 with 15 mM HEPES and wash the cells off of the well. Transfer the cell suspension to a 15 mL conical tube.
   d. Centrifuge at 300 x g for 5 minutes.
   e. Discard the supernatant and resuspend the cell pellet by gently flicking the tube.
   f. Add a suitable volume (e.g. 1 mL) of STEMdiff™ Astrocyte Differentiation Medium.
   g. Perform a cell count using Trypan Blue and a hemocytometer.
   h. Using a serological pipette or by aspiration, gently remove the excess Matrigel® solution from the 6-well plate (prepared in step 1). Ensure that the coated surface is not scratched. Immediately add 2 mL/well of warm (37°C) STEMdiff™ Astrocyte Differentiation Medium.
   i. Seed cells at a density of 1.5 - 2 x 10^5 cells/cm^2.
3. Incubate the plate at 37°C and 5% CO_2 for 6 - 8 days.
4. Passage cells every 6 - 8 days (at 80 - 100% confluence) until day 20 - 21 of astrocyte differentiation, performing full-medium changes every 2 - 3 days with warm STEMdiff™ Astrocyte Differentiation Medium.
5. On day 20 - 21 of astrocyte differentiation, astrocyte precursor cells will reach 80 - 100% confluence and be ready to passage. Proceed to section C for astrocyte maturation or section D to cryopreserve the astrocyte precursor cells.

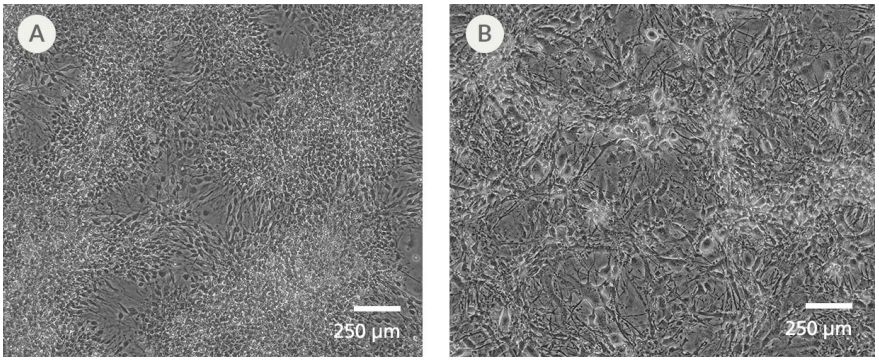


Figure 3. Representative images of ~90% confluent cultures of **(A)** astrocyte precursor cells after 14 days of astrocyte differentiation and **(B)** mature astrocytes after 21 days of astrocyte maturation. Scale bar = 250 µm.

C. ASTROCYTE MATURATION

The following instructions are for one well of a 6-well plate; if using other cultureware, adjust volumes accordingly. Refer to Figure 3B for a representative image of a 90% confluent culture of astrocyte precursor cells.

1. Prepare STEMdiff™ Astrocyte Serum-Free Maturation Medium (Preparation section C) and coat a 6-well plate with Matrigel® (Preparation section A). Warm (37°C) sufficient volumes of STEMdiff™ Astrocyte Serum-Free Maturation Medium, DMEM/F-12 with 15 mM HEPES, and ACCUTASE™.
2. Passage cells as directed in section B step 2, using warm (37°C) STEMdiff™ Astrocyte Serum-Free Maturation Medium as the resuspension and seeding medium.
3. Incubate the plate at 37°C and 5% CO_2 for 6 - 8 days.
4. Passage cells every 6 - 8 days (at 80 - 100% confluence) until day 20 - 21 of astrocyte maturation, performing full-medium changes every 2 - 3 days with warm STEMdiff™ Astrocyte Serum-Free Maturation Medium.

   NOTE: Recommended seeding density is 1.5 - 2 x 10^5 cells/cm^2; if mature astrocytes are to be used for immunocytochemistry, use a lower seeding density of 5 x 10^4 - 1 x 10^5 cells/cm^2.
5. After three weeks (day 20 - 21) of astrocyte maturation, mature astrocytes (S100β+/GFAP+) will be ready for use in downstream applications. Alternatively, proceed to section D to cryopreserve mature astrocytes.

D. CRYOPRESERVING ASTROCYTE PRECURSOR CELLS OR ASTROCYTES

1. Label cryogenic vials as needed.
2. Prepare a single-cell suspension of astrocyte precursor cells or mature astrocytes in a 15 mL conical tube.
3. Count viable cells using Trypan Blue and a hemocytometer.
4. Centrifuge at 300 x g for 5 minutes.
5. Carefully aspirate the supernatant and resuspend the cell pellet at 1 - 5 x 10^6 cells/mL using cold (2 - 8°C) CryoStor® CS10.
6. Transfer the cell suspension into cryogenic vials.

   NOTE: Minimize the amount of time the cells sit in the freezing medium at room temperature. Quickly transfer the cells to the cryovial and then to the -80°C freezer in a freezing container.
7. Cryopreserve cells using a standard slow rate-controlled cooling protocol (approximately -1°C/minute) on a controlled-rate freezer, or a freezing container (e.g. Corning® CoolCell® LX Cell Freezing Container [Catalog #200-0644]). If using a freezing container, place the cryogenic vials into the container and place in a -80°C freezer overnight or for a minimum of 4 hours.
8. For long-term storage, transfer cryovials into vapor phase liquid nitrogen (below -135°C). Long-term storage at -80°C is not recommended.

E. THAWING ASTROCYTE PRECURSOR CELLS OR ASTROCYTES

Thaw cryopreserved astrocyte precursor cells or mature astrocytes as directed in section 6.4 of the Technical Manual: Generation and Culture of Neural Progenitor Cells Using the STEMdiff™ Neural System (Document #10000005588). Plate cells on a Matrigel®-coated 6-well plate at a density of 1.5 - 2 x 10^5 cells/cm^2 in 2 mL/well of warm (37°C) STEMdiff™ Astrocyte Serum-Free Maturation Medium supplemented with 10 µM Y-27632 (Dihydrochloride).

NOTE: If thawing astrocyte precursor cells, proceed according to section C to mature the cells for 3 weeks in STEMdiff™ Astrocyte Serum-Free Maturation Medium.

NOTE: It is recommended to allow mature astrocytes to recover for 1 week with full-medium changes every 2 - 3 days before using for functional assays.

Assessment of Astrocyte Differentiation

Astrocyte differentiation may be assessed by immunochemistry using antibodies selective for the astrocyte-specific marker S100β (Dako, rabbit polyclonal). 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]) or doublecortin (DCX, Aves lab). Results may vary depending on the 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.