Human iPSC-Derived Astrocytes

Frozen astrocytes differentiated from human induced pluripotent stem cell (iPSC) line, SCTi003-A

Catalog #200-0980 1 x 10^6 cells

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Product Description

Human induced pluripotent stem cell (iPSC)-Derived Astrocytes were manufactured from Healthy Control Human iPSC Line, Female, SCTi003-A (Catalog #200-0511), using:

- STEMdiff™ SMADi Neural Induction Kit (Catalog #08581),
- STEMdiff™ Astrocyte Differentiation Kit (Catalog #100-0013), and
- STEMdiff™ Astrocyte Serum-Free Maturation Kit (Catalog #100-1666)

Human iPSC-Derived Astrocytes should be thawed and matured using STEMdiff™ Astrocyte Serum-Free Maturation Kit, which will result in a population of highly pure astrocytes (≥ 70% S100B-positive astrocytes, ≥ 60% glial fibrillary acidic protein (GFAP)-positive astrocytes, and < 15% Doublecortin (DCX)-positive neuron precursors). These astrocytes are functional and can be maintained long-term in culture. They are versatile tools for modeling human neurological development and disease, co-culture applications, drug screening, toxicity testing, and cell therapy validation.

Cells were obtained using Institutional Review Board (IRB)-approved consent forms and protocols.

Stability and Storage

Cells are frozen in a cryopreservation medium containing dimethyl sulfoxide (DMSO). The product is stable at -135°C or colder for 12 months from date of receipt. Thawed samples must be used immediately.

Precautions

Cell Screening: iPSC master cell banks are screened for AAV2, BK virus, Epstein-Barr Virus, Hepatitis A, Hepatitis B, Hepatitis C, Herpes Simplex 1 and 2, Herpes Virus Type 6, Herpes Virus Type 7, Herpes Virus Type 8, HIV-1, HIV-2, HPV-16, HPV-18, Human Adenovirus, Human Cytomegalovirus, Human Foamy Virus, Human T-Lymphotropic Virus, John Cunningham Virus, LCMV, Parvovirus B19, Sarbecovirus (SARS Virus), Seoul Virus, Corynebacterium Bovis, and Mycoplasma (Human Comprehensive CLEAR Panel) by PCR. As testing cannot completely guarantee that the donor was virus-free, THIS PRODUCT SHOULD BE TREATED AS POTENTIALLY INFECTIOUS and only used following appropriate handling precautions such as those described in biological safety level 2.

Storage of frozen cell products in the vapor phase of a liquid nitrogen storage tank is recommended. Storage in the liquid phase can result in cross-contamination if the vial breaks or is not sealed properly. Storage in the liquid phase also increases the potential for liquid nitrogen to penetrate the vial and cause it to explode when removed from storage. Use of a face shield is required as a safety precaution when transferring cells from one container to another. When handling this product, do not use sharps such as needles and syringes.

STEMCELL cannot guarantee the biological function or any other properties associated with performance of cells in a researcher's individual assay or culture systems. STEMCELL assures the cells will meet the specifications only when assessed immediately after thawing by our test methods.

FOR IN VITRO RESEARCH USE ONLY. NOT APPROVED FOR DIAGNOSTIC, THERAPEUTIC, OR CLINICAL APPLICATIONS.

NOT APPROVED FOR HUMAN OR VETERINARY USE IN VIVO.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
ACCUTASE™	07920
Corning® Matrigel® hESC-Qualified Matrix	Corning 354277
DMEM/F-12 with 15 mM HEPES	36254
Hausser Scientific™ Bright-Line Hemocytometer	100-1181
Serological pipettes, 2 mL	e.g. 38002
STEMdiff™ Astrocyte Serum-Free Maturation Kit	100-1666
Trypan Blue	07050
Y-27632 (Dihydrochloride; optional)	72302

Preparation of Reagents and Materials

A. COATING CULTUREWARE WITH CORNING® MATRIGEL® HESC-QUALIFIED MATRIX

Using sterile technique, coat cultureware with Corning® Matrigel® hESC-Qualified Matrix prior to initial plating and expansion of Human iPSC-Derived Astrocytes. 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 with 15 mM HEPES into a 50 mL conical tube and keep on ice.
3. Add thawed Matrigel® to the cold DMEM/F-12 with 15 mM HEPES (in the 50 mL conical 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 diluted 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 diluted Matrigel® solution evaporate.

   NOTE: If not used immediately, the cultureware must be sealed to prevent evaporation of the diluted 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 for Coating and Plating Various Cultureware

CULTUREWARE	APPROXIMATE SURFACE AREA	VOLUME OF DILUTED MATRIGEL® SOLUTION	VOLUME OF STEMdiff™ ASTROCYTE SERUM-FREE MATURATION MEDIUM
96-well plate	0.33 cm^2/well	50 µL/well	100 µL/well
48- or 24-well plate	2 cm^2/well	250 µL/well	500 µL/well
12-well plate	4 cm^2/well	500 µL /well	1 mL/well
6-well plate	10 cm^2/well	1 mL/well	2 mL/well
35 mm dish	10 cm^2	1 mL	2 mL
60 mm dish	20 cm^2	2.5 mL	5 mL

B. COMPLETE STEMdiff™ ASTROCYTE SERUM-FREE MATURATION MEDIUM

Use sterile technique to prepare complete STEMdiff™ Astrocyte Serum-Free Maturation Medium (STEMdiff™ Astrocyte Serum-Free Maturation Basal Medium + STEMdiff™ Astrocyte Maturation Supplement A + STEMdiff™ Astrocyte Serum-Free Maturation Supplement).

For complete instructions on preparing complete STEMdiff™ Astrocyte Serum-Free Maturation Medium, refer to Product Information Sheet (Document #10000028947) available at www.stemcell.com, or contact us to request a copy.

Directions for Use

IMPORTANT: To confirm the number of cells provided, a viable cell count must be done immediately after thawing (before washing). Work quickly once the cells have been thawed to ensure high viability and recovery. Use sterile technique when processing thawed cells.

NOTE: Use tissue culture-treated cultureware. Using sterile technique, coat cultureware with Matrigel® prior to initial plating of Human iPSC-Derived Astrocytes.

THAWING AND PLATING HUMAN iPSC-DERIVED ASTROCYTES

1. Coat the desired number of wells of a tissue culture-treated plate with Matrigel® (see Preparation section A).
2. Warm DMEM/F-12 with 15 mM HEPES and complete STEMdiff™ Astrocyte Serum-Free Maturation Medium (see Preparation section B) to 37°C before starting the protocol to ensure that the thawing procedure is done as quickly as possible.
3. Add 10 mL of warm DMEM/F-12 with 15 mM HEPES to a 15 mL conical tube.
4. Wipe the outside of the vial of cells with 70% ethanol or isopropanol.
5. In a biosafety hood, twist the cap a quarter-turn to relieve internal pressure and then retighten.
6. Quickly thaw cells in a 37°C water bath by gently shaking the cryovial continuously until only a small frozen cell pellet remains. Do not vortex cells.

   NOTE: ThawSTAR® CFT2 Automated Thawing System (Catalog #100-0650) may be used to quickly and efficiently thaw cells. For complete instructions, refer to the Product Information sheet (Document #10000010334), available at www.stemcell.com, or contact us to request a copy.
7. Remove the cryovial from the water bath and wipe it with 70% ethanol or isopropanol.

   NOTE: It is important to work quickly in the following steps to ensure high cell viability and recovery.
8. In a biosafety hood, measure and record the total volume of the cell suspension using a 2 mL serological pipette.
9. Remove a 20 µL aliquot of cells for pre-wash counting. If using Trypan Blue to assess viability, dilute with a minimum of 20 µL of medium and record the volume of medium added. Count cells using a hemocytometer.
10. Transfer cells from the cryovial to the tube containing 10 mL of warm DMEM/F-12 with 15 mM HEPES. Mix gently.
11. Rinse the vial with 1 mL of warm DMEM/F-12 with 15 mM HEPES and add it dropwise to the cells, while gently swirling the 15 mL tube.
12. Centrifuge cells at 300 x g for 5 minutes at room temperature (15 - 25°C).
13. Aspirate medium, leaving the cell pellet intact.
14. Gently resuspend the cell pellet in 1 mL of complete STEMdiff™ Astrocyte Serum-Free Maturation Medium.
15. Remove a 20 µL aliquot of cells for post-wash counting. If using Trypan Blue to assess viability, dilute with a minimum of 20 µL of medium and record the volume of medium added. Count cells using a hemocytometer. Use the final post-wash cell concentration to calculate the volume of cell suspension to plate per well in step 17.
16. Using a serological pipette or by aspiration, gently remove the Matrigel® solution from the coated cultureware (prepared in step 1). Ensure that the coated surface is not scratched.
17. Seed cells onto a Matrigel®-coated cultureware at a density of 1.5 - 2 x 10^5 cells/cm^2 in complete STEMdiff™ Astrocyte Serum-Free Maturation Medium. Refer to Table 1 for recommended volumes.

    NOTE: The seeding density of Human iPSC-Derived Astrocytes should be optimized for the application.

    NOTE: Post-thaw viability is typically 70 - 90%. If poor cell recovery is observed after plating, 10 µM of Y-27632 (Dihydrochloride) may be added to the medium during the plating step.
18. 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 cells across the surface of the wells.
19. Passage cells every 6 - 8 days (at 80 - 100% confluence), performing full-medium changes every 2 - 3 days with warm complete 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.

PASSAGING HUMAN iPSC-DERIVED ASTROCYTES

The following instructions are for passaging Human iPSC-Derived Astrocytes from one well of a 12-well plate and plating them onto matrix-coated wells of a new 12-well plate for expansion. Indicated volumes are for a single well; if using other cultureware, adjust volumes accordingly.

1. Prepare complete STEMdiff™ Astrocyte Serum-Free Maturation Medium (Preparation section B) and coat a 12-well plate with Matrigel® (Preparation section A). Warm (37°C) sufficient volumes of complete STEMdiff™ Astrocyte Serum-Free Maturation Medium, DMEM/F-12 with 15 mM HEPES, and ACCUTASE™.
2. Aspirate medium from the well and add 0.5 mL ACCUTASE™.
3. Incubate at 37°C and 5% CO_2 for 5 - 10 minutes.
4. Add 3 - 4 mL DMEM/F-12 with 15 mM HEPES and wash the cells off of the well. Transfer cell suspension to a 15 mL conical tube (e.g. Catalog #38009).
5. Centrifuge at 300 x g for 5 minutes. Remove and discard supernatant.
6. Resuspend cells in a suitable volume (e.g. 1 mL) of complete STEMdiff™ Astrocyte Serum-Free Maturation Medium. Perform a cell count using Trypan Blue and a hemocytometer.
7. Seed cells onto Matrigel®-coated cultureware at a density of 1.5 - 2 x 10^5 cells/cm^2 in complete STEMdiff™ Astrocyte Serum-Free Maturation Medium.
8. Incubate at 37°C and 5% CO_2 for 6 - 8 days, performing a full-medium change every 2 - 3 days with warm (37°C) complete STEMdiff™ Astrocyte Serum-Free Maturation Medium.
9. Passage cells on a weekly basis as desired. Astrocytes will typically expand 1 - 2 fold over 7 days in complete STEMdiff™ Astrocyte Serum-Free Maturation Medium and can be maintained for at least 10 passages without loss of astrocyte marker expression.

Assessment of Astrocytes 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 or neuron markers such as:

- Anti-GFAP Antibody, Polyclonal (Catalog #60128)
- Anti-GFAP Antibody, Clone 2E1.E9 (Catalog #60048)
- Anti-doublecortin antibody (DCX, Aves Labs)

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.