Applied StemCell, Inc.
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Datasheet

Astrocytes Mature (iPSC from Blood Cells; Male)

Product Information

Catalog Number **ASE-9322M (Male)**

Description Applied StemCell's Astrocytes Mature are cryo-preserved, pre-differentiated mature astrocytes derived from a footprint-free, karyotype normal human iPSC line. Mature astrocytes are a consistent and reliable source of cells that can be used in physiologically relevant models for studying CNS function, neurogenesis, and neurological diseases, as well as for drug and toxicity screening applications. They can also be co-cultured with neurons to improve neuronal viability in cell therapy studies.

Characterization of Mature Astrocytes The maturation of astrocytes can be assessed by their morphology and by immunostaining using astrocyte marker Glial Fibrillary Acidic Protein (GFAP). Percentage of astrocytes can be determined by a count of GFAP positive astrocytes divided by the total number of cells (DAPI staining of nuclei).
Neural Stem Cells (NSCs).

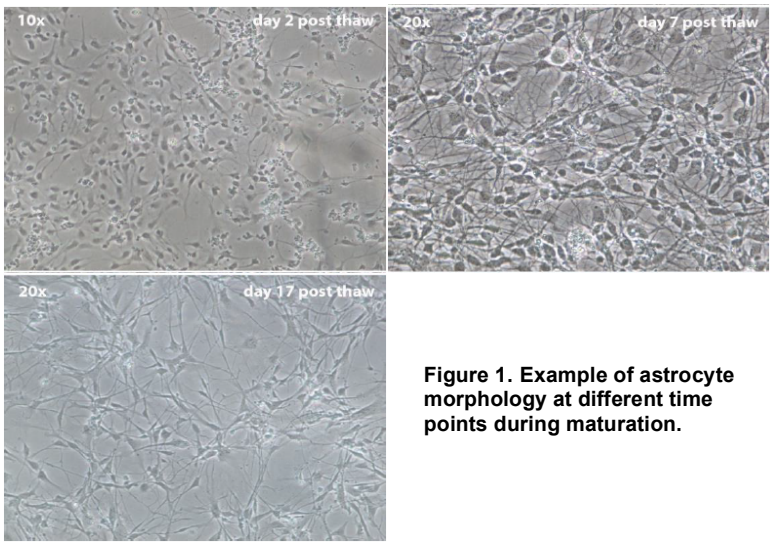

Figure 1. Example of astrocyte morphology at different time points during maturation.

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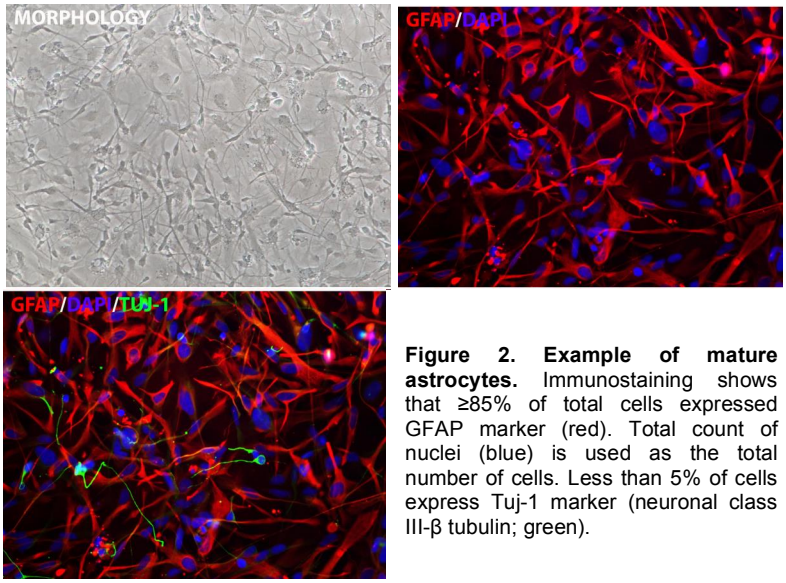

Figure 2. Example of mature astrocytes. Immunostaining shows that ≥85% of total cells expressed GFAP marker (red). Total count of nuclei (blue) is used as the total number of cells. Less than 5% of cells express Tuj-1 marker (neuronal class III-β tubulin; green).

Quality Control

Neuron purity: ≥85% GFAP positive, ≤5% Tuj positive
Recovery of frozen cells: ≥80% viability

Shipping

Dry ice

Storage and Stability

Store in liquid nitrogen freezer immediately upon receipt. This product is stable for at least 6 months from the date of receiving when stored as directed.

Safety Precaution

PLEASE READ BEFORE HANDLING ANY FROZEN VIALS. Please wear the appropriate Personal Protection Equipment (lab coat, thermal gloves, safety goggles and a face shield) when handling the cells. Handle the frozen vials with due caution. Please be aware that the following scenario can occur: Liquid nitrogen can leak into the vials when the vials are submerged in liquid nitrogen. Upon thawing, the liquid nitrogen returns to the gas phase, resulting in a dangerous build-up of pressure within the vial. This can result in the vial exploding and expelling not only the vial contents but also the vial cap and plastic fragments of the vial.

Restricted Use

This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.

Warranty

Performance of Applied StemCell's mature astrocytes has been extensively tested with other components. Applied StemCell will not hold responsibility if media other than the recommended media are used to culture mature astrocytes.

Media and Material Required but not Provided

- Astrocyte Maturation Media: Basal medium and supplements, Applied StemCell, Cat# ASE-9322AM
- D Matrigel™ hESC-qualified Matrix features, BD Biosciences, Cat# 354277
- Accutase, Cell Dissociation Reagent, Life Technologies, Cat# A11105-01
- DMEM, Dulbecco's Modified Eagle Medium, Life Technologies, Cat# 12491-015
- Primary antibodies:
  - Mouse anti-β III-tubulin isotype III clone SDL.3D10, Sigma, Cat# T8660
  - Rabbit anti-GFAP, Dako, Cat# M0761

Protocol

1. Handling Upon Receiving

The mature astrocyte cells are shipped on dry ice and at ambient temperature. A vial with cryopreserved mature astrocytes is packed in a small transparent bag, which is buried in dry ice. Upon receiving the product, check the integration of the packages and the presence of dry ice (contact Applied StemCell if the integrity of the package has been compromised, e.g. no dry ice in the package)

Cells can be plated immediately after arrival or transferred into liquid nitrogen. Do not remove the vials from dry ice during transportation to storage units. Immediately transfer components (especially the cryopreserved mature astrocytes) to storage units, avoiding exposure to room temperature where required.

After arrival, properly store the components as follows:

Component	Storage
Astrocytes, Mature	Liquid Nitrogen

2. Procedure

2.1 Coating Cell Culture Vessels with Matrigel

Please read producer's manual for handling of Corning Matrigel hESC-qualified Matrix.

Important producer's notes:

It is extremely important that BD Matrigel hESC- qualified Matrix and all culture ware or media coming in contact with Corning Matrigel hESC-qualified Matrix should be pre-chilled/ice-cold since BD Matrigel hESC-qualified Matrix will start to gel above 10°C.

The dilution is calculated for each lot based on the protein concentration. Prepare aliquots according to the dilution factor provided on the Certificate of Analysis (use 1.5-2.0 mL tubes). The volume of the aliquots is typically between 270-350 µL.

2.1.1. Pre-chill pipettes tips and dishes at 4°C.
2.1.2. Thaw an aliquot (typically between 270-350 µL) of Corning Matrigel hESC-qualified Matrix at 4°C (approximately 45 minutes).
2.1.3. Transfer the aliquot on ice into biological safety cabinet.
2.1.4. Prepare 25 mL aliquot of cold DMEM in 50 mL conical tube and keep on ice.
2.1.5. Using p1000 micropipette, transfer 1000 µL of the cold DMEM from the above tube into the tube with Matrigel and mix up several times. Transfer the Matrigel solution to the 50 mL conical tube containing cold DMEM and mix several times with a serological pipette (keep on ice).
2.1.6. Immediately, coat pre-chilled culture dishes with Matrigel/DMEM solution (volume 120-150 µL/cm^2).
2.1.7. Distribute coating matrix evenly and incubate the vessels at room temperature (15-25°C) for at least 1 hour before use.
2.1.8. Aspirate the remaining liquid from cell culture vessels just before use. Ensure that the tip of the pipet does not scratch the coated surface.
2.1.9. Coated vessels can be used immediately or can be stored at 4°C for up to 7 days (aseptic conditions).

Table 1. Recommended volumes of coating reagents for various vessels.

Vessel	Approx. Surface Area	Matrigel
96 well plate	0.33 cm^2/well	50 µL/well
4 or 24 well plate	2 cm^2/well	250 µL/well
35 mm dish	10 cm^2	1.5 mL
60 mm dish	20 cm^2	2.5 mL

2.2 Thawing and culturing astrocytes

2.2.1 One day before thawing mature astrocytes, place the 50 mL Astrocyte Medium bottle in 2°- 8°C fridge overnight.

2.2.2 On the day of thawing cryopreserved astrocytes (**Day 0**), transfer an aliquot of 25 mL of Astrocyte Medium from the 50 mL bottle into a 50 mL conical tube and add supplements to obtain complete medium as shown in Table 2.

Table 2. Preparation of an aliquot of complete Astrocyte Medium

Component	Volume
Astrocyte Medium	25 mL
Supplement A	2.25 mL
Supplement B	0.5 mL
Supplement C	25 µL

2.2.3 Transfer a 5 mL aliquot of complete medium prepared in step 1 into a 15 mL conical tube and pre-warm at 37°C. This aliquot will be used for recovery of the mature astrocytes from frozen stock.

2.2.4 Prepare another 2 mL aliquot of complete medium (volume for 35mm culture dish) and pre-warm at 37°C. Keep remaining medium at 2°- 8°C.

2.2.5 Shortly before thawing the cells, place pre-warmed medium and coated 35 mm dish in a biosafety cabinet.

2.2.6 To thaw cryopreserved mature astrocytes, remove the vial from LN2 storage unit and place immediately onto dry ice (the vial must be buried in the dry ice).

2.2.7 Bring the dry ice container with the vial to the site with the 37°C water bath.

2.2.8 Immerse the vial in the water bath (up to 2/3rd of the vial) and thaw cells until only a small piece of ice is still visible (approximately 1 minute).

Note: *Do not shake the vial during thawing.*

2.2.9 Immediately bring the vial to the biological cabinet, spraying it thoroughly with 70% ethanol and wiping it with an autoclaved paper towel.

2.2.10 Remove cells from the vial using a p1000 micropipette (or serological pipette) and transfer drop-wise while swirling into the 15 mL conical tube containing 5 mL of pre-warmed complete medium (step 2). Wash the vial with 1 mL of medium from the 15 mL conical tube and transfer it back to the tube.

Note: *Do not mix cells up and down and avoid generating bubbles.*

2.2.11 Centrifuge cells at 400 x g for 5 minutes at room temperature.

2.2.12 Aspirate the medium very carefully using a vacuum (or pipette if preferred), leaving only a drop of liquid in the tube. Take extra care not to remove or disturb the cell pellet during aspiration of medium.
2.2.13 Using a p1000 micropipette, add 1 mL of complete medium (step 3) into the tube and gently re-suspend cells by pipetting up and down 4-6 times.
2.2.14 Remove a 10 µL aliquot of cell suspension and mix it with 10 µL of Trypan blue solution.
2.2.15 Count the cells.
2.2.16 Aspirate Matrigel solution from pre-warmed 35 mm cell culture dish and immediately transfer 1 mL solution of thawed mature astrocytes into the dish. Wash the conical tube with an additional 1 mL of complete ASTROCYTE MEDIUM and transfer to the same culture dish. Cell density should be in range of 1-1.3 x 10^5/cm^2 (1-1.3 x 10^6cells/vial).
2.2.17 Distribute cells evenly and place the dish in the cell culture incubator (37°C/ 5% CO_2/humidity control). The day of seeding cells is called **day 0**.
2.2.18 Monitor the cells survival and attachment the following day (**Day 1**).
2.2.19 Change complete ASTROCYTE MEDIUM on day 2. Medium change should be done slowly (drop wise) pointing the pipette tip toward the wall of the cell culture vessel. Medium should be changed every other day.
2.2.20 Monitor cell growth every day.
2.2.21 Culture cells for an additional 2-3 days and use for experiments.

Table 3. Recommended seeding densities for Applied StemCell astrocytes in various types of cell culture vessels. Range: low to high.

Vessel	Surface/Well	Seeding
96-well plate	0.33 cm^2	1.6 x 10^4 – 3.3 x 10^4
4-well plate	2 cm^2	1 x 10^5 – 2 x 10^5
35 mm dish	10 cm^2	5 x 10^5 – 1 x 10^6
60 mm dish	20 cm^2	1 x 10^6 – 2 x 10^6

2.2.22 Culture cells for an additional 2-3 days and use for experiments.