amsbio

Astrocytes Precursors (iPSC from Blood Cells; Female)

Product Information

Catalog Number ASE-9322PF

Description

Astrocyte Precursors are cryo-preserved, pre-differenttiated precursor cells capable of yielding high, purity, mature and functional astrocytoes using an optimized astrocyte maturation medium (ASE-9322AM) and protocols.Shortly after seeding and recovery, the cells proliferate and need to be passaged a minimum of two times. On Day 17 post-seeding, the cell population will contain ≥ 80% Glial Fibrillary Acidic Protein (GFAP) positive astrocytes and ≤ 15% Tuj-1positive neurons (neuronal class III β-tubulin) The mature astrocytes are a reliable source of cells that can be used in physiologically relevant models forstudying CNS function, neurogenesis, and neurological diseases, as well as fordrug 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).

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 astrocytes precursors has been extensively tested with other components. AMSBIO will not hold responsibility if media other than the recommended astrocyte maturation media are used to culturea strocyte precursors.

Media and Material Required but not Provided

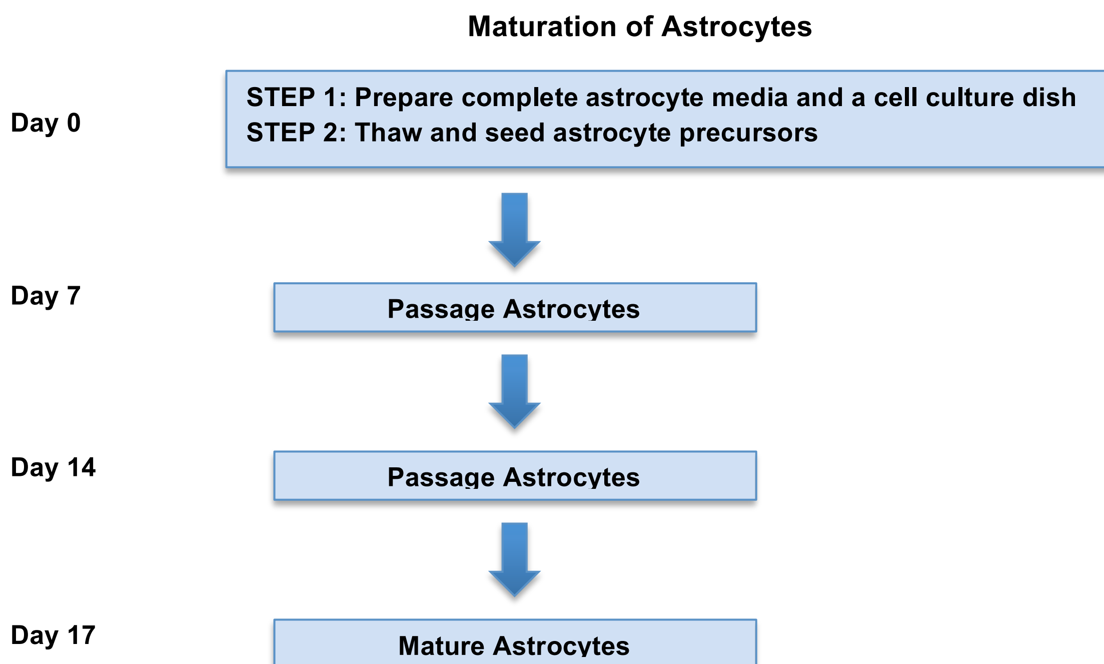
- Astrocyte Maturation Media: Basal medium and supplements, Cat# ASE-9322AM
- D Matrigel™ hESC-qualified Matrix features, BD Biosciences, Cat# 354277
- Accutase, Cell Dissociation Reagent, Life Technologies, Cat# A11105-01
- DMEM, Dulbecco's Modified Eagle Medium, Life Technologies, Cat# 12491-015
-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

Equipment required for astrocyte differentiation

Equipment	Specifications
Vertical laminar flow hood	
Incubator	Maintain 37°C and 95% humidity in an atmosphere of 5% CO2
Low speed centrifuge	(e.g. Thermo Centra CLS)
Pipette-aid	
Serological pipettes	
Hemacytometer or Automated Cell Counter	(e.g. BioRad TC 10)
Inverted microscope	(e.g. Nikon TS100)
Appropriate culture-ware	Tissue-treated plates (e.g. 430167, 430145, 430196, corning)

Protocol

Simplified diagram showing key steps in the process of culturing astrocytes



1. Handling Upon Receiving

Astrocytes Precursor cells are shipped on dry ice. Upon receiving the product, check the integration of the

packages and the presence of dry ice (contact AMSBIO 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 astrocyte precursors) to storage units, avoiding exposure to room temperature where required.

2. 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 Pre-chill pipettes tips and dishes at 4°C.
2.2 Thaw an aliquot (typically between 270-350 µL) of Corning Matrigel hESC-qualified Matrix at 4°C (approximately 45 minutes).
2.3 Transfer the aliquot on ice into biological safety cabinet.
2.4 Prepare 25 mL aliquot of cold DMEM in 50 mL conical tube and keep on ice.
2.5 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).
2.6 Immediately, coat pre-chilled culture dishes with Matrigel/DMEM solution (volume 120-150 µL/cm^2).
2.7 Distribute coating matrix evenly and incubate the vessels at room temperature (15-25°C) for at least 1 hour before use.
2.8 Aspirate the remaining liquid from cell culture vessels just before use. Ensure that the tip of the pipet does not scratch the coated surface.
2.9 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

3. Maturation of Astrocyte Precursor Cells

3.1 Preparation of Complete Astrocyte Maturation Media

Thaw media components overnight at 2°- 8°C. Complete medium shall be stored at 2°- 8°C and used within one week. Pre-warm an aliquot of complete medium at 37°C before use.

Table 2. Formulation of Complete Astrocyte Induction Medium (100 mL)

Component	Storage	Volume Provided	Formulation Per 50 mL	Optional one time re-freezing
Astrocyte Maturation Basal Medium	2°- 8°C	1x100 mL	50 mL	
Astrocyte Maturation Supplement A	2°- 8°C	1x8 mL	4 mL	
Astrocyte Maturation Supplement B	-20°C	1x2 mL	1 mL	yes
Astrocyte Maturation Supplement C	-20°C	1x100 µL	50 µL	yes

3.2 Thawing and Culturing Astrocytes

All steps described here must be performed in accordance with aseptic cell culture standards. All media and vessels used for cell culture must be pre-warmed to 37°C prior to use. For the entire process of astrocyte maturation, one type of complete medium is required: Astrocyte Maturation Medium + Supplement A + Supplement B + Supplement C (ASE-9322AM).

3.2.1 One day before thawing precursor cells, place the 50 mL Astrocyte Maturation Medium bottle in 2°- 8°C fridge overnight.
3.2.2 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 precursor cells from frozen stock.
3.2.3 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.
3.2.4 Shortly before thawing the cells, place pre-warmed medium and coated 35 mm dish in a biosafety cabinet.
3.2.5 To thaw cryopreserved astrocyte precursors, remove the vial from LN2 storage unit and place immediately onto dry ice (the vial must be buried in the dry ice).
3.2.6 Bring the dry ice container with the vial to the site with the 37°C water bath.
3.2.7 Immerse the vial in the water bath (up to $2/3^{rd}$ 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.

3.2.8 Immediately bring the vial to the biological cabinet, spraying it thoroughly with 70% ethanol and wiping it with an autoclaved paper towel.
3.2.9 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.

3.2.10 Centrifuge cells at 400 x g for 5 minutes at room temperature.
3.2.11 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.
3.2.12 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.
3.2.13 Remove a 10 µL aliquot of cell suspension and mix it with 10 µL of Trypan blue solution.
3.2.14 Count the cells.

3.2.15 Aspirate Matrigel solution from pre-warmed 35 mm cell culture dish and immediately transfer 1 mL solution of thawed astrocyte precursors into the dish. Wash the conical tube with an additional 1 mL of complete Astrocyte Maturation 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 10^6 cells/vial).

3.2.16 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**.

3.2.17 Monitor the cells survival and attachment the following day (**Day 1**).

3.2.18 Change complete Astrocyte Maturation 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.

3.2.19 Monitor cell growth every day.

3.2.20 On **Day 7**, the astrocytes in the 35 mm dish will reach full confluence. Passage the cells as described below.

a. Aspirate medium from the dish and add 1 mL of fresh pre-warmed accutase.
b. Keep the dish in the cell culture incubator until cells detach (3-5 minutes).
c. Add 1 mL of pre-warmed DMEM medium into the dish and re-suspend floating cell layer with p1000 micro-pipette by pipetting up and down several times.
d. Transfer astrocytes into a 15 mL conical tube containing 5 mL of pre-warmed complete Astrocyte Maturation Medium.
e. Centrifuge the cells at 400 x g for 5 minutes.
f. Carefully aspirate liquid and gently re-suspend cell pellet in 2 mL of complete medium.
g. Remove 10 µL aliquot of cell suspension and mix it with 10 µL of Trypan blue solution.
h. Perform an accurate live cell count.

3.2.21 Remove Matrigel from pre-warmed 60 mm dish and immediately transfer 2 mL of the astrocyte solution into it. The appropriate density of cells is $8x10^4$–$1x10^5$ live cells/cm^2 (per 60mm dish $1.6x10^6$ –$2x10^6$).

3.2.22 Monitor the cells the next day (**Day 8**).

3.2.23 On **Day 9**, change complete medium and keep monitoring the cells. Change the medium every other day.

3.2.24 On **Day 14**, the astrocytes growing in the 60 mm dish will reach full confluence. Passage the cells as described below.

a. Aspirate medium from the dish and add 2 mL of fresh pre-warmed accutase.
b. Keep the dish in the cell culture incubator until cells detach (2-5 minutes).
c. Add 2 mL of pre-warmed DMEM medium into the dish and re-suspend floating cell layer with p1000 micro-pipette by pipetting up and down several times.
d. Transfer astrocytes into a 15 mL conical tube containing 5 ml of pre-warmed DMEM medium.
e. Centrifuge the cells at 400 x g for 5 minutes.
f. Carefully aspirate liquid and gently resuspend cell pellet in 5 mL of complete medium.
g. Remove 10 µL aliquot of cell suspension and mix it with 10 µL of Trypan blue solution.
h. Perform an accurate live cell count.
i. Plate astrocytes in complete medium on desirable vessels coated with Matrigel at the density ranging from low to high (5 x 10^4 – 1 x 10^5 live cells/cm^2). Refer to table 3 for quick guidance.

Table 3. Recommended seeding densities for 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

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