Astrocytes Precursors Starter Kit (iPSC from Blood Cells; Female)

Product Information

Catalog Number

ASE-9322PKF

Description

The Astrocytes Precursors Starter Kit contains cryo-preserved, pre-differentiated astrocyte precursors derived from a footprint-free, karyotype normal human iPSC line. It is designed for customers to generate mature astrocytes using optimized maturation medium and supplements. Mature astrocytes can be obtained within 17 days. Shortly after seeding and recovery, the cells proliferate and need to be passaged a minimum of two times. In general, on Day 17 post-seeding, the cell population will contain ≥80% Glial Fibrillary Acidic Protein (GFAP) positive astrocytes and ≤ 15% Tuj-1 positive neurons (neuronal class III β-tubulin).

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).

Shipping

Dry ice at ambient temperature

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 has been extensively tested with othercomponents of the Astrocytes Precursors Starter Kit. AMSBIO will not holdresponsibility if components other than those provided with Astrocytes PrecursorsStarter Kit are used to culture astrocyte precursors.

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Media and Material

Astrocytes Precursors Starter Kit (ASE-9322PKF)

Component	Amount	Storage	Shelf Life
Astrocyte Precursors	1 vial ≥1 x 10^6 viable cells	Liquid Nitrogen	12 months
Astrocyte Medium	2 x 50 mL	2-8°C	12 months
Supplement A	2 x 4.5 mL	2-8°C	12 months
Supplement B	2 vials x 1 mL	-20°C	12 months
Supplement C	2 vials x 50 µL	-20°C	12 months

Media and Material Required but not Provided

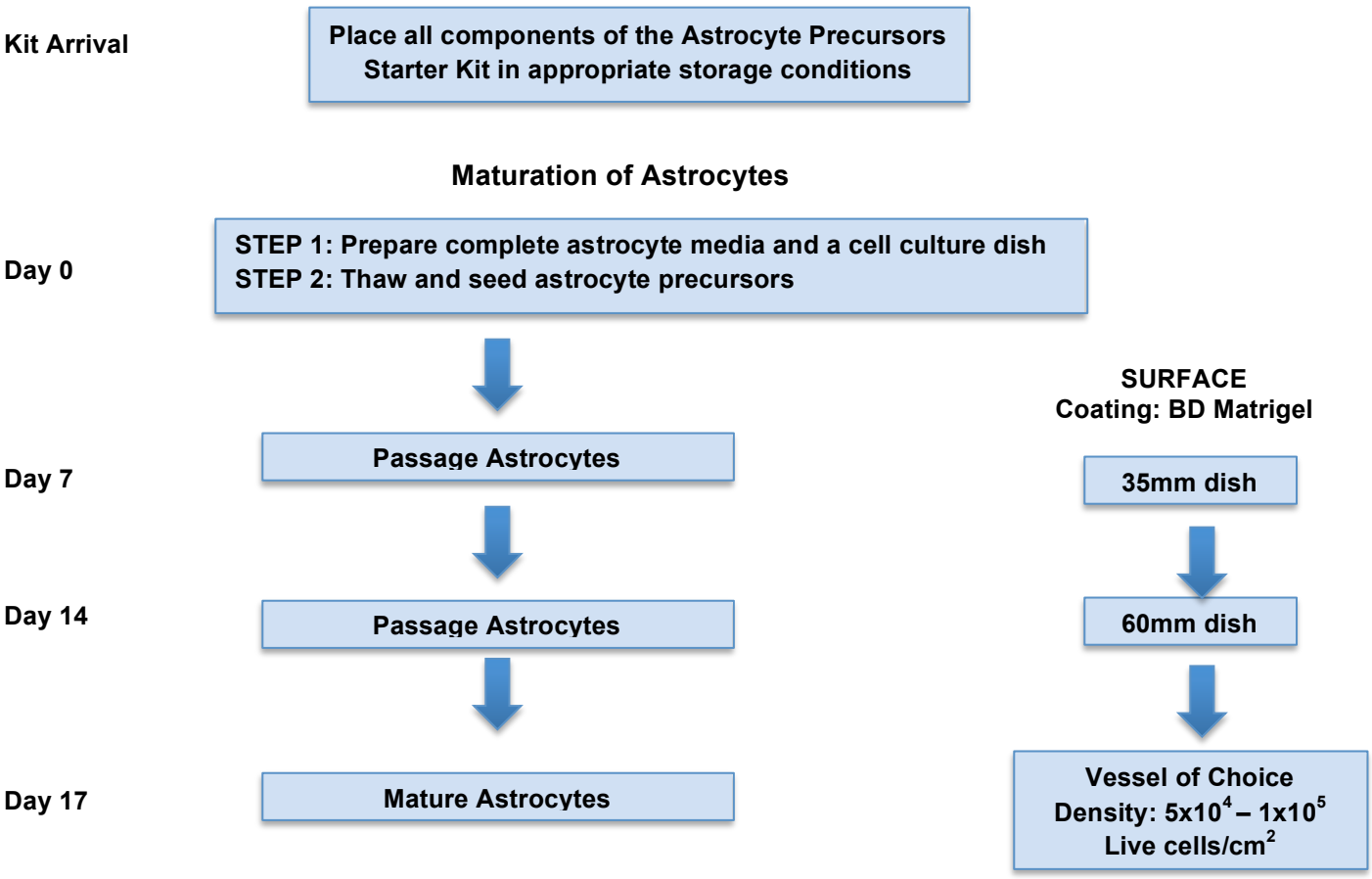
- D Matrigel™ hESC-qualified Matrix features, BD Biosciences, Cat# 354277
- Accutase, Cell Dissociation Reagent, Life Technologies, Cat# A11105-01
- DMEM, Dulbecco's Modified Eagle Medium, Life Technologies, Cat# 11965-084
- Primary antibodies:
  - Mouse anti-β III-tubulin isotype III clone SDL.3D10, Sigma, Cat# T8660
  - Rabbit anti-GFAP, Dako, Cat# M0761

Protocol

Notes:

- *We do not recommend re-freezing supplements and medium provided with the Astrocytes Precursors Starter Kit.*
- *We do not recommend cryopreserving Astrocytes Precursors Starter Kit cultured astrocytes.*

Simplified diagram showing key steps in the process of culturing astrocytes



1. Handling Upon Receiving

Astrocytes Precursors Starter Kit is shipped on dry ice and at ambient temperature. The components are packed in zip-lock bags. A vial with cryopreserved astrocyte precursors 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 AMSBIO if the integrity of the package has been compromised, e.g. no dry ice inthe 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 Astrocytes Mature Starter Kit components (especially the cryopreserved astrocytes) to storage units, avoiding exposure to room temperature where required.

After arrival, properly store the components as follows:

Component	Storage
Astrocyte Mature	Liquid Nitrogen
Astrocyte Medium	2-8°C
Supplement A	2-8°C
Supplement B	-20°C

Supplement C	-20°C

2. Procedure

This procedure has been extensively tested with the Astrocytes Precursors. The user should follow this procedure closely. The user assumes all responsibility for the failure of the experiment should there be any deviation from this procedure.

2.1 Coating Cell Culture Vessels with Matrigel

Cell culture vessels should be coated one day before or on the day of plating the cells.
Please read producer's manual for handling of BD 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 BD 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 BD Matrigel hESC-qualified Matrix at 4°C (approximately 45-60 minutes).
2.1.3. Transfer the aliquot on ice into biological safety cabinet.
2.1.4. Prepare 25mL aliquot of cold DMEM/F12 in 50 mL conical tube and keep on ice.
2.1.5. Using p1000 micropipette and cold tips, transfer 1000 µL of the cold DMEM/F12 from the above tube to the tube with Matrigel and mix up and down several times. Transfer the Matrigel solution to the 50 mL conical tube containing cold DMEM/F12 and mix several times with a serological pipette (keep on ice).
2.1.6. Immediately, coat pre-chilled culture dishes with Matrigel/DMEM F12 solution (volume µL/cm^2).
2.1.7. Distribute coating matrix evenly and incubate at room temperature (15-25°C) for at least 1 hour before use.
2.1.8. Coated dishes can be used immediately or can be stored at (4°C) for up to 7 days (aseptic conditions).
2.1.9. Pre-warm vessels at 37°C before use.
2.1.10. Aspirate Matrigel just before seeding astrocyte precursors. Do not let the surface dry.

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

Vessel Type	Matrigel
96 well plate	50 µL/well
4 or 24 well plate	250 µL/well
35 mm dish	1.5 mL
60 mm dish	2.5 mL

2.2 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 Medium + Supplement A + Supplement B + Supplement C. Thawed mature astrocyte cells will be seeded onto Matrigel coated 35

mm dish (1.0 - 1.4 x 10^5 live cells/cm^2).

2.2.1 One day before thawing precursor cells, 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 precursor cells 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 astrocyte precursors, 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 astrocyte precursors 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 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 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.

2.2.22 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 $1.6x10^6$ –$2x10^6$).

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

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

2.2.25 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 min.
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 (5×10^4 – 1×10^5 live cells/cm^2). Refer to table 3 for quick guidance.

Table 3. Recommended seeding densities for astrocytes in varioustypes of cell culture vessels. Range: low to high.

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

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