Applied StemCell, Inc.
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Datasheet

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

## Product Information

**Catalog Number**

**ASE-9322PKF**

**Description**

Applied StemCell's Astrocytes Precursors Starter Kit contains cryo-preserved, pre-differentiated astrocyte precursors derived from a footprint-free, karyotype normal human iPSC line. It is designed to generate mature astrocytes using our 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

**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 astrocytes has been extensively tested with other components of the Astrocytes Precursors Starter Kit. Applied StemCell will not hold responsibility if components other than those provided with Astrocytes Precursors Starter Kit are used to culture astrocyte precursors.

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## Media and Material

**Astrocytes Precursors Starter Kit (ASE-9322PKF)**

| Catalog # | Component | Amount | Storage | Shelf Life |
|---|---|---|---|---|
| ASE-9322PF | Astrocyte Precursors | 1 vial ≥1 x $10^6$ viable cells | Liquid Nitrogen | 12 months |
| ASE-9322AM-d | Astrocyte Maturation Basal Medium | 1 x 100 mL | 2-8°C | 18 months |
| ASE-9322AM-a | Astrocyte Matruation Supplement A | 1 x 9 mL | 2-8°C | 18 months |
| ASE-9322AM-b | Astrocyte Matruation Supplement B | 1 x 2 mL | -20°C | 18 months |
| ASE-9322AM-c | Astrocyte Matruation Supplement C | 1 x 100 µL | -20°C | 18 months |

## Media and Material Required but not Provided

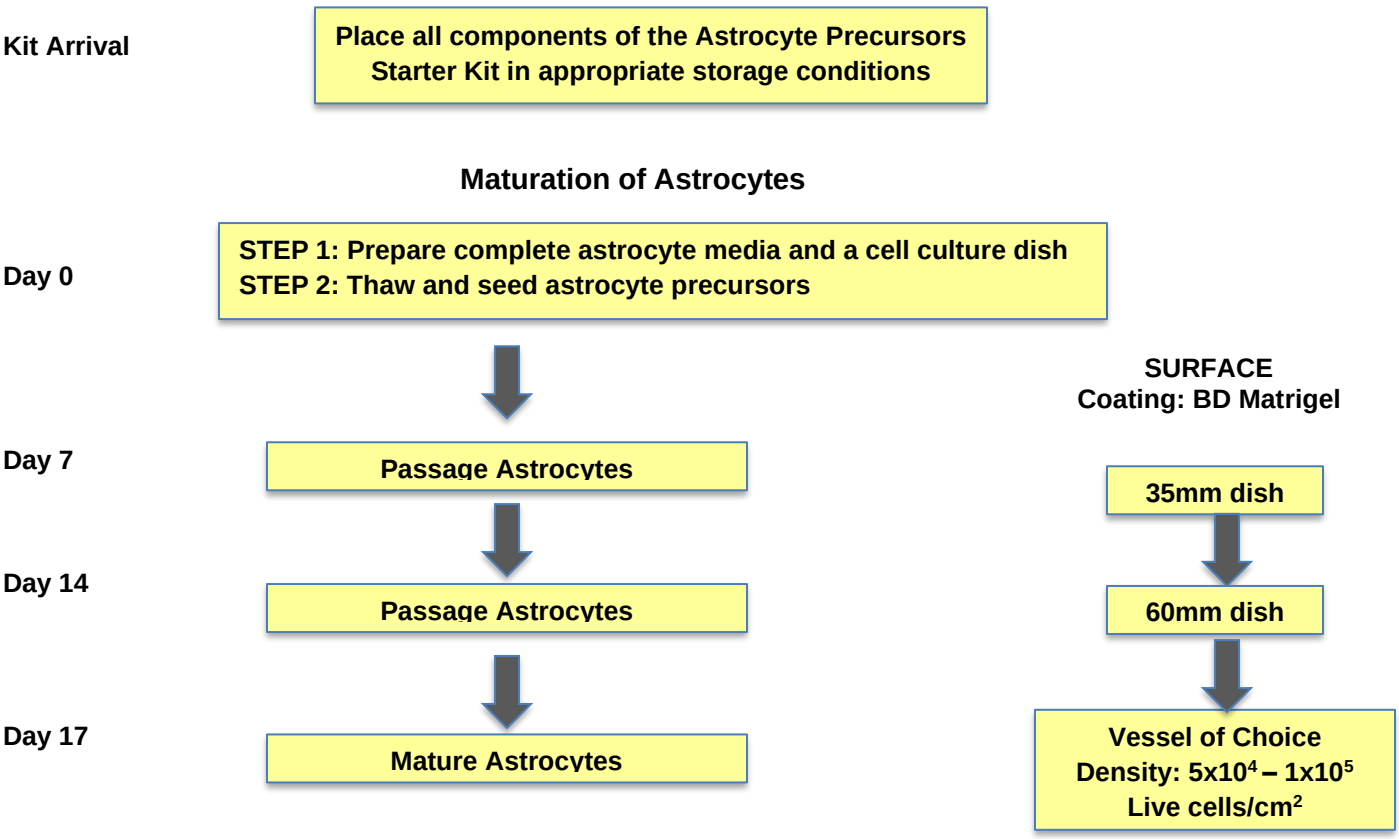
- D-Matrigel™ hESC-Qualified Matrix, BD Biosciences, Cat# 354277
- StemPro™ Accutase™ Cell Dissociation Reagent, ThermoFisher, Cat# A1110501
- Dulbecco's Modified Eagle Medium (DMEM), ThermoFisher, Cat# 11965084
- Primary antibodies: Rabbit anti-GFAP, Dako, Cat# M0761

## 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

Components of Astro Maturation Media are shipped as two separate packages: The -20°C components are shipped on dry ice; whereas the 2-8°C components are shipped in cooler containing cold inserts (8-15°C). Upon receiving the product, check the integrity of the packages and the presence of dry ice (contact Applied StemCell if the integrity of a package has been compromised, e.g. no dry ice in the package). Store components as specified in the Media and Material table.

### 2. Preparation of Culture Vessels and Media

#### 2.1 Coating Cell Culture Vessels with Matrigel

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

***Important 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 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 and down 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 Coated vessels 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 astrocytes. Do not let the surface dry.

**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. Astrocyte Maturation

The astrocyte differentiation process is comprised of two phases. I) During the induction phase, differentiation of NSC into astrocyte precursors will be initiated, which will be hallmarked by morphological changes in NSC (cell polarization and elongation), however the cells will still proliferate. II) During the maturation phase, cell divisions will gradually decrease, however not stop, and astrocyte precursors will elongate significantly and mature.

All steps described below 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: Astro Maturation Basal Medium + SUPPLEMENT A + SUPPLEMENT B + SUPPLEMENT C. Thawed astrocyte precursor cells will be seeded onto Matrigel-coated 35mm dish (1.0 – 1.4 x 10^5 live cells/cm$^2$).

**Preparation of complete Astro Maturation Media:**
Use sterile techniques when preparing reagents and materials.

**Table 2. Formulation of Complete Astro Maturation Medium**

| Component | Volume |
|---|---|
| Astro Maturation Basal Medium | 25 mL |

| | |
|---|---|
| Astro Maturation Supplement A | 2.25 mL |
| Astro Maturation Supplement B | 0.5 mL |
| Astro Maturation Supplement C | 25 µL |

3.1 One day before thawing precursor cells, place the 100 mL Astro Maturation Basal Medium bottle in 2°-8°C fridge overnight.
3.2 On the day of thawing cryopreserved astrocytes (**Day 0**), transfer an aliquot of 50 mL of Astro Maturation Basal Medium from the 100 mL bottle into a 50 mL-conical tube and add supplements to obtain complete medium as shown in Table 2.
3.3 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.
3.4 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.
3.5 Shortly before thawing the cells, place pre-warmed medium and coated 35-mm dish in a biosafety cabinet.
3.6 To thaw cryopreserved astrocyte precursors, remove the vial from liquid nitrogen storage unit and place immediately onto dry ice (the vial must be buried in the dry ice).
3.7 Bring the dry ice container with the vial to the site with the 37°C water bath.
3.8 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.*
3.9 Immediately bring the vial to the biological cabinet, spraying it thoroughly with 70% ethanol and wiping it with an autoclaved paper towel.
3.10 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*

3.11 Centrifuge cells at 300 X g for 5 minutes at room temperature.
3.12 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.
3.13 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.
3.14 Remove a 10 µL aliquot of cell suspension and mix it with 10 µL of Trypan blue solution.
3.15 Count the cells.
3.16 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 Astro Maturation Medium and transfer to the same culture dish. Cell density should be in range of 1 x 10^5-to-1.3 x 10^5/cm$^2$ (1 x 10^6-to-1.3 x 10^6 cells/vial)
3.17 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**.
3.18 Monitor the cells survival and attachment the following day (**Day 1**).
3.19 Change complete Astro 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.
3.20 Monitor cell growth every day.
3.21 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 Astro 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.

3.22 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 8 x 10^4 -to– 1 x 10^5 live cells/cm$^2$ (per 60-mm dish 1.6 x 10^6 -to- 2 x 10^6).

3.23 Monitor the cells the next day (**day 8**).

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

3.25 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 minutes.
f. Carefully aspirate liquid and gently re-suspend 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/cm2). 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$^2$ | 1.6x10^4 - 3.3x10^4 |
| 4-well plate | 2 cm$^2$ | 1x10^5 - 2x10^5 |
| 35-mm dish | 10 cm$^2$ | 5x10^5 - 1x10^6 |
| 60-mm dish | 20 cm$^2$ | 1x10^6 - 2x10^6 |