



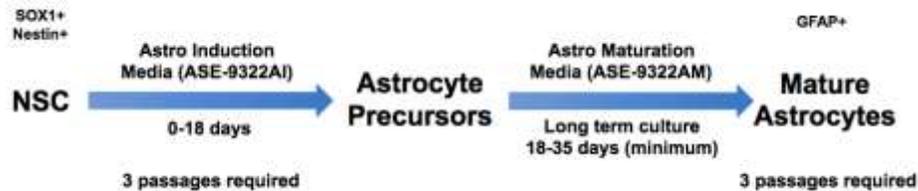
## Astro Maturation Media

### Product Information

Catalog Number **ASE-9322AM**

#### Description

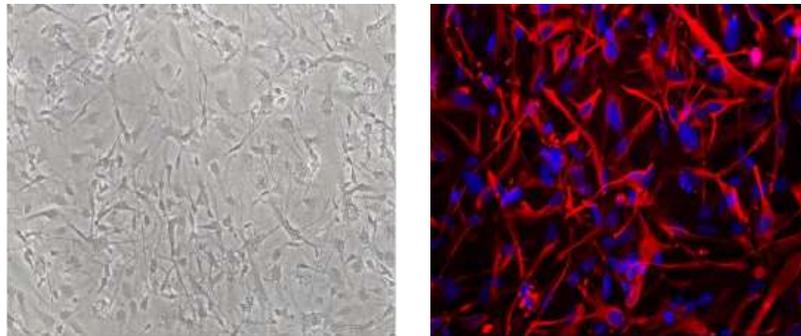
Astro Maturation Media is a serum-free media to enable researchers to differentiate astrocyte precursor cells into functional astrocytes and to maintain mature astrocytes in long-term culture (up to 60 days). The astrocyte differentiation process is divided into two stages. In the first stage, the Astro Induction Medium (ASE-9322AI) is used to differentiate NSC into astrocyte precursors. In the second stage, the Astro Maturation Medium is used to further differentiate astrocyte precursors into mature, functional astrocytes (see Fig 1). Astro Induction Medium (ASE-9322AI) can be purchased separately or as part of the Astrocytes Mature Starter Kit (ASE-9322MK/ ASE-9322MKF).



**Figure 1.** The process of astrocyte differentiation using Astro Induction and Maturation Media.

#### 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).



**Figure 2.** Example of mature astrocytes. Immunostaining shows that >80% of total cells expressed GFAP marker. Total count of nuclei (blue) is used as the total number of cells. Red: GFAP Glial Fibrillary Acid Protein); green: Tuj-1 (Neuronal Class III  $\beta$ -Tubulin).

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**Figure 3.** An example of densities of NSC, astrocyte precursors and mature astrocytes at different stages of differentiation using Astro Induction and Astro Maturation Media.

- Shipping** The components of the Astro Maturation Media are shipped as two packages: the -20°C components are shipped on dry ice and the 2-8°C components are shipped in cooler containing cold inserts (8-15°C).
- Storage** Maturation Basal Medium and Maturation Supplement A: store at 2-8°C; Maturation Supplement B and Maturation Supplement C: store at -20°C.
- 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 Astro Maturation Media has been extensively tested and optimized using astrocytes derived at Applied StemCell and the recommended media and materials; however, performance of these media may vary depending upon the quality of NSCs used to derive astrocytes from different sources. Applied StemCell will not hold responsibility if components other than the provided and recommended components are used.

## Media and Material

Components included with Astro Maturation Media (ASE-9322AM):

Cat. Number	Component	Amount	Storage
ASE-9322AM-d	Astro Maturation Basal Medium	100 mL	2-8°C
ASE-9322AM-a	Astro Maturation Supplement A	9 mL	2-8°C
ASE-9322AM-b	Astro Maturation Supplement B	2 mL	-20°C
ASE-9322AM-c	Astro Maturation Supplement C	100 µL	-20°C

## Additional Reagents 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

### 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<sup>2</sup>).
- 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 <sup>2</sup> /well	50 μL/well
4 or 24 well plate	2 cm <sup>2</sup> /well	250 μL/well
35 mm dish	10 cm <sup>2</sup>	1.5 mL
60 mm dish	20 cm <sup>2</sup>	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<sup>5</sup> live cells/cm<sup>2</sup>).

#### 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<sup>rd</sup> 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  $1 \times 10^5$ -to- $1.3 \times 10^5$ /cm<sup>2</sup> ( $1 \times 10^6$ -to- $1.3 \times 10^6$  cells/vial)
- 3.17 Distribute cells evenly and place the dish in the cell culture incubator (37°C/ 5% CO<sub>2</sub> /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  $8 \times 10^4$  -to-  $1 \times 10^5$  live cells/cm<sup>2</sup> (per 60-mm dish  $1.6 \times 10^6$  -to-  $2 \times 10^6$ ).
- 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  $\mu$ L aliquot of cell suspension and mix it with 10  $\mu$ L 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 ( $5 \times 10^4$  –  $1 \times 10^5$  live cells/cm<sup>2</sup>). 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.**

<b>Vessel</b>	<b>Surface/Well</b>	<b>Seeding</b>
96-well plate	0.33 cm <sup>2</sup>	$1.6 \times 10^4$ - $3.3 \times 10^4$
4-well plate	2 cm <sup>2</sup>	$1 \times 10^5$ - $2 \times 10^5$
35-mm dish	10 cm <sup>2</sup>	$5 \times 10^5$ - $1 \times 10^6$
60-mm dish	20 cm <sup>2</sup>	$1 \times 10^6$ - $2 \times 10^6$