Datasheet

Astro Induction Media

Product Information

Catalog Number **ASE-9322AI**

Description Astro Induction Media is a serum- free media produced using Applied StemCell's proprietary formulations to allow researchers to differentiate neural stem cells (NSC) into functional astrocytes. Applied StemCell's induction and maturation media have been extensively tested and optimized using NSC and astrocytes derived from a variety of human pluripotent cells (hESC and hiPSC). The astrocyte differentiation process is divided into two stages. In the first stage, the Astro Induction Medium is used to differentiate NSC into astrocyte precursors. In the second stage, the Astro Maturation Medium (ASE-9322AM) is used to further differentiate astrocyte precursors into mature, functional astrocytes and to maintain mature astrocytes in long-term culture (up to 60 days) (See Fig 1). Astro Maturation Medium can be purchased separately (ASE-9322AM) or as part of the Astrocytes Mature Starter Kit (ASE-9322MK/ ASE-9322MKF).

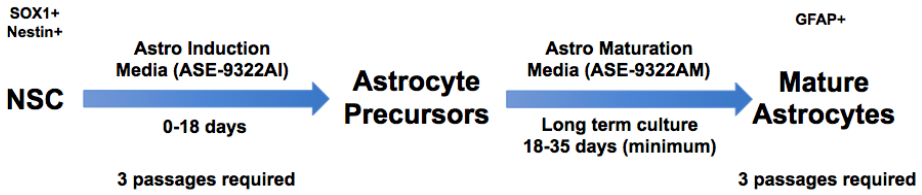

Figure 1. The process of astrocyte differentiation using Astro Induction and Maturation Media.

Quantity Astro Induction Basal Medium: 100 mL; Induction Supplement A: 8 mL; Induction Supplement B: 2 mL; Induction Supplement C: 50 µL

Shipping The components of the Astro Induction 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.

Storage and Stability Store the Induction Basal Medium and Induction Supplement A: Store at 2-8°C; Store Induction Supplement B and Induction Supplement C: Store at -20°C. 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.

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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 Neuron Induction Media has been extensively tested with other components. Applied StemCell will not hold responsibility if components other than the recommended components is used.

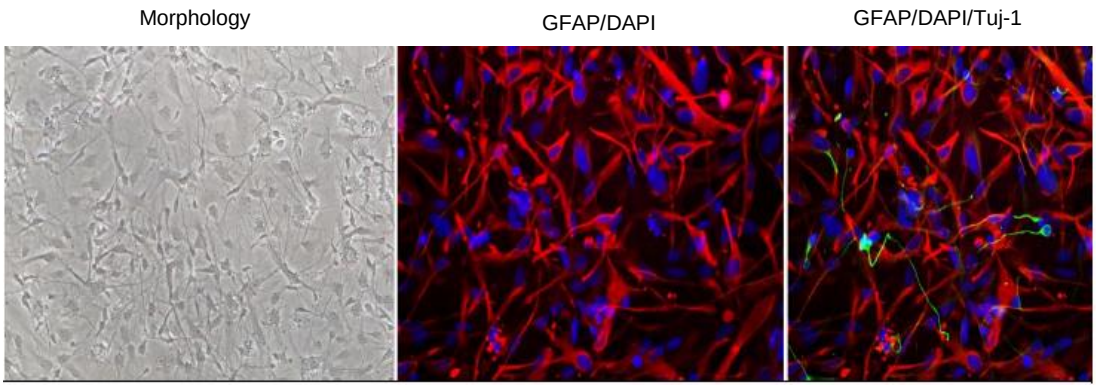

Figure 2. Astrocyte morphology and immunostaining data at 17 days post-seeding: GFAP Glial Fibrillary Acid Pro-tein) – red, Tuj-1 (Neuronal Class III β-Tubulin) – green

Media and Material

Components included with Astro Induction Media (ASE-9322AI):

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

Additional Reagents Required but not Provided

- Matrigel hESC-Qualified Matrix, Corning, Cat# 354277
- Accutase (cell dissociation reagent), Life Technologies, Cat# A11105-01
- DMEM, Life Technologies, Cat# 12491-015

Protocol

1. Handling Upon Receiving

Components of Applied StemCell's Induction 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.

2. Preparation of Culture Vessels and Media

The handling procedures described below have been extensively tested for all of Applied StemCell's NSC line

(ASE-9234 and ASE-9234F) using specified substrate coating and Applied StemCell's optimized maintenance media. The user should follow these procedures closely. The user assumes all responsibility for the failure of the experiment should there be any deviation from these procedures.

3. 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.

3.1 Pre-chill pipettes tips and dishes at 4°C.
3.2 Thaw an aliquot (typically between 270-350 µL) of Corning Matrigel hESC-qualified Matrix at 4°C (approximately 45 minutes).
3.3 Transfer the aliquot on ice into biological safety cabinet.
3.4 Prepare 25 mL aliquot of cold DMEM in 50 mL conical tube and keep on ice.
3.5 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).
3.6 Immediately, coat pre-chilled culture dishes with Matrigel/DMEM solution (volume 120-150 µL/cm^2).
3.7 Distribute coating matrix evenly and incubate the vessels at room temperature (15-25°C) for at least 1 hour before use.
3.8 Aspirate the remaining liquid from cell culture vessels just before use. Ensure that the tip of the pipet does not scratch the coated surface.
3.9 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

4. Differentiation of NSC into Functional Astrocytes

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 were optimized using NSC produced by Applied StemCell. If NSC from other sources

are used, the protocols might need further optimization according to performance of these cells. All operations should 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.

Preparation of complete Astro Induction Media:
Use sterile techniques when preparing reagents and materials. Thaw frozen supplement at room temperature (15-25°C) or overnight at 2°-8°C. It is advised to use thawed components within 2 days to formulate complete medium, however if desired, thawed components can be re- frozen once. Complete medium shall be stored at 2°-8°C and used within 7-10 days. Pre- warm complete medium at 37°C before use.

Table 2. Formulation of Complete Astro Induction Medium (e.g. 100 mL size)

Component	Storage	Volume Provided	Formulation per 50 mL	Optional One-time Re-freezing
Astro Induction Basal Medium	2°-8°C	1 x 100 mL	50 mL	
Astro Induction Supplement A	2°-8°C	1 x 8 mL	4 mL	
Astro Induction Supplement B	-20°C	1 x 2 mL	1 mL	yes
Astro Induction Supplement C	-20°C	1 x 100 µL	50 µL	yes

4.1 At **day 0**, aspirate Matrigel solution from cell culture vessels and immediately seed NSC at density ranging from $4x10^4$ to $6x10^4$ live cells /cm^2 in culture medium used for NSC (Figure 3).

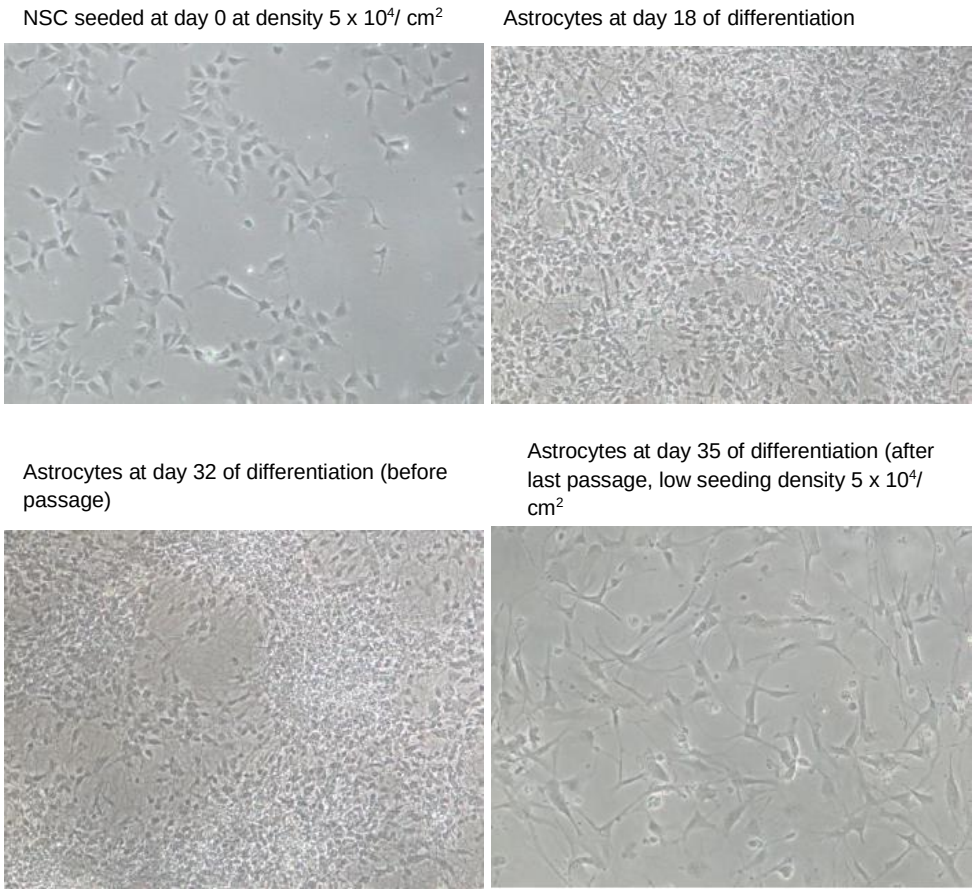

Figure 3. An example of NSC, neuronal precursors and mature astrocytes at different stages of differentiation using Astro Induction and Astro Maturation Media.

4.2 The following day, switch to Astro Induction Medium.
4.3 Change medium every alternate day.
4.4 At **day 5**, the cells will reach confluence*. Passage astrocyte precursors using Accutase and seed them onto new Matrigel coated vessels in Astro Induction Medium at a density ranging from 4 x 10^4 to 6 x 10^4/cm^2 live cells.

a. Aspirate induction medium and add Accutase to the vessel with cells (100µL/cm^2)
b. Incubate 5 minutes in the cell culture incubator
c. Add equal volume of DMEM to dilute Accutase and wash/mix the cells off of the vessel
d. Centrifuge cells at 400xg for 5 minutes
e. Re- suspend cells in desired volume of Astro Induction Medium (e.g. 5 mL) and perform cell count.
f. Seed the cells onto new Matrigel coated vessels

Note: *NSC lines from alternate sources may show variable differentiation dynamics. If cells are not confluent at day 5, adjust protocol according to cell performance (e.g. increase seeding densities).*

4.5 Proceed with astrocyte induction changing medium every alternate day, until **day 10**. Passage cells as described below and increase seeding density of live cells onto new vessels (1.5 x 10^5/ cm^2).
4.6 Proceed with astrocyte induction changing medium every alternate day, until **day 15**. Passage cells as described below and increase seeding density of live cells onto new vessels (1.5 x 10^5/ cm^2)

a. Aspirate induction medium and add Accutase to the vessel with cells (100µL/cm^2)
b. Incubate 5-10 minutes in the cell culture incubator and shake the vessel periodically. If the astrocyte

precursors do not detach within 10 minutes, proceed as described in "c"

c. Add equal volume of DMEM to dilute Accutase and wash/mix the cells off of the vessel using p1000 micropipette
d. Centrifuge cells at 400xg for 5 minutes
e. Re- suspend cells in desired volume of Astro Induction Medium or DMEM (e.g. 10-15 mL) and perform cell count.
f. Seed the cells onto new Matrigel coated vessels

4.7 At **day 18**, switch to Astro Maturation Medium (ASE-9322AM) and proceed according to astrocyte maturation protocol.

4.8 Proceed with astrocyte maturation, changing medium every alternate day.

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