Applied StemCell, Inc.
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Datasheet

iPSC-Derived Human Astrocytes Kit (African-American, Male Line)

Product Information

Catalog Number **ASE-9743**

Description

Applied StemCell has developed an efficient integration-free, small molecule-based method to differentiate high-quality astrocytes from human iPSCs. The differentiated astrocytes recapitulate the phenotype and functional parameters of primary and *in vivo* astrocytes.

We provide astrocytes differentiated from an integration-free, control human iPSC line (ASE-9211), reprogrammed from the fibroblasts of an African-American male donor. These high-purity (≥95%) cells express high levels of astrocyte biomarkers, GFAP and s100beta (Figure 2).

To harness the full potential of our astrocytes, we also provide optimized Astrocyte Basal Culture Media (ASE-9743MM) and Astrocyte Culture Media Supplement A (100X) (ASE-9743MM-A) that support the robust maintenance and functionality of the astrocytes in culture.

These iPSC-differentiated astrocytes can be used as control lines to compare phenotype and functionality of patient-derived, genome edited iPSC-derived astrocytes for drug screening applications. These astrocytes can also be used in neurotoxicity assays.

Parental Tissue

Control human iPSC (ASE-9211); p15
Age: Neonate
Gender: Male
Ethnicity: African-American
Tissue Source: Dermal Fibroblasts
Reprogramming Method: Episomal
Culture Conditions: Feeder-free

Clinical information

Healthy (with no known disease phenotypes)

Shipping

Dry ice

Storage and Stability

Store the components of the kit at the appropriate storage conditions as indicated in the media and materials table, immediately upon arrival. Shelf-life of the product is contingent upon proper storage conditions.

Quality Control

Each lot of iPSC-derived astrocytes has been tested for growth, viability and purity (≥95%) following recovery from cryopreservation. In addition, each lot has been tested for expression of astrocyte markers, and for the absence of mycoplasma and pathogens.

Safety Precaution

PLEASE READ BEFORE HANDLING ANY FROZEN VIALS. Please wear appropriate Personal Protection Equipment (lab coat, thermal gloves, safety goggles and a face shield) when handling frozen vials. 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.

Warranty

The performance of Applied StemCell's iPSC-derived astrocytes has been validated with the Astrocyte Basal Culture Media (ASE-9743MM) and Astrocyte Culture Media Supplement A (100X) provided in the Astrocytes Kit and the recommended additional reagents. Applied StemCell will not hold responsibility if components other than the components provided with the Astrocytes Kit and those recommended are used to culture the Applied StemCell astrocytes.

Restricted Use

This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.

Characterization of the ASE-9743 Astrocytes

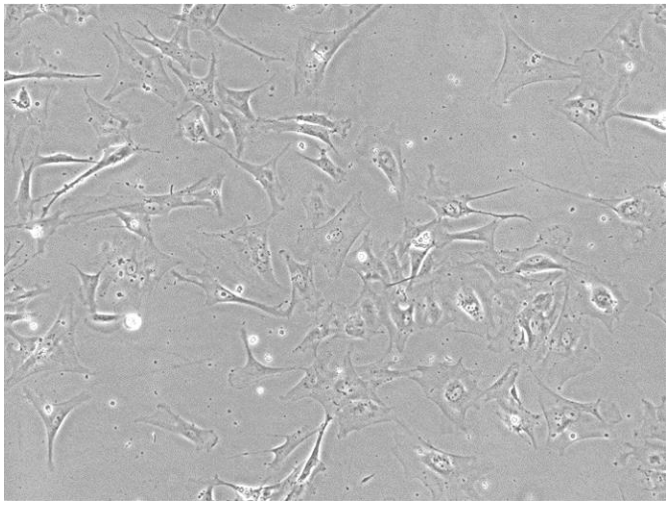

Figure 1. Bright Field Image of iPSC-derived Astrocytes from Applied StemCell's (ASC's) Control Line, ASE-9211.

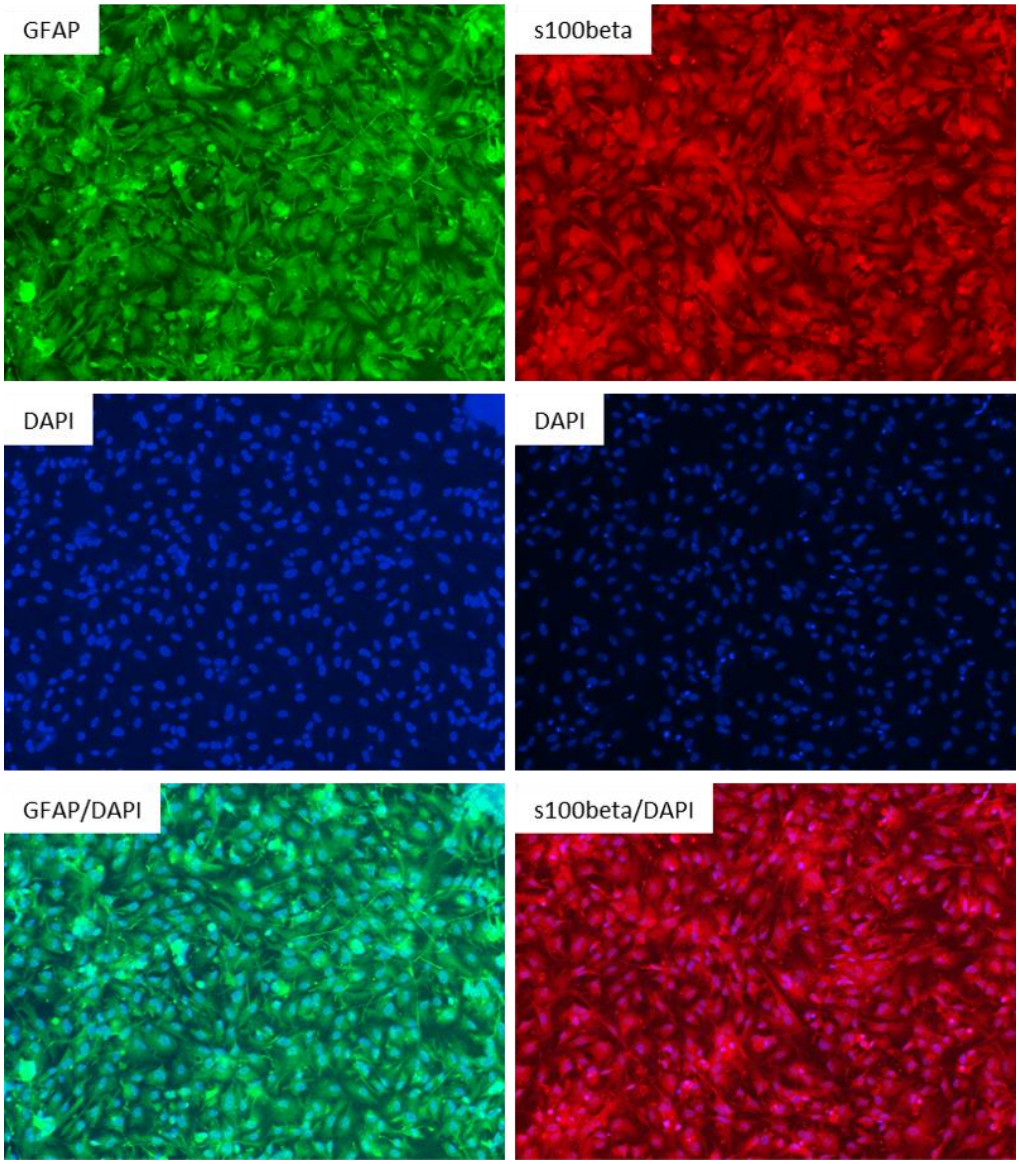

Figure 2: Antibody Staining of the Astrocytes differentiated from the NIST Control Line, ASE-9211. The astrocytes differentiated from ASC's iPSC line were stained with the astrocyte markers GFAP and s100beta. Left Three Images: Top Image: GFAP (Green), Middle Image: DAPI (Blue), Bottom Image: GFAP (Green) & DAPI (Blue); Right Three Images: Top Image: s100beta (Red), Middle Image: DAPI (Blue), Bottom Image: s100beta (Red) & DAPI (Blue)

Media and Materials

Astrocytes Kit (ASE-9743)

Catalog #	Component	Amount	Storage	Shelf Life
ASE-9743-C	iPSC-derived Astrocytes; African-American, Male Line	≥1x10^6 cells/ vial	Liq. N2	12 months
ASE-9743MM	Astrocyte Basal Culture Media	100 mL	-20°C	12 months
ASE-9743MM-A	Astrocyte Culture Media Supplement A (100X)	50 µL	-20°C	12 months

Additional Reagents Required

The below reagents are recommended for use with the astrocytes. If you use reagents other than those recommended, we suggest that you do a batch-test to validate quality of the cells and culture protocol.

- Matrigel®, Corning, Cat# 354230
- Antibodies:
  - GFAP R&D Systems, Cat# AF2594
  - S100B R&D Systems, Cat# AF1820
- Secondary Antibodies: corresponding secondary antibodies were purchased from ThermoFisher

Protocol

1. Coating Cell Culture Vessels with Coating Matrix

1.1 Coat the plates with 80 µg/mL Matrigel®.
Note: Please follow manufacturer's instructions in coating plates using Matrigel®.

1.2 Incubate at room temperature for at least 1 hour before use.

2. Preparation of Astrocyte Culture Media

2.1 Thaw the Astrocyte Basal Culture Media at room temperature before thawing the cryopreserved astrocytes.
2.2 The Basal Culture Media should be aliquoted and stored at -20°C if it will not be used immediately.
Note: The media can be stored at 4°C for up to 2 weeks or at -20°C for up to 12 months.

3. Thawing and Culturing Cryopreserved Astrocytes

3.1 To thaw the cryopreserved Astrocytes, remove one vial from the storage unit.
3.2 Immerse the vial in the water bath (up to 2/3rd of the vial) and thaw the cells rapidly until only a small piece of ice is still visible (approximately one minute).
Note: Do not shake the vial during thawing.

3.3 Bring the vial to the biological cabinet immediately and spray the outside of the vial thoroughly with 70% ethanol and wipe it with an autoclaved paper towel.
3.4 Remove the cells from the vial using a p1000 micropipette (or serological pipette) and transfer it slowly, drop-wise while swirling into a 15 mL conical tube containing 5 mL of pre-warmed Astrocyte Basal Culture Medium. Wash the vial with 1 mL 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.

3.5 Centrifuge the cells at 250 x g for 5 minutes at room temperature.
3.6 Aspirate the medium very carefully using a vacuum (or pipette if preferred), leaving only a drop of liquid in the tube.
Note: Take extra care not to remove or disturb the cell pellet during aspiration of medium.

3.7 Using a p1000 micropipette, add 5 mL of the pre-warmed astrocyte complete culture medium (5 mL Astrocyte Basal Culture Media + 50 uL Astrocyte Culture Media Supplement A (100X)) into the tube and gently re-suspend cells by pipetting up and down 2-3 times.
3.8 Remove a 10 µL aliquot of the cell suspension and mix it with 10 µL of Trypan blue solution.
3.9 Count the cells.
3.10 Aspirate the coating matrix from the pre-warmed cell culture vessel.
3.11 Seed the Astrocytes at a density ranging from 100,000-150,000 live cells/cm^2 in astrocyte complete culture medium.
3.12 Distribute the cells evenly.
3.13 Place the cell culture vessels in the incubator (37°C/ 5% CO_2/ humidity control) overnight.
3.14 Completely change the media on the second day using the Astrocyte Basal Culture Medium.