Applied StemCell, Inc.
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Datasheet

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

Product Information

Catalog Number **ASE-9741**

Description

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

We provide cortical neurons differentiated from an integration-free, control human iPSC line (ASE-9211), reprogrammed from the fibroblasts of an African-American male donor. These high-purity (≥80%) cells express high levels of the cortical neuron biomarker Tuj1 (Figure 1).

To harness the full potential of our cortical neurons, we also provide optimized Cortical Neuron Culture Media (ASE-9741MM) that supports robust maintenance and functionality of the cortical neurons in culture.

These iPSC-differentiated cortical neurons can be used as control lines to compare phenotype and functionality of patient-derived, genome edited iPSC-derived cortical neurons for drug screening applications. The cortical neurons can also be used for 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 human cortical neurons has been tested for growth, viability and purity (≥80%) following recovery from cryopreservation. In addition, each lot has been tested for expression of a cortical neuron marker 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 cortical neurons has been validated with the Cortical Neuron Culture Media provided in the Cortical Neuron Kit and the recommended additional reagents. Applied StemCell will not hold responsibility if components other than the components provided with the Cortical Neuron Kit and those recommended are used to culture the Applied StemCell cortical neurons.

Restricted Use

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

Characterization of the ASE-9741 Cortical Neurons

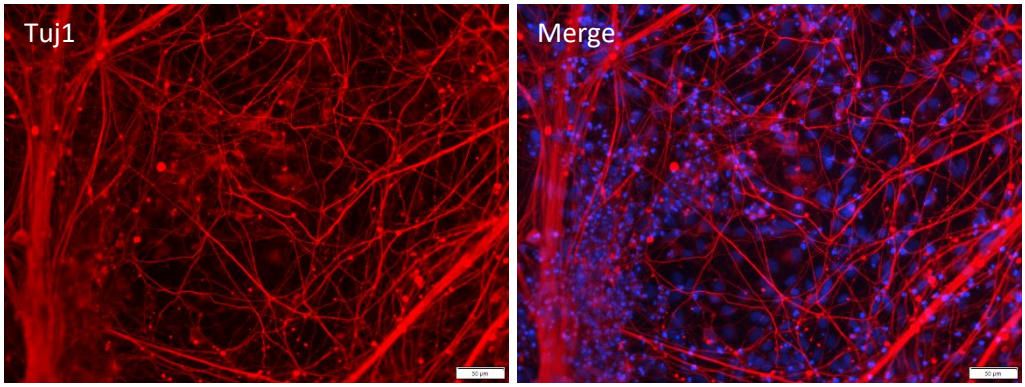

Figure 1. Immunostaining of ASE-9741 iPSC-derived Cortical Neurons for a cortical neuron biomarker. Cryopreserved cortical neurons, differentiated from Applied StemCell's control iPSC line ASE-9211, were stained with Tuj1. (Red: Tuj1; Blue: DAPI)

Media and Material

Cortical Neuron Kit (ASE-9741)

Catalog #	Component	Amount	Storage	Shelf Life
ASE-9741-C	iPSC-Derived Cortical Neurons; African-American Male Line	≥1x10^6 cells/ vial	Liq. N2	12 months
ASE-9741MM	Cortical Neuron Culture Media	100 mL	-20°C	12 months

Additional Reagents Required

The below reagents are recommended for use with the cortical neurons. 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
- Primary antibodies:
  - Tuj1 R&D systems MAB1195
- 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 Cortical Neuron Culture Media

2.1 Thaw the Cortical Neuron Culture Media at room temperature before thawing the cryopreserved Cortical Neurons.
2.2 The 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 Cortical Neurons

3.1 To thaw the cryopreserved Cortical Neurons, 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 cortical neuron 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 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 1 mL of the pre-warmed cortical neuron culture medium 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 Cortical Neurons at a density ranging from 150,000-200,000 live cells/cm^2 in Cortical Neuron Culture Media.
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 We recommend changing half of the media every 3-4 days.