Applied StemCell, Inc.
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Datasheet

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

Product Information

Catalog Number **ASE-9742**

Description

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

We provide dopaminergic 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 (≥70%) cells express high levels of the dopaminergic neuron biomarker TH (Figure 1).

To harness the full potential of our DOPA neurons, we also provide optimized DOPA Neuron Basal Media (ASE-9742MM) and DOPA Neuron Supplement (ASE-9742MM-A) that support robust maintenance and functionality of the dopaminergic neurons in culture.

These iPSC-differentiated DOPA neurons can be used as control lines to compare phenotype and functionality of patient-derived, genome edited iPSC-derived DOPA neurons for drug screening applications. The dopaminergic 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 dopaminergic neurons has been tested for growth, viability and purity (≥70%) following recovery from cryopreservation. In addition, each lot has been tested for expression of DOPA neuron 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 dopaminergic neurons has been validated with the DOPA Neuron Basal Media and DOPA Neuron Supplement provided in the Dopaminergic Neuron Kit and the recommended additional reagents. Applied StemCell will not hold responsibility if components other than the components provided with the Dopaminergic Neuron Kit and those recommended are used to culture the Applied StemCell DOPA 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-9742 Dopaminergic Neurons

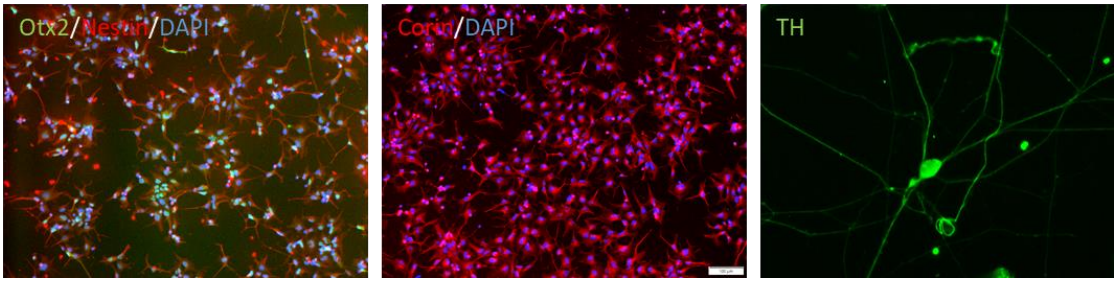

Figure 1. **Immunostaining of the ASE-9742 Dopaminergic Neurons derived from the human iPSC control line ASE-9211.** The dopaminergic neuron precursors (provided separately) were identified by Otx2, Nestin and Corin (Left, Middle). The mature dopaminergic neurons were identified by TH (Right).

Media and Material

Dopaminergic Neuron Kit (ASE-9742)

Catalog #	Component	Amount	Storage	Shelf Life
ASE-9742-C	iPSC-Derived Dopaminergic Neurons; African-American Male Line	≥1x10^6 cells/ vial	Liq. N2	12 months
ASE-9742MM	DOPA Neuron Basal Media	100 mL	-20°C	12 months
ASE-9742MM-A	DOPA Neuron Supplement	1 mL	-20°C	12 months

Additional Reagents Required

The below reagents are recommended for use with the DOPA 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:
  - Otx2 antibody R&D systems AF1979
  - Nestin antibody R&D systems MAB1259
  - Corin antibody R&D systems MAB2209
  - TH antibody R&D systems MAB7566
- 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 on coating plates using Matrigel®.

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

2. Preparation of Dopaminergic Neuron Culture Media

2.1 Thaw the Dopaminergic Neuron Basal Media and Supplement at room temperature before thawing the cryopreserved Dopaminergic Neurons.
2.2 Mix 100 mL of the Basal Media and 1 mL of the Supplement to make the Complete Culture Media.
2.3 The Complete 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 Dopaminergic Neurons

3.1 To thaw the cryopreserved Dopaminergic 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 dopaminergic neuron complete 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 dopaminergic neuron complete 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 Dopaminergic Neurons at a density ranging from 50,000-100,000 live cells/cm^2 in Dopaminergic Neuron Complete 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 using the recovered Dopaminergic Neurons within 14 days after recovery. Change half of the media every 3-4 days.