Applied StemCell, Inc.
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Datasheet

iPSC-Derived Human Motor Neurons Starter Kit (African-American, Male Line)

Product Information

Catalog Number **ASE-9701**

Description

Motor neurons (MN) are a specialized type of neurons originating from the spinal cord that are responsible for integrating signals from the brain and the muscles to control and coordinate voluntary and involuntary contraction of muscles and movement..Motor neuron degeneration or dysfunction has been implicated in serious and debilitating diseases such as spinal muscular atrophy (SMA), amyotrophic lateral scleoris (ALS), post-polio syndrome (PPS) and others. iPSC-derived motor neurons provide a convenient, consistent, reliable and physiologically relevant source of cell line mdels to model diseases and to test novel therapies.

Applied StemCell has developed an efficient integration-free, small molecule-based method to differentiate high-quality motor neuron cells from human iPSCs. Our proprietary differentiation protocol involves both the activation of the Wnt pathway, SHH signaling, and the inhibition of the activin-nodal and BMP signaling. The differentiated motor neurons recapitulate the phenotype and functional parameters of primary and *in vivo* motor neurons.

We provide motor neurons differentiated from an integration-free, control human iPSC line (ASE-9211), reprogrammed from fibroblasts of an African-American male donor. These high-purity (≥90%) cells show distinct neurite outgrowth in 2-7 days after thaw (Figure 1) and express late-stage motor neuron precursor biomarkers, HB9 and ChAT at day 2 after thaw (Figure 2), and mature motor neuron biomarkers, Tuj1 and MAP2 at day 5 (Figure 3). The cells are provided as cryopreserved, late-stage precursors that mature in 5 days after recovery.

To harness the full potential of our motor neurons, we also provide optimized, serum-free Motor Neuron Culture Media (ASE-9701MM) that supports robust maintenance and functionality of the motor neurons in culture.

These iPSC-differentiated motor neurons can be used as control lines to compare phenotype and functionality of patient-derived and genome edited iPSC-derived motor neurons, for co-culture models with other neurons, glia and skeletal muscle cells, as well as for neurotoxicity and drug screening.

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)

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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 motor neuron cells has been tested for growth, viability and purity (≥90%) following recovery from cryopreservation. In addition, each lot has been tested for expression of motor neuron markers (HB9 and Tuj1 at day2; ChAT and MAP2 at day 5), 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	Performance of Applied StemCell's motor neurons has been validated with the motor neuron culture media provided in the motor neuron Starter Kit and the recommended additional reagents. Applied StemCell will not hold responsibility if components other than the components provided with the Motor Neurons Starter Kit and those recommended are used to culture the Applied StemCell motor 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-9701 Motor Neurons

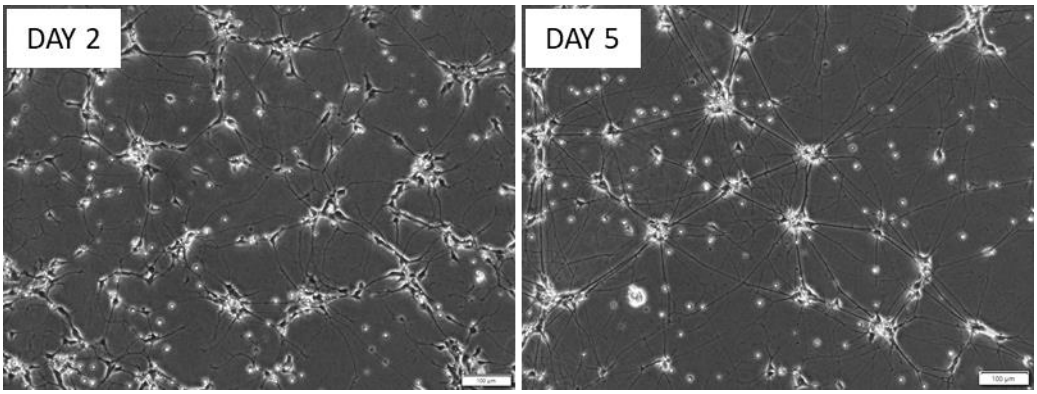

Figure 1. **Recovery of cryopreserved ASE-9701 iPSC-derived Motor Neurons.** Cryopreserved motor neurons, differentiated from Applied StemCell's control iPSC line, ASE-9211 were recovered in motor neuron culture media and undergo fast maturation in 5 days. Images at 20x magnification.

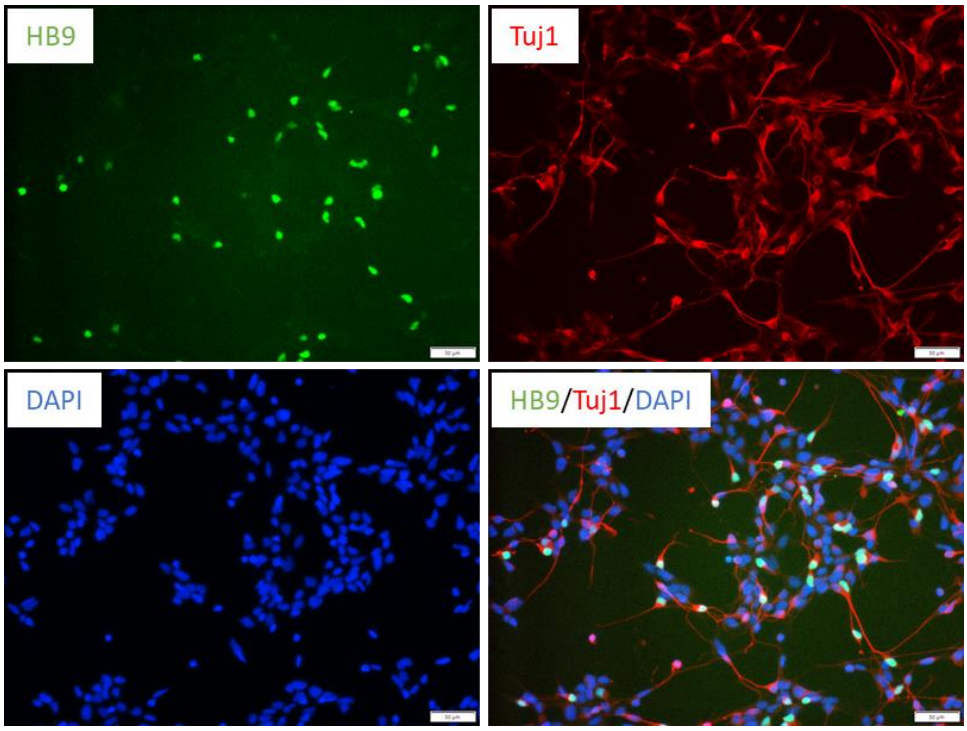

Figure 2. **Immunostaining of ASE-9701 iPSC-derived Motor Neurons for motor neuron biomarkers.** Cryopreserved motor neurons, differentiated from Applied StemCell's control iPSC line, ASE-9211 were recovered in motor neuron culture media The cells were stained for motor neuron late-stage precursor markers, HB9 (green) and TUJ1 (red) at day2,. DAPI: nuclear counterstain (blue). Images at 20x magnification.

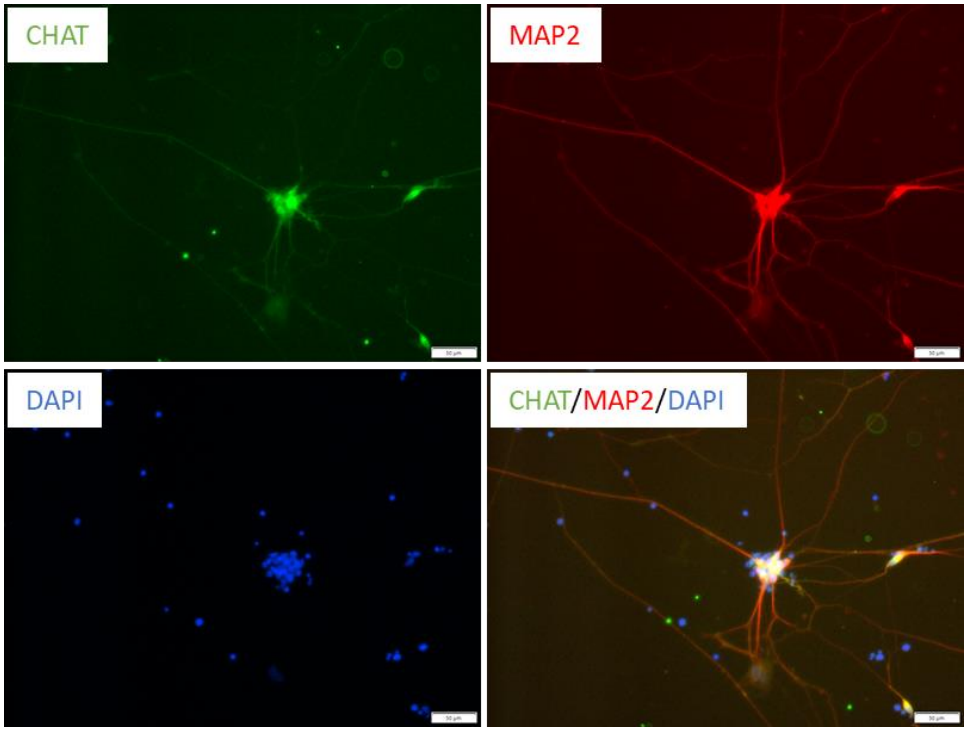

Figure 3. **Immunostaining of ASE-9701 iPSC-derived Motor Neurons for motor neuron biomarkers.** Cryopreserved motor neurons, differentiated from Applied StemCell's control iPSC line, ASE-9211 were recovered in motor neuron culture media The cells were stained for mature motor neuron markers, CHAT (green) and pan-neuronal marker MAP2 (red) at day5. DAPI: nuclear counterstain (blue). Images at 20x magnification.

Media and Material

Motor Neurons Starter Kit (ASE-9701)

Catalog #	Component	Amount	Storage	Shelf Life
ASE-9701-C	iPSC-derived Motor Neurons; African-American Male Line	≥2 x 10^6 cells/ vial	Liq. N_2	12 months
ASE-9701MM	Motor Neuron Basal Culture Media	95 mL	-20°C	12 months
ASE-9701MM-A	Motor Neuron Culture Media Supplement A	5 mL	-20°C	12 months

Additional Reagents Required

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

- Matrigel®, Corning, Cat# 354230
- Primary antibodies:
  - HB9 Antibody, ThermoFisher Cat# PA5-67195
  - beta-III Tubulin/Tuj1Antibody, R&D system MAB1195
  - Choline Acetyltransferase/ChAT Antibody, R&D system AF3447
  - MAP2 Antibody, R&D system MAB8304
- 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 Motor Neuron Culture Media

2.1 Thaw the motor neuron basal culture media and supplement at room temperature before thawing the cryopreserved motor neurons.

2.2 Mix 95 mL of the basal culture media and 5 mL of the supplement to make the motor neuron complete media.
Note: [Optional] add 1mL penicillin/streptomycin to 100 mL of the complete media to prevent bacterial contamination.

2.3 The complete media should be aliquoted and stored at -20°C if it will not be used immediately.
Note: The complete media can be stored at 4°C for up to 2 weeks or at -20°C for up to 6 months.

3. Thawing and Culturing Cryopreserved Motor Neurons

3.1 To thaw the cryopreserved motor neurons, remove one vial from the storage unit.

3.2 Immerse the vial in the 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 motor 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 generation of 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 motor 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 motor neurons at a density ranging from 50,000 – 100,000 live cells/cm^2. Distribute the cells evenly.
3.12 Place cell culture vessels in the incubator (37°C/ 5% CO_2/ humidity control) overnight.
3.13 A half media change is recommended for every 2-3 days.
3.14 The cells express HB9 and Tuj1 right after thaw, and express ChAT and MAP2 in 5 days.
3.15 We recommend using the recovered motor neurons within 7-10 days after recovery. Prolonged culture will give rise to non-neuronal cell outgrowth.
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