Applied StemCell, Inc.
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Datasheet

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

Product Information

Catalog Number **ASE-9706**

Description

Applied StemCell has developed an efficient integration-free method to differentiate high-quality myoblasts from human iPSCs with further differentiation into myotubes and skeletal muscle cells. The differentiated myoblasts recapitulate the phenotype and functional parameters of primary and *in vivo* myoblasts.

We provide cryopreserved, myoblasts 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 express high level of myoblast biomarkers (CD56, Pax7, Myogenin) (Figure 1) and form mature myotubes (skeleton muscle) characterized by elongated, multi-nucleated structures and the expression of myotube marker alpha-MHC (Figure 2) in 4-6 days.

To harness the full potential of our myoblasts, we also provide optimized, serum-free Myoblast Culture Media (ASE-9706MBM) and Myotube Formation Media (ASE-9706MTM) that supports robust maintenance and functionality of the myoblasts/myotubes in culture.

These differentiated myoblasts/myotubes can be used as control lines to compare phenotype and functionality of patient-derived and genome edited iPSC-derived myoblasts/myotubes, for co-culture models with motor neurons (ASE-9701), as well as for toxicity 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)

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 myoblasts has been tested for growth, viability and purity (≥90%) following recovery from cryopreservation. In addition, each lot has been tested for expression of myoblast markers, and for the absence of mycoplasma and pathogens.

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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 myoblasts has been validated with the Myoblast Culture Media provided in the Myoblast Kit and the recommended additional reagents. Applied StemCell will not hold responsibility if components other than the components provided with the Myoblast Kit and those recommended are used to culture the Applied StemCell Myoblasts.
Restricted Use	This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.

Characterization of the ASE-9706 Myoblasts

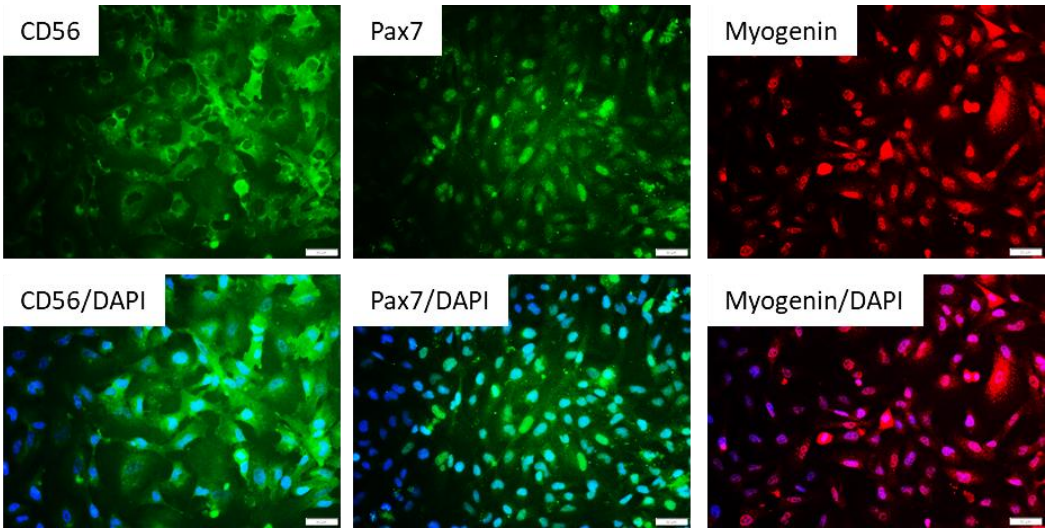

Figure 1. **Immunostaining of ASE-9706 iPSC-derived Myoblasts for myoblast biomarkers.** Cryopreserved myoblasts, differentiated from Applied StemCell's control iPSC line, ASE-9211 were recovered in myoblast culture media. The cells were stained with myoblast markers, CD56, Pax7, Myogenin on day 2 after recovery. DAPI: nuclear counterstain; blue. Images at 20x magnification.

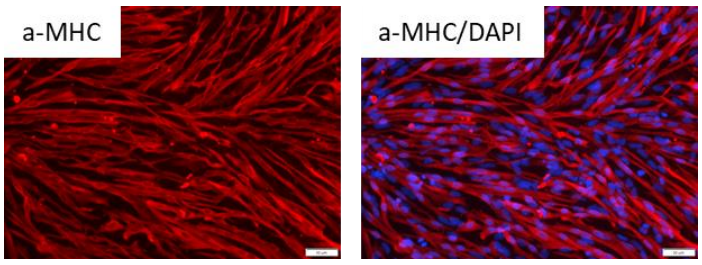

Figure 2. **Immunostaining of Myotubes derived from ASE-9706 iPSC-derived Myoblasts for myotube biomarkers.** Cryopreserved myoblasts, differentiated from Applied StemCell's control iPSC line, ASE-9211 were recovered in myoblast culture media until they reached confluency and then switched to Myotube Formation Media for 4 days. The cells were stained with myotube marker, alpha-MHC (a-MHC) on day 4. DAPI: nuclear counterstain; blue. Images at 20x magnification.

Media and Material

Myoblast Kit (ASE-9706)

Catalog #	Component	Amount	Storage	Shelf Life
ASE-9706-C	iPSC-derived Myoblast; African-American Male Line	≥1 x 10^6 cells/ vial	Liq. N2	12 months
ASE-9706MBM	Myoblast Culture Media	100 mL	-20°C	12 months
ASE-9706MTM	Myotube Formation Media	100 mL	-20°C	12 months

Additional Reagents Required

The below reagents are recommended for use with the myoblasts. 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:
  - CD56 antibody, R&D systems, AF2408
  - Pax7 antibody, Invitrogen, PA1-117
  - Myogenin antibody, R&D systems, MAB6686
  - alpha-MHC antibody, R&D systems, MAB4470
- 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 Myoblast Culture Media

2.1 Thaw the Myoblast Culture Media at room temperature before thawing the cryopreserved myoblasts.
2.2 The Myoblast Culture 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 12 months.

3. Thawing and Culturing Cryopreserved Myoblast

3.1 To thaw the cryopreserved myoblasts, 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 Myoblast 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 Myoblast 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 myoblasts at a density ranging from 50,000-100,000 live cells/cm^2 in Myoblast Culture Medium. Distribute the cells evenly.
3.12 Place cell culture vessels in the incubator (37°C/ 5% CO_2/ humidity control) overnight.
3.13 Change media every other day.
3.14 Once the cells are confluent, change the media to Myotube Formation Media.
3.15 Change media every other day.
3.16 The myotubes should form in 4-6 days.