Applied StemCell, Inc.
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Datasheet

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

Product Information

Catalog Number **ASE-9703**

Description

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

We provide cardiomyocytes 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 beat in 2-3 days after recovery and express high levels of cTnT (Figure 1). These cryopreserved cardiomyocytes are ready-to-use in 2-3 days after recovery and are functionally viable for 7-14 days after recovery.

To harness the full potential of our cardiomyocytes, we also provide optimized, serum-free Cardiomyocyte Culture Media (ASE-9703MM) and Cardiomyocyte Recovery Supplement A (1,000x) (ASE-9703MM-A) that supports robust maintenance and functionality of the cardiomyocytes in culture.

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

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 cardiomyocytes has been tested for growth, viability and purity (≥80%) following recovery from cryopreservation. In addition, each lot has been tested for expression of a cardiomyocyte marker (cTnT at day 2) 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	The performance of Applied StemCell's iPSC-derived cardiomyocytes has been validated with the Cardiomyocyte Culture Media and Cardiomyocyte Recovery Supplement A (1,000x) provided in the Cardiomyocytes Kit and the recommended additional reagents. Applied StemCell will not hold responsibility if components other than the components provided with the Cardiomyocytes Kit and those recommended are used to culture the Applied StemCell cardiomyocytes.
Restricted Use	This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.

Characterization of the ASE-9703 Cardiomyocytes

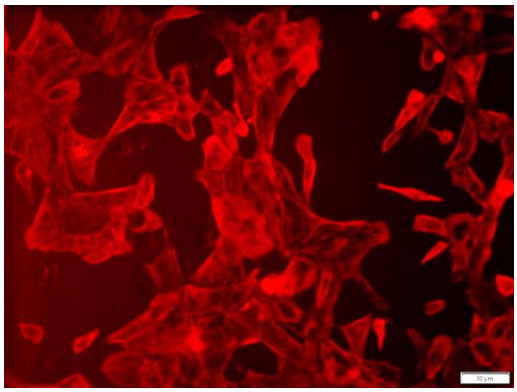

Figure 1. Immunostaining of ASE-9703 iPSC-derived cardiomyocytes for a cardiomyocyte biomarker. Cryopreserved cardiomyocytes, differentiated from Applied StemCell's control iPSC line, ASE-9211 were recovered in cardiomyocyte culture media. The cells were stained with cardiomyocyte marker cTnT (red) on day 2 after recovery. Image at 20x magnification.

Media and Material

Cardiomyocytes Kit (ASE-9703)

Catalog #	Component	Amount	Storage	Shelf Life
ASE-9703-C	iPSC-derived Cardiomyocytes; African-American Male Line	≥1 x 10^6 cells/ vial	Liq. N2	12 months
ASE-9703MM	Cardiomyocyte Culture Media	100 mL	-20°C	12 months
ASE-9703MM-A	Cardiomyocyte Recovery Supplement A (1,000x)	10 µL	-20°C	12 months

Additional Reagents Required

The below reagents are recommended for use with the cardiomyocytes. 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:
  - cTnT antibody: R&D systems MAB1874
- 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 Cardiomyocyte Culture Media

2.1 Thaw the Cardiomyocyte Culture Media and Supplement at room temperature before thawing the cryopreserved cardiomyocytes.
2.2 The Culture Media should be aliquoted and stored at -20°C if it will not be used immediately.
Note: The culture 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 Cardiomyocytes

3.1 To thaw the cryopreserved cardiomyocytes, 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 Cardiomyocyte 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 Cardiomyocyte 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 cardiomyocytes at a density ranging from 200,000 live cells/cm^2 in Cardiomyocyte Culture Medium supplemented with 1x Cardiomyocyte Recovery Supplement A.
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 Change media the next day with Cardiomyocyte Culture Medium.
3.15 We recommend using the recovered cardiomyocytes within 7-14 days after recovery. Fully change media every 2-3 days.
3.16 The cells express cTnT right after thawing and may start beating in the dish 2-3 days after thawing.