Applied StemCell, Inc.
(866) 497-4180
www.appliedstemcell.com

Datasheet

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

Product Information

Catalog Number ASE-9745-1

Description

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

We provide monocytesdifferentiated 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 levels of monocyte biomarkers, CD14(Figure 1).

To harness the full potential of our monocytes, we also provide optimized Monocyte Culture Media (ASE-9745MM) that supports robust maintenance and functionality of the monocytes in culture.

These iPSC-differentiated monocytes can be used as control lines to compare phenotype and functionality of patient-derived, genome edited iPSC-derived monocytes 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 monocytes has been tested for growth, viability and purity (≥90%) following recovery from cryopreservation. In addition, each lot has been tested for expression of monocyte 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.

Applied StemCell, Inc.
521 Cottonwood Dr. #111, Milpitas, CA 95035
Phone: 866-497-4180 (US Toll Free); 408-773-8007 Fax: 408-773-8238
info@appliedstemcell.com www.appliedstemcell.com

Warranty The performance of Applied StemCell's iPSC-derived monocytes has been validated with the Monocyte Culture Media provided in the Monocyte Kit and the recommended additional reagents. Applied StemCell will not hold responsibility if components other than the components provided with the Monocyte Kit and those recommended are used to culture the Applied StemCell monocytes.

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

Characterization of the ASE-9745 Monocytes

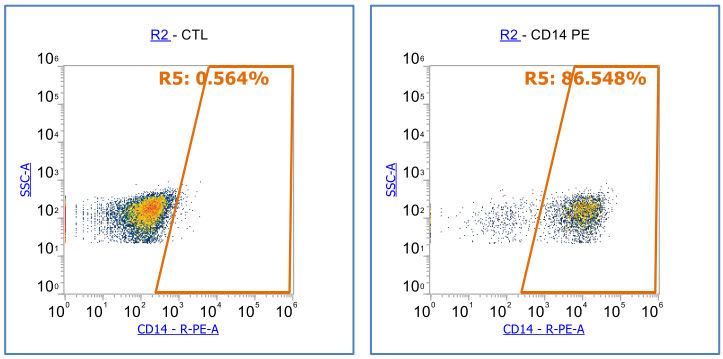

Figure 1. Immunostaining of ASE-9745 iPSC-derived Monocytes for monocyte biomarkers. Cryopreserved monocytes, differentiated from Applied StemCell's control iPSC line, ASE-9211 were recovered in MonocyteCulture Media. The cells were stained with Monocyte markers, CD14.

Media and Material

Monocyte Kit (ASE-9745)

Catalog #	Component	Amount		Storage	Shelf Life
ASE-9745-C	iPSC-derived Monocytes;	≥1x10^6 cells/ vial		Liq. N2	3 years
ASE-9745MM	Monocyte Basal Culture Media	100 mL		-20°C	12 months

Additional Reagents Required

The below reagents are recommended for use with the monocytes. 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.

- monocyte expansion supplement (not provided)
- monocyte differentiation supplement (not provided)
- antibodies:
  - CD14: Biolegend # 301806

Protocol

1. Preparing Cell Culture Surfaces

1.1 Please use ultra-low attachment vessels for best results.

2. Preparation of Monocyte Culture Media

2.1 Thaw the Monocyte Culture Basal Media at room temperature before thawing the cryopreserved monocytes.
2.2 Add required hematopoietic cytokines and growth factors into Monocyte Culture Basal Media for intended use.

Note: [Optional] add 1mL penicillin/streptomycin to 100 mL of the complete media to prevent bacterial contamination.

3. Thawing and Culturing Cryopreserved Monocytes

3.1 To thaw the cryopreserved Monocytes, 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 Monocyte 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 Monocyte 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 Monocytes at desired plating density.
3.12 Distribute the cells evenly.
3.13 Place the cell culture vessels in the incubator (37°C/ 5% CO_2/ humidity control).