

iPSC-Derived Monocytes With Media

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

We provide endothelial cells differentiated from an integration-free, control human iPSC line (ASE9211), reprogrammed from fibroblasts of an African-American male donor. These high-purity ($\geq 90\%$) cells express high levels of endothelial cell biomarkers, CD144 and CD31 (Figure 1).

To harness the full potential of our endothelial cells, we also provide optimized Endothelial Cell Culture Media (ASE-9744MM) that supports robust maintenance and functionality of the endothelial cells in culture.

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

Parental Tissue Control human iPSC (ASE-9211)
Age: Neonate
Gender: Male
Ethnicity: African-American
Tissue Source: Dermal Fibroblasts
Reprogramming Method: Episomal
Culture Conditions: Feeder-free

Catalog #	Product	Size
ASE-9744	iPSC-Derived Monocytes With Media	1 Kit

Kit Components

Catalog #	Component	Amount	Storage
ASE-9744-C	iPSC-derived Endothelial Cells	$\geq 1 \times 10^6$ cells/ vial	Liq. N ₂
ASE-9744MM	Endothelial Cell Culture Media	100 mL	-20°C

Intended Use

For research or further manufacturing purposes. Not for injection or diagnostic procedures.

Quality Assurance

Each lot of iPSC-derived human endothelial cells has been tested for growth, viability and purity ($\geq 90\%$) following recovery from cryopreservation. In addition, each lot has been tested for expression of endothelial cell markers and for the absence of mycoplasma and pathogens.

Storage Instructions 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.

Precautions

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.

Directions for Use

1. Coating Cell Culture Vessels with Coating Matrix

- 1.1. Coat the plates with 80 µg/mL Matrigel®.
Note: Please follow manufacture's instructions in coating plates using Matrigel®.
- 1.2. Incubate at room temperature for at least 1 hour before use.

2. Endothelial Cell Culture Media Preparation

- 2.1. Thaw the Endothelial Cell Culture Media at room temperature before thawing the cryopreserved endothelial cells.
- 2.2. The 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 two weeks or at -20°C for up to 12 months.

3. Thawing and Culturing Cryopreserved Endothelial Cells

- 3.1. To thaw the cryopreserved Endothelial cells, 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 Endothelial Cell Culture Media. 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 Endothelial Cell Culture Media into the tube and gently resuspend 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 Endothelial cells at a density ranging from 100,000-150,000 live cells/cm² in Endothelial Cell Culture Media.
- 3.12. Distribute the cells evenly.
- 3.13. Place the cell culture vessels in the incubator (37°C/ 5% CO₂/ humidity control).

3.14. Change media every 2-3 days.

Characterization of the ASE-9744 Endothelial Cells

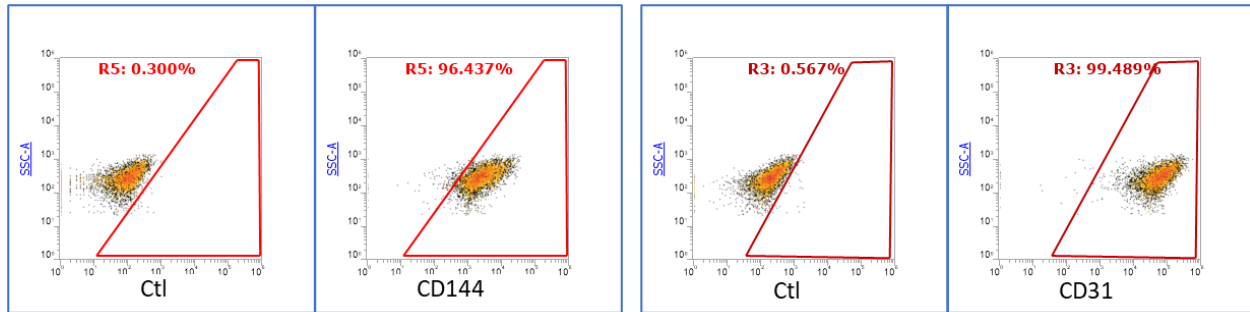


Fig 1. Immunostaining of ASE-9744 iPSC-derived Endothelial Cells for endothelial cell biomarkers.

Cryopreserved endothelial cells, differentiated from Applied StemCell's control iPSC line, ASE-9211 were recovered in Endothelial Cell Culture Media. The cells were stained with Endothelial Cell markers, CD144 and CD31.

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

Technical Support

CONTACT US

For more information or assistance, contact Customer Service at:

- Email: info@appliedstemcell.com
- Direct line: (408) 773-8007

WEBSITE RESOURCES

Visit appliedstemcell.com for technical resources and information including:

- Safety Data Sheets (SDS)
- Certificates of Analysis (when available)
- FAQs
- Product literature
- Complete contact information

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Effective Date: 15 September 2026