

## iPSC-Derived Monocytes With Media

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 monocytes differentiated from an integration-free, control human iPSC line (ASE-9211), reprogrammed from fibroblasts of an African-American male donor. These high-purity ( $\geq 90\%$ ) 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.

|                        |                                                                                                                                                                                                             |
|------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Parental Tissue</b> | Control human iPSC (ASE-9211); p15<br>Age: Neonate<br>Gender: Male<br>Ethnicity: African-American<br>Tissue Source: Dermal Fibroblasts<br>Reprogramming Method: Episomal<br>Culture Conditions: Feeder-free |
|------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

| Catalog # | Product                           | Size  |
|-----------|-----------------------------------|-------|
| ASE-9745  | iPSC-Derived Monocytes With Media | 1 Kit |

## Kit Components

| Catalog #  | Component                    | Amount                           | Storage             |
|------------|------------------------------|----------------------------------|---------------------|
| ASE-9745-C | iPSC-derived Monocytes       | $\geq 1 \times 10^6$ cells/ vial | Liq. N <sub>2</sub> |
| ASE-9745MM | Monocyte Basal 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 monocytes has been tested for growth, viability and purity ( $\geq 90\%$ ) following recovery from cryopreservation. In addition, each lot has been tested for expression of monocyte markers and for the absence of mycoplasma and pathogens.

## Storage Instructions and Stability

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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

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**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

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### 1. Coating Cell Culture Vessels with Coating Matrix

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

### 2. Preparation of Motor Neuron Culture Media

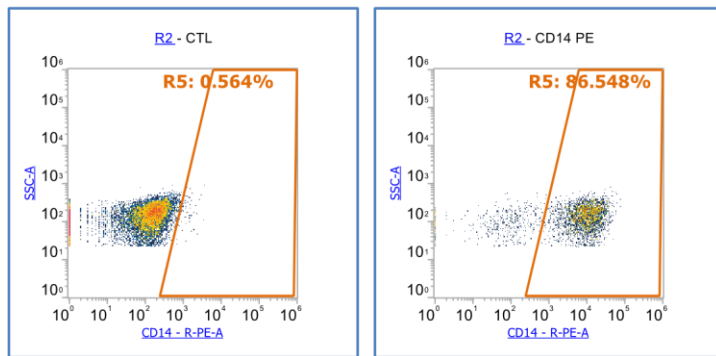
- 2.1. Thaw the Monocyte Culture Basal Media at room temperature before thawing the cryopreserved monocytes.
- 2.2. Add required hematopoietic cytokines and growth factors into Monocyte Culture Basal Media for intended use.  
**Note:** [Optional] Add 1 mL penicillin/streptomycin to 100 mL of the complete media to prevent bacterial contamination.

### 3. Thawing and Culturing Cryopreserved Motor Neurons

- 3.1. To thaw the cryopreserved Monocytes, remove one vial from the storage unit.
- 3.2. Immerse the vial in the bath (up to 2/3<sup>rd</sup> 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  $\mu$ L aliquot of the cell suspension and mix it with 10  $\mu$ L 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 cell culture vessels in the incubator (37°C/ 5% CO<sub>2</sub>/ humidity control).

## Characterization of the ASE-9475 Monocytes



**Fig 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 Monocyte Culture Media. The cells were stained with Monocyte markers, CD14.

### 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

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### CONTACT US

For more information or assistance, contact Customer Service at:

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

### WEBSITE RESOURCES

Visit [appliedstemcell.com](http://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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#### Applied StemCell, Inc.

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