

## iPSC-Derived Human Retinal Pigment Epithelium Kit (African-American, Male Line)

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

We provide RPE cells differentiated from an integration-free, control human iPSC line (ASE-9211), reprogrammed from the fibroblasts of an African-American male donor. These high-purity ( $\geq 90\%$ ) cells express high levels of RPE biomarkers, PMEL1, MITF, ZO-1, and RPE65 (Figure 1).

To harness the full potential of our RPE cells, we also provide optimized RPE Culture Media (ASE-9710MM) that supports robust maintenance and functionality of the RPE cells in culture.

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

| Catalog # | Product                                                               | Size  |
|-----------|-----------------------------------------------------------------------|-------|
| ASE-9710  | <b>iPSC-Derived Retinal Pigment Epithelium (RPE) Cells With Media</b> | 1 Kit |

### Kit Components

| Catalog #  | Product                                             | Amount                           |
|------------|-----------------------------------------------------|----------------------------------|
| ASE-9710-C | <i>iPSC-derived RPE, African-American Male Line</i> | $\geq 1 \times 10^6$ cells/ vial |
| ASE-9710MM | <i>RPE culture Media</i>                            | 100 mL                           |

### Intended Use

For research use only and not intended for human or animal diagnostic or therapeutic uses.

### Quality Assurance

Each lot of iPSC-derived human RPE 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 RPE 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

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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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### PROTOCOL FOR iSPC-Derived Human Retinal Pigment Epithelium Kit

- 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.

#### Retinal Pigment Epithelium Culture Media Preparation

- 2.1 Thaw the RPE Culture Media at room temperature before thawing the cryopreserved RPE 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 2 weeks or at -20°C for up to 12 months.*

#### Thawing and Culturing Cryopreserved RPE Cells

- 3.1 To thaw the cryopreserved RPE cells, remove one vial from the storage unit.
- 3.2 Immerse the vial in the water 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 RPE 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 RPE Culture Medium supplemented with 10 µM Y27632 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 RPE cells at a density ranging from 100,000-150,000 live cells/cm<sup>2</sup> in RPE Culture Medium supplemented with 10 µM Y27632.
- 3.12 Distribute the cells evenly.

- 3.13 Place the cell culture vessels in the incubator (37°C/ 5% CO<sub>2</sub>/ humidity control) overnight.
- 3.14 Change media the next day with RPE culture media without Y27632.
- 3.15 Change media every 2-3 days.
- 3.16 The cells may start showing ZO-1 and RPE65 staining 3-4 weeks after thaw.

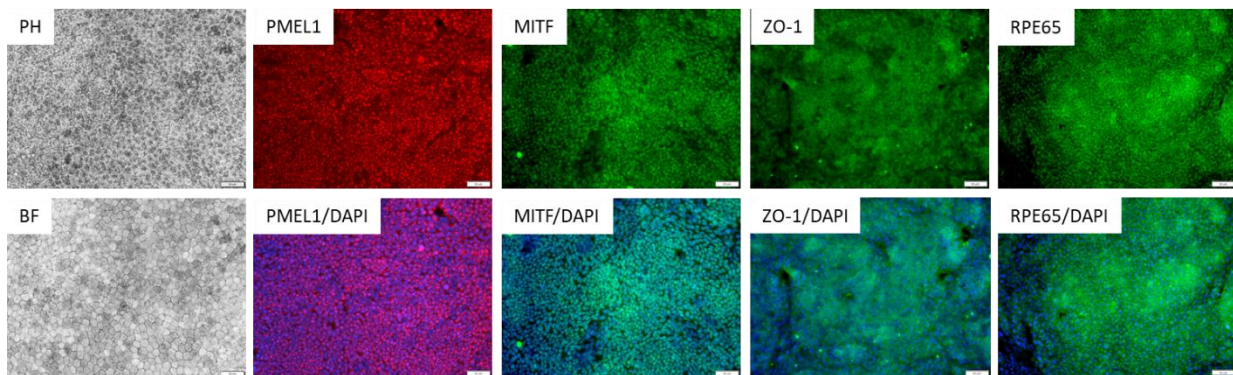
### Expected Results

- 4.1. The cells start to form tight junction and get pigmented in 3-4 weeks.
- 4.2. The cells can be passaged.
- 4.3. After passaging, the cells will lose pigmentation and seed another 3-4 weeks to get pigmentated again.

## Data

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### Characterization of the ASE-9710 Retinal Pigment Epithelium Cells



**Fig 1. Immunostaining of ASE-9710 iPSC-derived Retinal Pigment Epithelium Cells for retinal pigment epithelium biomarkers.** Cryopreserved RPE cells, differentiated from Applied StemCell's control iPSC line, ASE-9211 were recovered in RPE culture media. The cells were stained with RPE markers, PMEL1, MITF, ZO-1, and RPE65.

# 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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Effective Date: 15 September 2026