Applied StemCell, Inc.
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Datasheet

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

Product Information

Catalog Number **ASE-9710**

Description

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 (≥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. These RPE cells can also be used as effector cells for cytotoxicity assays.

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 RPE cells has been tested for growth, viability and purity (≥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.

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 RPE cells has been validated with the RPE Culture Media provided in the Retinal Pigment Epithelium Kit and the recommended additional reagents. Applied StemCell will not hold responsibility if components other than the components provided with the Retinal Pigment Epithelium Kit and those recommended are used to culture the Applied StemCell RPE cells.

Restricted Use

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

Characterization of the ASE-9710 Retinal Pigment Epithelium Cells

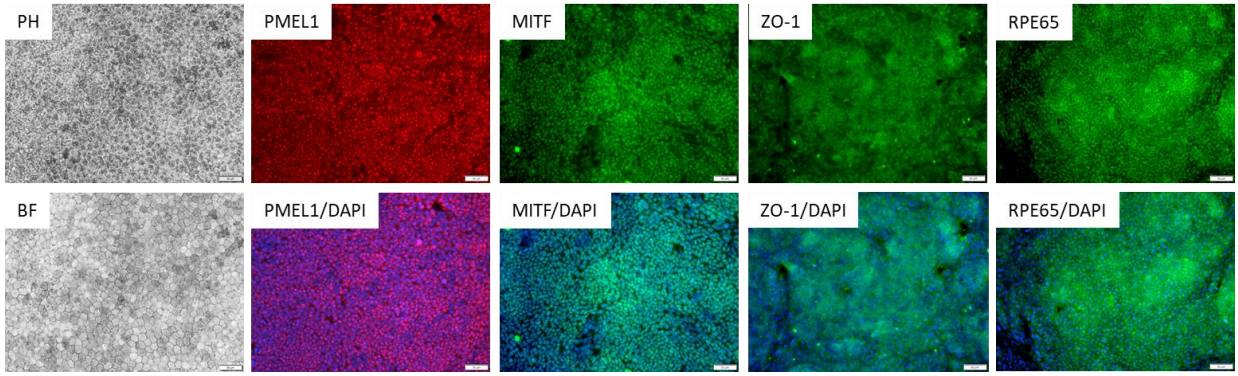

Figure 1. Immunostaining of ASE-9710 iPSC-derived Retinal Pigment Epithelium Cells for retinal pigment epithelium biomarkers. Cryopreserved RPE cells, differentiated from Applied StemCell's control iPSC line, ASE-9211 were recovered in RPE culture media. The cells were stained with RPE markers, PMEL1,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

Media and Material

Retinal Pigment Epithelium Kit (ASE-9710)

Catalog #	Component	Amount	Storage	Shelf Life
ASE-9710-C	iPSC-derived RPE; African-American Male Line	≥1x10^6 cells/ vial	Liq. N2	12 months
ASE-9710MM	RPE Culture Media	100 mL	-20°C	12 months

Additional Reagents Required

The below reagents are recommended for use with the RPE cells. 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
- Rock inhibitor Y27632 (Stem Cell Technology)
- Primary antibodies:
  - PMEL-1 Invitrogen #PA5-101023
  - MITF R&D systems #AF5769
  - ZO-1 Invitrogen #33-9100
  - RPE65 Abcam #ab235950
- 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. 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.

3. 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 2/3rd 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 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/cm2 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% CO2/ 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.

4. 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.