Applied StemCell, Inc.
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Datasheet

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

Product Information

Catalog Number **ASE-9715**

Description

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

We provide photoreceptor cells differentiated from an integration-free, control human iPSC line (ASE-9211), reprogrammed from fibroblasts of an African-American male donor. These cryopreserved, high-purity (≥90%) cells express high levels of photoreceptor cell biomarkers, CRX and NR2E3 (Figure 1), as well as Tuj1, Rhodopsin, and PDE6a (Figure 2).

To harness the full potential of our photoreceptor cells, we also provide optimized Photoreceptor Maturation Basal Media (ASE-9715MM) and Photoreceptor Culture Maturation Media Supplement (100x) (ASE-9715-A) that support the robust maintenance and functionality of the photoreceptor cells in culture.

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

Performance of Applied StemCell's photoreceptor cells has been validated with the Photoreceptor Maturation Basal Media and Photoreceptor Culture Maturation Media Supplement (100x) provided in the Photoreceptor Kit and the recommended additional reagents. Applied StemCell will not hold responsibility if components other than the components provided with the Photoreceptor Kit and those recommended are used to culture the Applied StemCell photoreceptor 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-9715 Photoreceptor Cells

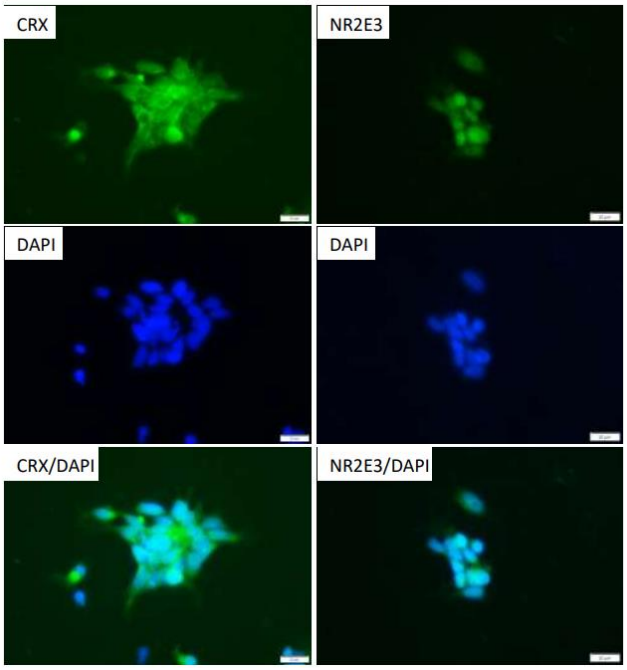

Figure 1. **Immunostaining of iPSC-derived Photoreceptor Precursors for photoreceptor biomarkers.** Photoreceptor precursors (ASE-9715), derived from Applied StemCell's control iPSC line, ASE-9211 were verified by antibody staining with photoreceptor precursor markers, CRX and NR2E3.

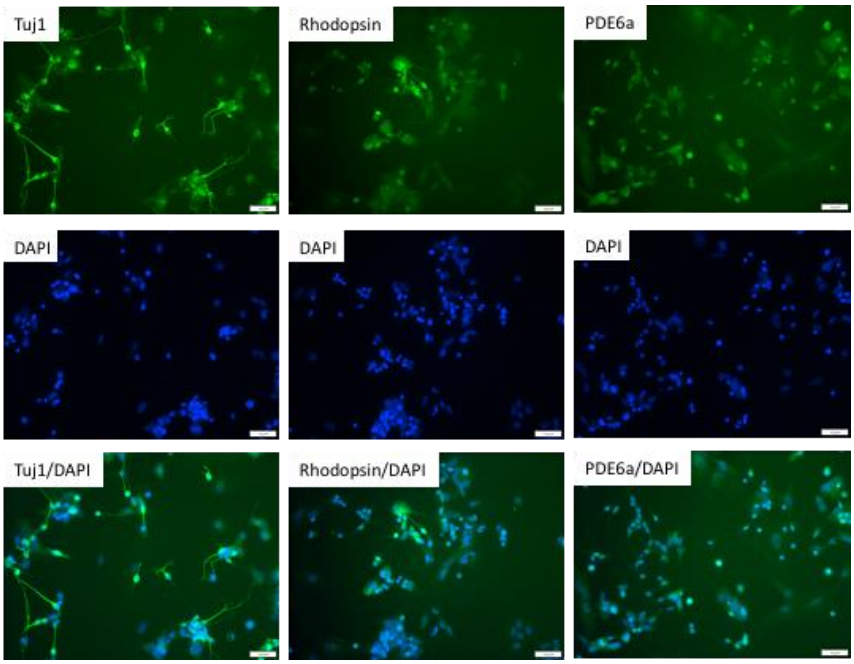

Figure 2. Immunostaining of Mature Photoreceptors derived from human iPSCs for photoreceptor biomarkers. Photoreceptor precursors (ASE-9715), derived from Applied StemCell's control iPSC line, ASE-9211 can be further differentiated in photoreceptor maturation media in 1-2 weeks. The mature photoreceptors were verified by antibody staining with photoreceptor markers, Tuji, Rhodopsin, and PDE6a.

Media and Material

Photoreceptor Kit (ASE-9715)

Catalog #	Component	Amount	Storage	Shelf Life
ASE-9715-C	iPSC-derived Photoreceptor Progenitors; African-American Male Line	≥1x10^6 cells/ vial	Lq. N2	12 months
ASE-9715MM	Photoreceptor Maturation Basal Media	100 mL	-20°C	6 months
ASE-9715-A	Photoreceptor Culture Maturation Media Supplement (100x)	1 mL	-20°C	6 months

Additional Reagents Required

The below reagents are recommended for use with the photoreceptor progenitor 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® Growth Factor Reduced: Corning, Cat# 354230
- DMEM/F-12, no glutamine: ThermoFisher, Cat# 21331020
- Primary antibodies:
  - CRX antibody: R & D system, Cat# AF7085
  - NR2E3 antibody: Proteintech, Cat#: I4246-1-AP
  - Tuj1 antibody: R&D systems, Cat# MAB1195
  - Rhodopsin antibody: ThermoFisher, Cat# MA5-11741
  - PDE6a antibody: ThermoFisher, Cat# PA1-720
- Secondary Antibodies: corresponding secondary antibodies were purchased from ThermoFisher

Protocol

1. Coating Cell Culture Vessels with Coating Matrix

1.1 Thaw the frozen vial of Matrigel® overnight at 4°C. Adjust the concentration into 1mg/mL with pre-chilled DMEM/F12. Aliquot the media into 1mL/15mL conical tube. Before use, thaw one aliquot in 4°C for at least 2 hours and add 11 mL pre-chilled DMEM/F12. Mix well. Add 1mL per well of a 6-well plate.
Note: Please follow manufacturer's instructions in coating plates using Matrigel®.

1.2 Incubate at room temperature for at least 1 hour before use.
Note: The coated plates can be stored in 4°C for up to two weeks.

2. Preparation of Photoreceptor Culture Media

2.1. Thaw the Photoreceptor Maturation Media Basal and Supplement over night at 4°C temperature before thawing the cryopreserved photoreceptor progenitors.
2.2. Mix 100 mL of the basal culture media and 1 mL of the supplement to make complete media.
2.3. The Photoreceptor Complete Media should be aliquoted and stored at -20°C if it will not be used immediately.
Note: The complete media can be stored at -20°C for up to six months.

3. Thawing and Culturing Cryopreserved Photoreceptor Progenitors

3.1 Remove one vial of cells from liquid nitrogen and transfer vial to a 37°C water bath.
Caution: Cryogenic gloves and safety glasses should be worn when working with liquid nitrogen.

3.2 Keep hold of the top of the sealed vial, and gently swirl around the water bath to ensure even thawing of frozen cells.
Note: Do not shake the vial during thawing.

3.3 Once only a small pellet of ice remains, remove the vial from water bath, spray the sealed vial with 70% ethanol, and transfer to laminar flow hood.
3.4 Transfer cell suspension to a 15 mL conical tube.
3.5 Add 4 mL of pre-warmed complete media into the conical tube and gently mix by pipetting up and down 1–2 times.
3.6 Centrifuge the conical tube at 200x g for 3 minutes.
3.7 Carefully aspirate supernatant with a sterile pipette tip while avoiding contact with the pellet.
Note: Take extra care not to remove or disturb the cell pellet during aspiration.

3.8 Resuspend the pellet with appropriate volume of complete media and plate suspension into Matrigel-coated plate.
Note: We recommend to seed 15k cells/cm^2 (e.g., 25k cells/500µL media/well in 24 well plate).

3.9 Change media every other day.
3.10 Cells can be stained with PDE6a and Rhodopsin after 5 days in culture.