Human iPSC-Derived Retinal Pigment Epithelial Cells

Frozen retinal pigment epithelial cells differentiated from human induced pluripotent stem cell lines

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Product Description

Human iPSC-Derived Retinal Pigment Epithelial (RPE) Cells are highly pure cells derived and manufactured from Healthy Control Human iPSC Line, SCTi003-A, Female (Catalog #200-0511) or SCTi004-A, Male (Catalog #200-0769) using STEMdiff™-ACF RPE Differentiation Kit (Catalog #100-1367).

RPE cells should be thawed and matured using xeno-free STEMdiff™-XF RPE Maturation Medium (Catalog #100-1365) to generate fully functional and mature retinal pigment epithelium. Mature RPE cells are ready for assessment and use in downstream applications after ≥ 5 weeks of culture post-thaw. The addition of STEMdiff™-ACF RPE Plating Supplement (Catalog #100-1364) is required to enhance the survival and attachment of RPE cells after thawing and replating.

Mature RPE cells express high levels of key maturity markers PMEL17, RPE65, EZRIN, and CRALBP, display polygonal morphology, are pigmented, polarized, and able to phagocytose photoreceptor outer segments. Additionally, these cells may be maintained long-term (≥ 9 weeks) while maintaining functionality and polarity. Mature RPE cells can be used for retinal disease modeling, drug discovery, and the development of regenerative medicine.

Cells were obtained using Institutional Review Board (IRB)-approved consent forms and protocols.

Ordering Information

iPSC SOURCE	CATALOG #	SIZE
Healthy Control Human iPSC Line, Female, SCTi003-A	200-0912	1 Vial, 1 x 10^6 cells/vial
Healthy Control Human iPSC Line, Female, SCTi003-A	100-2150	3 Vials, 1 x 10^6 cells/vial
Healthy Control Human iPSC Line, Male, SCTi004-A	200-0913	1 Vial, 1 x 10^6 cells/vial
Healthy Control Human iPSC Line, Male, SCTi004-A	100-2151	3 Vials, 1 x 10^6 cells/vial

Stability and Storage

Cells are frozen in a cryopreservation medium containing dimethyl sulfoxide (DMSO). Product stable at -135°C or colder for 12 months from date of receipt. Thawed samples must be used immediately.

Precautions

Cell Screening: iPSC master cell banks are screened for AAV2, BK virus, Epstein-Barr Virus, Hepatitis A, Hepatitis B, Hepatitis C, Herpes Simplex 1 and 2, Herpes Virus Type 6, Herpes Virus Type 7, Herpes Virus Type 8, HIV-1, HIV-2, HPV-16, HPV-18, Human Adenovirus, Human Cytomegalovirus, Human Foamy Virus, Human T-Lymphotropic Virus, John Cunningham Virus, LCMV, Parvovirus B19, Sarbecovirus (SARS Virus), Seoul Virus, Corynebacterium Bovis, and Mycoplasma (Human Comprehensive CLEAR Panel) by PCR. As testing cannot completely guarantee that the donor was virus-free, THIS PRODUCT SHOULD BE TREATED AS POTENTIALLY INFECTIOUS and only used following appropriate handling precautions such as those described in biological safety level 2.

Storage of frozen cell products in the vapor phase of a liquid nitrogen storage tank is recommended. Storage in the liquid phase can result in cross-contamination if the vial breaks or is not sealed properly. Storage in the liquid phase also increases the potential for liquid nitrogen to penetrate the vial and cause it to explode when removed from storage. Use of a face shield is required as a safety precaution when transferring cells from one container to another. When handling this product, do not use sharps such as needles and syringes.

STEMCELL cannot guarantee the biological function or any other properties associated with performance of cells in a researcher's individual assay or culture systems. STEMCELL assures the cells will meet the specifications only when assessed immediately after thawing by our test methods.

FOR IN VITRO RESEARCH USE ONLY. NOT APPROVED FOR DIAGNOSTIC, THERAPEUTIC, OR CLINICAL APPLICATIONS.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
12-well cell culture inserts	e.g. Sterlitech 9310422
12-well plate, tissue culture-treated	e.g. 200-0624
24-well plate, tissue culture-treated	e.g 38017
70 µm Reversible Strainer, Large	27260
Cell lifter	e.g 200-0596
Corning® Matrigel® hESC-Qualified Matrix OR Vitronectin XF™	Corning 354277 OR 07180
Polypropylene conical tubes, 15 mL and 50 mL	e.g. 38009 and 38010
STEMdiff™-ACF RPE Plating Supplement	100-1364
STEMdiff™-XF RPE Maturation Medium	100-1365
Trypan Blue	07050
TrypLE™ Express Enzyme (1X), no phenol red	Thermo Fisher Scientific 12604013

Preparation of Reagents and Materials

A. COATING CULTUREWARE WITH CORNING® MATRIGEL®

Use sterile technique when coating cultureware with Corning® Matrigel® hESC-Qualified Matrix. Corning® Matrigel® should be aliquoted and frozen. Consult the Certificate of Analysis supplied with Matrigel® for the recommended aliquot size ("Dilution Factor") to prepare 24 mL of diluted matrix. Make sure to always keep Matrigel® on ice when thawing and handling to prevent it from gelling. For complete instructions for coating cultureware with Corning® Matrigel®, refer to the Technical Manual: Maintenance of Human Pluripotent Stem Cells in mTeSR™ Plus, available at www.stemcell.com, or contact us to request a copy.

NOTE: One vial of Human iPSC-Derived RPE Cells (i.e. 1 x 10^6 cells) is enough to seed three wells of a 24-well plate at the recommended seeding density.

NOTE: Use tissue culture-treated cultureware with Corning® Matrigel®.

1. Thaw one aliquot of Corning® Matrigel® on ice.
2. Dispense 24 mL of cold DMEM/F-12 with 15 mM HEPES into a 50 mL conical tube and keep on ice.
3. Add thawed Matrigel® to the cold DMEM/F-12 with 15 mM HEPES (in the 50 mL tube) and mix well. The vial may be washed with cold medium if desired.
4. Immediately use the diluted Matrigel® solution to coat tissue culture-treated cultureware. Refer to Table 1 for recommended coating volumes.
5. Swirl the cultureware to spread the solution evenly across the surface.

   NOTE: If the cultureware's surface is not fully coated by the Matrigel® solution, it should not be used.
6. Incubate at room temperature (15 - 25°C) for at least 1 hour before use. Do not let the Matrigel® solution evaporate.

   NOTE: If not used immediately, the cultureware must be sealed to prevent evaporation of the Matrigel® solution (e.g. with Parafilm®) and can be stored at 2 - 8°C for up to 1 week after coating. Allow stored coated cultureware to come to room temperature before proceeding to step 7.
7. Immediately prior to seeding cells, gently tilt the cultureware onto one side and allow the excess Matrigel® solution to collect at the edge. Remove the excess solution using a serological pipette or by aspiration. Ensure that the coated surface is not scratched.

B. COATING CULTUREWARE WITH VITRONECTIN XF™

Use sterile technique when coating cultureware with Vitronectin XF™.

NOTE: Use tissue culture-treated cultureware for thawing and culturing RPE cells with Vitronectin XF™.

1. Thaw Vitronectin XF™ at room temperature (15 - 25°C).

   NOTE: For storage and stability information, refer to the Product Information Sheet (PIS) for Vitronectin XF™, available at www.stemcell.com, or contact us to request a copy.
2. In a 50 mL polypropylene conical tube, dilute Vitronectin XF™ in CellAdhere™ Dilution Buffer (Catalog #07183) to reach a final concentration of 10 µg/mL.

   For example, use 40 µL of Vitronectin XF™ per mL of CellAdhere™ Dilution Buffer.
3. Gently mix the diluted Vitronectin XF™. Do not vortex.
4. Immediately use the diluted Vitronectin XF™ solution to coat tissue culture-treated cultureware. Refer to Table 1 for recommended coating volumes.
5. Gently rock the cultureware back and forth to spread the solution evenly across the surface.

   NOTE: If the cultureware surface is not fully coated by the Vitronectin XF™ solution, it should not be used.
6. Incubate at room temperature (15 - 25°C) for at least 1 hour before use. Do not let the Vitronectin XF™ solution evaporate.
7. Immediately prior to seeding cells, gently tilt the cultureware onto one side and allow the excess Vitronectin XF™ solution to collect at the edge. Remove the excess solution using a serological pipette or by aspiration. Ensure that the coated surface is not scratched.

   NOTE: Washing Vitronectin XF™-coated cultureware before seeding cells is not required for RPE culture.

Table 1. Recommended Volumes for Coating Cultureware

CULTUREWARE	VOLUME OF DILUTED MATRIX
96-well plate	100 µL/well
24-well plate	250 µL/well
12-well cell culture inserts*	250 µL (apical side)
12-well plate	500 µL/well
6-well plate	1 mL/well

* Do not seed thawed RPE cells directly onto cell culture inserts. Refer to section B for instructions on preparing RPE cells for culture on inserts.

C. PREPARATION OF RPE PLATING MEDIUM

Use sterile technique to prepare RPE Plating Medium (STEMdiff™-XF RPE Maturation Medium + STEMdiff™-ACF RPE Plating Supplement). The following example is for preparing 25 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw STEMdiff™-ACF RPE Plating Supplement at room temperature (15 - 25°C). Mix thoroughly.

   NOTE: Once thawed, use immediately or aliquot and store at -20°C. Do not exceed the shelf life of the supplement. After thawing the aliquots, use immediately. Do not re-freeze.
2. Add 250 µL of STEMdiff™-ACF RPE Plating Supplement to 24.75 mL of STEMdiff™-XF RPE Maturation Medium. Mix thoroughly. Warm to room temperature before use.

   NOTE: Use on day of preparation; do not store.

Directions for Use

Use sterile technique when performing the following protocols:

A. Thawing and Plating RPE Cells

B. Harvesting RPE Cells and Plating onto Cell Culture Inserts

C. RPE Maturation and Long-Term Maintenance

A. THAWING AND PLATING RPE CELLS

Generally, 1 x 10^6 of Human iPSC-Derived RPE Cells is enough to seed three wells of a 24-well plate at the recommended seeding density. The following instructions are for thawing and seeding RPE cells into coated 24-well plates. If using other cultureware, adjust volumes accordingly (see Table 2).

NOTE: It is not recommended to seed RPE cells onto cell culture inserts immediately after thawing. RPE cells should be thawed and cultured for approximately one week in RPE Plating Medium before harvesting and plating onto cell culture inserts, as described in section B.

1. Coat the desired number of wells of a 24-well tissue culture-treated plate with Corning® Matrigel® or Vitronectin XF™ (Preparation section A or B) and prepare fresh RPE Plating Medium (Preparation section C).
2. Warm RPE Plating Medium, a sufficient volume of STEMdiff™-XF RPE Maturation Medium, and coated cultureware to room temperature (15 - 25°C) before starting the protocol to ensure that the thawing procedure is done as quickly as possible.
3. Add 8 mL of warm RPE Maturation Medium to a 15 mL conical tube.
4. Wipe the outside of the vial of cells with 70% ethanol or isopropanol.
5. In a biosafety hood, twist the cap a quarter-turn to relieve internal pressure and then retighten.
6. Quickly thaw cells in a 37°C water bath by gently shaking the cryovial. Remove the cryovial when a small frozen cell pellet remains. Do not vortex cells.

   NOTE: ThawSTAR® CFT2 Automated Thawing System (Catalog #100-0650) may be used to quickly and efficiently thaw cells.
7. Wipe the outside of the cryovial with 70% ethanol or isopropanol.

   NOTE: It is important to work quickly in the following steps to ensure high cell viability and recovery.
8. Use a 1 mL pipette to transfer the contents of the cryovial to the 15 mL conical tube prepared in step 3.
9. Rinse the cryovial with 1 mL of warm STEMdiff™-XF RPE Maturation Medium and transfer the rinse to the same conical tube.
10. Centrifuge cells at 300 x g for 5 minutes at room temperature.
11. Carefully remove the supernatant with a pipette, leaving a small amount of medium to ensure the cell pellet is not disturbed. Resuspend the cell pellet by gently flicking the tube.
12. Add 2 mL of warm RPE Plating Medium to the tube. Mix gently.
13. Remove a 20 µL aliquot of cells to perform a viable cell count using Trypan Blue and a hemocytometer (e.g. Catalog #100-1181).
14. Using a serological pipette or by aspiration, gently remove the matrix solution from the 24-well tissue culture-treated plate (prepared in step 1). Ensure that the coated surface is not scratched.
15. Plate 2.9 x 10^5 cells in 1 mL of warm RPE Plating Medium per well of the matrix-coated 24-well plate (i.e. 1.5 x 10^5 cells/cm^2). Refer to Table 2 for recommended volumes and plating densities for other cultureware.

Table 2. Recommended Volumes and Plating Densities for Various Cultureware

CULTUREWARE	VOLUME OF MEDIUM	NUMBER OF CELLS PER WELL
96-well plate	200 µL/well	5 x 10^4 cells
24-well plate	1 mL/well	2.9 x 10^5 cells
12-well plate	2 mL/well	5.7 x 10^5 cells
6-well plate	4 mL/well	1.5 x 10^6 cells

16. Incubate at 37°C and 5% CO_2. After 3 - 4 days, perform a full-medium change as follows:
    a. Prepare a sufficient volume of RPE Plating Medium and warm to room temperature.
    b. Remove and discard spent medium. Add 1 mL of warm RPE Plating Medium per well.
    c. Incubate cells at 37°C and 5% CO_2 for an additional 3 - 4 days.

Proceed to section B to harvest and plate RPE cells onto cell culture inserts. For applications that do not require replating RPE cells onto other cultureware, proceed to section C to continue RPE maturation.

B. HARVESTING RPE CELLS AND PLATING ONTO CELL CULTURE INSERTS

The following instructions are for harvesting and plating a 24-well plate of RPE cells onto cell culture inserts in a 12-well plate format. If harvesting RPE cells from other cultureware, adjust volumes according to Table 3.

Table 3. Recommended Volumes for Harvesting RPE Cells From Various Cultureware

CULTUREWARE	D-PBS WASH	TrypLE™ EXPRESS ENZYME	STEMDIFF™-XF RPE MATURATION MEDIUM WASH
96-well plate	100 µL/well	100 µL/well	400 µL/well
24-well plate	250 µL/well	250 µL/well	1 mL/well
12-well plate	500 µL/well	500 µL/well	2 mL/well
6-well plate	1 mL/well	1 mL/well	4 mL/well

1. Prepare a 12-well plate containing the desired number of cell culture inserts. Coat each cell culture insert with 250 µL of Corning® Matrigel® or Vitronectin XF™ as directed in Preparation section A or B, respectively.
2. Prepare fresh RPE Plating Medium (Preparation section C). Warm RPE Plating Medium, coated cultureware, and a sufficient volume of STEMdiff™-XF RPE Maturation Medium to room temperature (15 - 25°C).
3. Harvest the RPE cell culture as follows:
   a. Remove and discard the medium. Gently wash each well with 250 µL of D-PBS (Without Ca++ and Mg++). Discard the wash.
   b. Add 250 µL of TrypLE™ Express Enzyme per well and incubate the plate at 37°C for 10 minutes.
   c. Gently tap the side of the plate to detach the monolayer. If needed, use a cell lifter to detach any remaining cells.
   d. Pipette the cells up and down several times to generate a uniform single-cell suspension and transfer to a 50 mL conical tube.
   e. Wash each well with 1 mL of warm STEMdiff™-XF RPE Maturation Medium to collect any remaining cells and transfer the wash to the same tube.
   f. Pass the cell suspension through a 70 µm Reversible Strainer attached to a new 50 mL conical tube and collect the flow-through.
   g. Centrifuge the tube at 300 x g for 5 minutes at room temperature.
   h. Discard the supernatant and resuspend the cells in 2 mL of warm RPE Plating Medium (prepared in step 1).
   i. Perform a viable cell count using Trypan Blue and a hemocytometer (e.g. Catalog #100-1181).
4. Using a serological pipette or by aspiration, gently remove the matrix solution from the apical side of each cell culture insert (prepared in step 1). Ensure that the coated surface is not scratched.
5. Immediately plate RPE cells as follows:
   a. Add 4 x 10^5 cells in 0.5 mL of warm RPE Plating Medium (8 x 10^5 cells/mL) to each cell culture insert (apical chamber).
   b. Add 1.5 mL of warm RPE Plating Medium to each well of the 12-well plate containing an insert (basal chamber).
6. Incubate the plate at 37°C and 5% CO_2.
7. After 3 - 4 days of incubation, perform a full-medium change as follows:
   a. Prepare a sufficient volume of RPE Plating Medium and warm to room temperature.
   b. Gently remove and discard medium from the apical and basal chambers. Add 0.5 mL and 1.5 mL of warm RPE Plating Medium to the apical and basal chambers of each well, respectively.
   c. Incubate cells at 37°C and 5% CO_2 for an additional 3 - 4 days.

Proceed to section C to continue RPE maturation.

C. RPE MATURATION AND LONG-TERM MAINTENANCE

RPE cells must be cultured in STEMdiff™-XF RPE Maturation Medium for at least 5 weeks post-thaw to generate fully functional and mature retinal pigment epithelium. The following instructions are for maturing RPE cells on 12-well cell culture inserts. If using other cultureware (e.g. the 24-well plate RPE culture from section A), adjust volumes according to Table 2.

1. Warm a sufficient volume of STEMdiff™-XF RPE Maturation Medium to room temperature (15 - 25°C).
2. Gently remove and discard RPE Plating Medium from the apical and basal chambers. Add 0.5 mL and 1.5 mL of warm STEMdiff™-XF RPE Maturation Medium to the apical and basal chambers of each well, respectively.
3. Incubate cells at 37°C and 5% CO_2. Perform full-medium changes every 3 - 4 days using warm STEMdiff™-XF RPE Maturation Medium.
4. Mature RPE cells are ready for assessment and use in downstream applications after ≥ 5 weeks of culture post-thaw. For long-term maintenance, mature RPE cells may be cultured in STEMdiff™-XF RPE Maturation Medium for ≥ 9 weeks while maintaining functionality and polarity.

Assessment of Mature RPE Cells

Mature RPE cells can be assessed by immunocytochemistry or flow cytometry after labelling with the following antibodies:

- Anti-human PMEL17 antibody, clone HMB-45 (BioLegend #911505)
- Anti-human RPE65 antibody, clone 401.8B11.3D9 (Thermo Fisher Scientific #MA1-16578)
- Anti-human EZRIN antibody, clone 3C12 (Santa Cruz Biotechnology #sc-58758)
- Anti-human CRALBP antibody, clone B2 (Santa Cruz Biotechnology #sc-59487)
- Anti-human BEST1 antibody, clone E6-6 (Millipore Sigma #ZMS1043)
- Anti-human ZO-1 polyclonal antibody (Thermo Fisher Scientific #40-2200)

Antibodies against RPE65, EZRIN, CRALBP, BEST1, and ZO-1 require staining with a secondary antibody. Refer to Table 4 below for expected expression levels for mature RPE.

Table 4. Expected Expression Levels for Assessment of Mature RPE Cells

ANTIBODY TARGET	EXPECTED EXPRESSION
PMEL17	≥ 90%
RPE65	> 80%
EZRIN	> 80%
CRALBP	> 80%
BEST1	Positive
ZO-1	Positive

RPE maturity may also be assessed by functional assays, such as melanin production, polarized secretion of VEGF and PEDF (as determined by ELISA), transepithelial resistance, and ability to phagocytose bovine or porcine photoreceptor outer segments.

Related Products

For related products, including specialized cell culture and storage media, supplements, antibodies, cytokines, and small molecules, visit www.stemcell.com, or contact us at techsupport@stemcell.com.

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