phenocell

stem cells for better drugs

Human Retinal Pigment Epithelium cells
PCi-RPE

User's guide

PRODUCT INFORMATION

Product Ref. PCi-RPE
Additional Ref.: PhenoCULT®-RPE culture medium ; PhenoCULT®-RPE KIT (cells and culture medium)
Thank you for purchasing PCi-RPE, Phenocell's human iPSC-derived Retinal Pigment Epithelium cells. After receiving a batch of PCi-RPE, you may follow this guide for successful culture of your sample.
PCi-RPE are provided in CryoStor® CS10 cryopreservation medium (StemCell Technologies).CS10 contains 10% DMSO.

Product	Catalog No.	Quantity
Human iPSC-derived Retinal Pigment Epithelium cells	PCi-RPE_2M	$2 * 10^6$ cell/vial
Human iPSC-derived Retinal Pigment Epithelium cells	PCi-RPE_6M	$6 * 10^6$ cell/vial

- Each lot is tested for absence of mycoplasma, HBV, HCV, HIV1/2.
- Expiration:
  - Guaranteed for up to 12 months from date of receipt if properly stored. Use cells immediately after thawing.

STORAGE

PCi-RPE should be kept below -135°C, either in a deepfreezer (-145°C) or in the vapor phase of liquid nitrogen. Long-term storage at -80°C is not recommended. PCi-RPE are provided in CryoStor® CS10 cryopreservation medium (StemCell Technologies, e.g. #07959). CS10 contains 10% DMSO

PRODUCT USE

PCi-RPE are intended for in vitro research use only and are not to be used for any other purpose, which includes, but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.
Products are covered by issued and pending patents. The purchase of the Product does not include nor carry any right or license to use, develop or otherwise exploit the Product commercially. Contact Phenocell for more information on Product Limited License Use.

SAFETY PRECAUTIONS

Wear the appropriate personal protection equipment and handle the frozen vials with due caution. This product should be treated as potentially infectious and only used in adequate biological safety premises and conditions.
Do not ingest. In case of contact with eyes, rinse immediately with water for at least 15 min and seek medical advice. Environmental measures: soak up with inert absorbent material. Clean with bleach and rinse thoroughly. Prevent further leakage or spillage if safe to do so.
Phenocell can not be held liable for any damage or losses resulting from the handling or from contact with the product.

BEFORE YOU START

If you perform PCi-RPE culture for the first time, you might feel more confident with a little help. Our skilled technical support staff is fully available at contact@phenocell.com and by phone or online at `www.phenocell.com`. Do not hesitate to contact us to get personalized help and fully achieve your goals with PCi-RPE.
Phenocell cannot guarantee the biological function or any other properties associated with performance of the product in researchers' individual culture systems. Phenocell guarantees that the product will meet the specifications only when assessed immediately after thawing using the recommended Protocol.

FOR RESEARCH USE ONLY

Not intended for human or animal diagnostic, therapeutic or clinical applications.

PROTOCOL

IMPORTANT NOTICE

This protocol has been validated using the **Reagents and medium** references mentioned.

All steps should be performed in a sterile culture environment using adequate handling procedures. Cells should be maintained in a 37°C-5% CO2 incubator.
PCi-RPE are human cells and, as such, should be handled with required ethical and safety rules.

NOTE ON MATRIGEL® COATING:

For best performance with PCi-RPE, we advise to prepare the Matrigel® coating at a final density of 8-10 µg/cm2 of tissue culture surface, and to incubate for at least 6h (best overnight) in 37°C incubator before use. Unused Matrigel®-coated plates can be stored at 4°C for a maximum of 5 days.
DO NOT USE if Matrigel® coating has dried.

THAWING

Reagents and medium

- PhenoCULT®-RPE medium (See online)
- Matrigel® (Corning Life Sci., Cat. No. 354230) coated tissue culture plates Not included

Procedure

Important: If you are using matrigel for the first time, please carefully review matrigel product sheet and associated coating protocols.

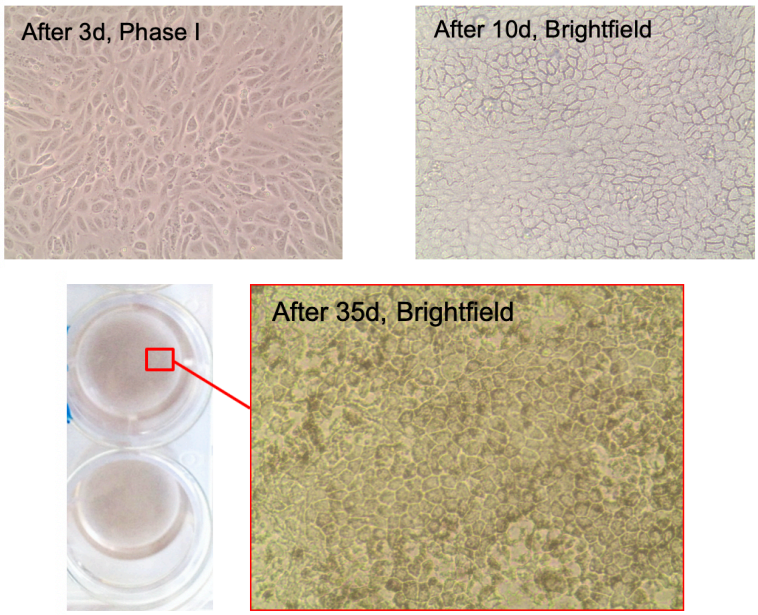
1. Pre-warm RPE medium in a 37°C water-bath.
2. Quickly thaw PCi-RPE cells vial in 37°C water bath until a small frozen cell pellet remains. Do not vortex cells.
3. Wipe out the outside of the vial of cells with 70% ethanol.
4. Transfer the cells to a 15 mL conical tube and add 2 mL of RPE medium.
5. Centrifuge cell suspension at 150 g x 3 min at room temperature.
6. Carefully remove the supernatant, leaving a small amount of medium to ensure the cell pellet is not disturbed.

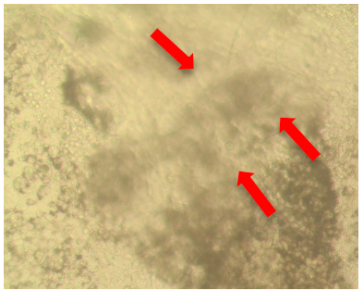
7. Gently add 1 mL of RPE medium to resuspend the cell pellet. Dissociate the pellet by gentle pipetting until no visible clump remain.
8. Count cells and plate on Matrigel®-coated tissue culture surface at a density of 100,000 cells/cm2. Use 3 mL of RPE medium for each 10 cm2 of culture surface.
9. Note: because of RPE pigmentation, image-based trypan blue viability determination may be skewed towards lower percentages. For best results, use a fluorescent-based approach such as propidium iodide exclusion method.
10. Place the plate into the incubator (37°C-5%CO2). To evenly distribute the cells, move the plate twice forward to backward and side-to-side, in quick motions.
11. Feed every day with 3 mL RPE medium for each 10 cm2 of culture surface. For week-ends, use 4mL medium on Saturday (no need to feed on Sunday).

Note: once PCi-RPE cells establish a confluent monolayer, they will quickly consume the nutrients in the culture medium, and produce metabolic waste which will turn the medium yellowish. Feeding everyday allows best results when culture is performed on regular flat surface (such as plates or flasks). If you use an insert, then medium feeding can be done every other day.

Morphology

Within the first 15 days after plating at 100,000 cell/cm2, PCi-RPE will acquire their characteristic polygonal morphology. After one month, pigmentation is visible to the naked eye (against a white background).





Note that after one month of culture in a standard tissue culture dish, swollen areas will appear (red arrows). These swollen areas are fluid-filled domes. They confirm that RPE cells are functional and transport fluid from apical to basal sides.
Would domes presence impair your studies, you might avoid them by culturing PCi-RPE on Transwell® inserts.

PASSAGING

PCi-RPE passage is usually performed every other week, when the cells have acquired their polygonal morphology and are lightly pigmented (usually only observable in the cell pellet during passaging).

Reagents and medium

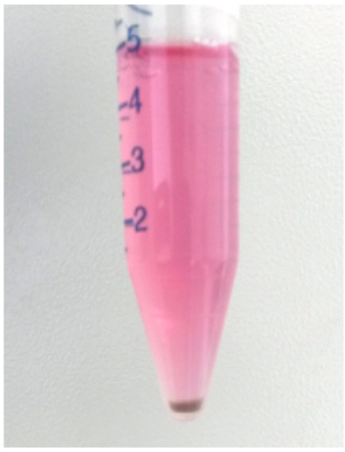
- PhenoCULT®-RPE medium (See online)
- Accumax™ (Sigma, Cat. A7089) Not included
- Matrigel® (Corning Life Sci., Cat. 354230) coated tissue culture plates. Not included

Procedure

1. Remove culture medium and add 1 mL Accumax™ for each 10 cm2 of culture surface.
2. Incubate at 37°C-5%CO2 for 15-20 min. Regularly check the cells and proceed to the next step when all the cells look rounded.
3. Thoroughly flush the PCi-RPE cell layer using the Accumax™ already in the dish. If cells do not readily detach, incubate at 37°C for additional 5 min.
4. Transfer the cells to a Falcon tube of the appropriate size pre-loaded with 2 mL RPE medium for each 1 mL of Accumax™ added.
5. Centrifuge at RT, 150 g, 3 min.
6. Eliminate supernatant and re-suspend in RPE medium. With a 1 mL pipette, gently triturate until a single cell solution is achieved.

7. Count cells and plate on Matrigel®-coated tissue culture surface at a density of 100,000 cells/cm2. Use 3 mL of RPE medium for each 10 cm2 of culture surface.
8. Note: because of RPE pigmentation, image-based trypan blue viability determination may be skewed towards lower percentages. For best results, use a fluorescent-based approach such as propidium iodide exclusion method.
9. Place the plate into the incubator (37°C-5%CO2). To evenly distribute the cells, move the plate twice forward to backward and side-to-side, in quick motions.
10. Feed every day with 3 mL RPE medium for each 10 cm2 of culture surface. For week-ends, use 4mL medium on Saturday (no need to feed on Sunday).

Note: once PCi-RPE cells establish a confluent monolayer, they will quickly consume the nutrients in the culture medium, and produce metabolic waste which will turn the medium yellowish. Feeding everyday allows best results when culture is performed on regular flat surface (such as plates or flasks). If you use an insert, then medium feeding can be done every other day.



Presence of a pigmented pellet upon passaging PCi-RPE 15 days after plating