Phenocell
Stem Cells for better Drugs

Human iPSC-derived Keratinocytes PCi-KER

User's guide

PRODUCT INFORMATION

Product Ref. PCi-KER_CAU
Additional Ref.: PhenoCULT®-KER culture medium.
Thank you for purchasing PCi-KER, Phenocell's human iPS cell-derived Keratinocytes. After receiving a batch of PCi-KER, you may follow this guide for successful culture from your sample.
PCi-KER are provided in 1-million cell format frozen in cryopreservation medium and are shipped in dry ice.

Product	Catalog No.	Quantity	Donor
Human iPSC-derived Keratinocytes	PCi-KER_CAU	10^6 cell/vial	Caucasian
PhenoCULT®-KER culture medium	PhenoCULT®-KER	100 mL	

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

STORAGE

PCi-KER 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-KER are provided in CryoStor® CS10 cryopreservation medium (StemCell Technologies, e.g. #07959). CS10 contains 10% DMSO.
Medium: keep at 4°C and use within 2 weeks after thawing.

PRODUCT USE

PCi-KER 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.

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-KER 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-KER.
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. PCi-KER are human cells and, as such, should be handled with required ethical and safety rules.

THAWING

IMPORTANT: work quickly after the cells have been thawed to ensure high viability and recovery.

Reagents and medium

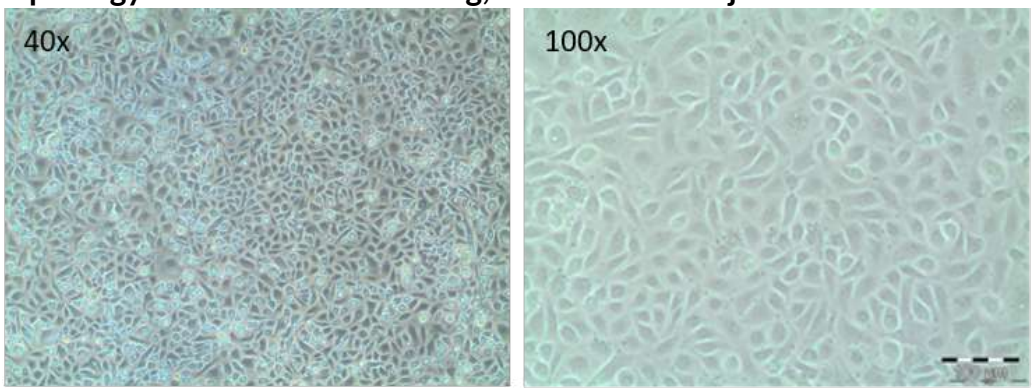
- PhenoCULT®-KER complete medium Only in kit
- CellAdhere™ Laminin-521 (StemCell Technologies, Cat #77003) coated tissue culture plates Not included

Procedure

1. Coat tissue culture plate with Laminin diluted to 1/50 in dPBS (0.1mL per cm2). Incubate for at least 2h in a 37°C incubator. Remove Laminin solution before use.
2. Pre-warm PhenoCULT®-KER.
3. Quickly thaw PCi-KER in a 37°C water bath, gently swirling the tube for less than a minute until only a small piece of ice remains. Do not vortex cells.
4. Transfer the vial to a biosafety hood and gently wipe the outside of the vial with 70% ethanol.
5. Transfer the cells to a conical tube with 6 mL of complete culture medium.
6. Centrifuge at 300g for 3 min, discard supernatant and resuspend in 1mL of medium
7. Count cells and directly plate on Laminin-coated surface at a density of 25,000 cells/cm2. Use 2 mL of complete culture medium per 10 cm2 of culture surface.
8. 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.

9. Replace the medium with complete PhenoCULT®-KER every other day.
10. Cells can be passaged when 90% confluence is reached.

Morphology evolution after thawing, when cells have just reached confluence



PASSAGING

Reagents and medium

- PhenoCULT®-KER complete medium Only in kit
- CellAdhere™ Laminin-521 (StemCell Technologies, Cat #77003) coated tissue culture plates
- Trypsin-EDTA (0.05%) (ThermoFischer, cat. #25300) Not included

Procedure

For PCi-KER amplification, passage every 5-6 days.

1. Coat tissue culture plates with Laminin diluted at 1/50 in dPBS. Incubate for at least 2 h in 37°C incubator. Before use, remove Laminin solution.
2. Pre-warm PhenoCULT®-KER and Trypsin-EDTA (0.05%).
3. Discard culture medium, briefly wash cells once with PBS.
4. Add 1 mL Trypsin-EDTA (0.05%) for each 10 cm2 of culture surface and incubate at 37°C for 5-10 min. Regularly check cell digestion: when PCi-KER are rounding up, detach them by gently flushing with the culture medium present in the plate.
5. Transfer to a 15 mL tube pre-loaded with complete culture medium (anticipate at least a 1/3 dilution ratio to stop Trypsin-EDTA (0.05%) action).
6. Centrifuge at room temperature, 300 g for 3 min.
7. Eliminate supernatant and re-suspend in complete culture medium. Gently triturate until a single cell solution is achieved.

8. Count cells and plate on Laminin-coated culture surface at a density of 25,000 cells/cm2.
9. Place the plate into the incubator. To evenly distribute the cells, move the plate twice forward to backward and side to side, in quick motions.
10. Replace medium every other day using 2mL/10cm² (add 3 mL/10 cm² culture surface for week-ends).