Phenocell
Stem Cells for better Drugs

Human iPSC-derived Melanocytes
PCi-MEL

User's guide

PRODUCT INFORMATION

Product Ref. PCi-MEL_CAU / PCi-MEL_ASI / PCi-MEL_AFR
Additional Ref.: PhenoCULT®-MEL culture medium.
Thank you for purchasing PCi-MEL, Phenocell's human iPS cell-derived Melanocytes. After receiving a batch of PCi-MEL, you may follow this guide for successful culture from your sample.
PCi-MEL 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 Melanocytes	PCi-MEL_CAU	10^6 cell/vial	Caucasian
Human iPSC-derived Melanocytes	PCi-MEL_ASI	10^6 cell/vial	Asian
Human iPSC-derived Melanocytes	PCi-MEL_AFR	10^6 cell/vial	African
PhenoCULT®-MEL culture medium	PhenoCULT®-MEL	100 mL	

- Each lot is tested for expression of keratinocytes markers and for absence of 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-MEL 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-MEL 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; use within 1 week after addition of Supplement.

PRODUCT USE

PCi-MEL 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-MEL 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-MEL.
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-MEL 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

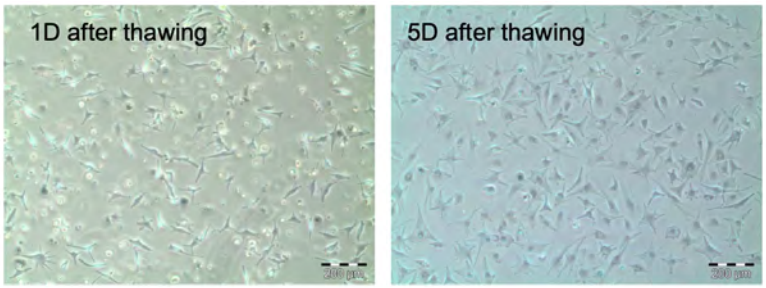
- PhenoCULT®-MEL medium + Supplement or standard medium for primary melanocyte culture
- Fibronectin (Sigma, cat. #F1141) - If you are performing this protocol for the first time, please make sure to use this specific reference.
- TrypLE Express (ThermoFischer, cat. #12605)

Procedure

1. Coat tissue culture plate with fibronectin diluted to 1/100 in PBS (0.1mL per cm2). Incubate for at least 2h in a 37°C incubator. Remove fibronectin solution before use.
2. Pre-warm PhenoCULT®-MEL.
3. Prepare complete culture medium by adding 200 µL Supplement to 50 mL PhenoCULT®-MEL (stable at 4° C for one week after reconstitution).
4. Quickly thaw PCi-MEL 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.
5. Transfer the vial to a biosafety hood and gently wipe the outside of the vial with 70% ethanol.
6. Transfer the cells to a conical tube with 6 mL of complete culture medium.
7. Count cells and directly plate on fibronectin-coated surface at a density of 30,000 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. About 4 h later, verify that the cells have attached and carefully replace culture medium with fresh, pre-warmed complete culture medium.
10. Replace medium every other day (add 3 mL/10 cm2 culture surface for week-ends).

Morphology evolution after plating: Some floating cells are observed one day after thawing.



Over the first 10-15 days, PCi-MEL will mature and pigmentation will become more prominent.

PASSAGE

Reagents and medium

- PhenoCULT®-MEL medium + Supplement or standard medium for primary melanocyte culture.
- Fibronectin (Sigma, cat. #F1141)
- TrypLE Express (ThermoFischer, cat. #12605)

Procedure

For PCi-MEL amplification, passage every 7-15 days.

1. Coat tissue culture plates with fibronectin diluted at 1/100 in PBS. Incubate for at least 2 h in 37°C incubator. Before use, remove fibronectin solution.
2. Pre-warm PhenoCULT®-MEL and TrypLE™ Express.
3. Discard culture medium, briefly wash cells once with PBS.
4. Add 1 mL TrypLE Express for each 10 cm2 of culture surface and incubate at 37°C for 5-10 min. Regularly check cell digestion: when PCi-MEL 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 TrypLE Express action).

6. Centrifuge at room temperature, x 200 g, 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 fibronectin-coated culture surface at a density of 20,000 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 (add 3 mL/10 cm2 culture surface for week-ends).