

Human iPSC derived Melanocytes

PCi-MEL

Description

Product Ref.: PCi-MEL-1M

Additional Ref.: PhenoCULT-MEL® culture medium.

Thank you for purchasing PCi-MEL, Phenocell's human iPSC 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 cells format frozen in cryopreservation medium and are shipped in dry ice.

Product Information

Product	Catalog No.	Quantity	Donor
Human iPSC-derived Melanocytes	PCi-MEL_1M	10 ⁶ cells	Male Caucasian (Phototype III-IV)

- Storage conditions : Product stable at -135°C or colder. Storage in the vapor phase of a liquid nitrogen storage tank is recommended.
- Expiration : Guaranteed for up to 12 months from date of receipt if properly stored. Use cells immediately after thawing.

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 biological safety Level 2 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

PCi-MEL behave like primary melanocytes in culture. However, 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. Do not hesitate to contact us to get personalized help and fully achieve your goals with PCi-MEL.

Protocol starts on next page.

FOR RESEARCH USE ONLY. Not intended for human or animal diagnostic or therapeutic use.

Phenocell can not 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.

Thawing and Culture recommendations

Culture medium and reagents

- PhenoCULT®-MEL basal (100 mL).
- PhenoCULT®-MEL supplements (400 µL, 250x).
- Fibronectin (Sigma, cat. #F1141)
- TrypLE™ Express (ThermoFischer, cat. #12605)

Preparing PhenoCULT-MEL® complete medium

To the entire content of a bottle of PhenoCULT®-MEL basal (100 mL), add the entire content of the supplements (400 µL). PhenoCULT®-MEL complete is stable for 2 weeks at 4°C.

It is possible to thaw and freeze once both basal medium and supplements for re-aliquoting purposes.

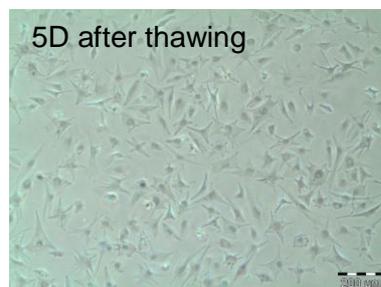
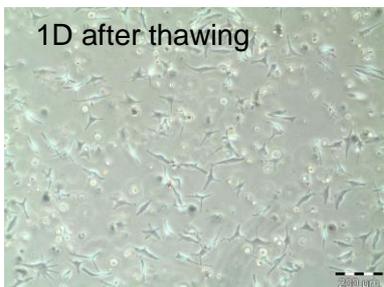
In the protocol below, PhenoCULT®-MEL complete medium will be named PhenoCULT-MEL® for the sake of clarity

1. Thawing

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

1. Coat tissue culture plate with fibronectin diluted to 1/100 in PBS. Incubate for at least 2h in a 37°C incubator. Before use, remove fibronectin solution.
2. Pre-warm PhenoCULT®-MEL.
3. 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.
4. Wipe the vial with 70% ethanol to sterilize and transfer it to a biosafety hood.
5. Transfer the cells to a conical tube with 10 mL of PhenoCULT®-MEL.
6. Directly plate on fibronectin-coated surface at a density of 30,000 cells/cm². Use 2 mL of PhenoCULT®-MEL per 10 cm² of culture surface.
7. To evenly distribute the cells, move the plate twice forward to backward and side to side, in quick motions.
8. Incubate at 37°C with 20% O₂- 5% CO₂ and humidity control.
9. About 4 h later, verify that the cells have attached and carefully replace culture medium with fresh, pre-warmed PhenoCULT®-MEL. Use 2 mL medium per 10 cm² of culture surface.
10. Replace medium every other day (add 3 mL/10 cm² culture surface for week-ends).

Morphology evolution after plating: Note that one day after thawing, some floating cells are observed.



Over the first week, PCi-MEL will gradually proliferate and pigmentation will become more prominent.

2. Passaging PCi-MEL

To obtain the best amplification, we advise to passage PCi-MEL about once a week.

1. For PCi-MEL amplification, passage once a week.
2. Coat tissue culture plates with fibronectin diluted at 1/100 in PBS. Incubate for at least 2h in 37°C incubator. Before use, remove fibronectin solution.
3. Pre-warm PhenoCULT®-MEL and TrypLE™ Express.
4. Discard culture medium, briefly wash cells once with PBS.
5. 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.
6. Transfer to a 15 mL tube pre-loaded with PhenoCULT®-MEL (anticipate at least a 1/3 dilution ratio to stop TrypLE™ Express action).
7. Centrifuge at room temperature, x 200 g, 3 min.
8. Eliminate supernatant and re-suspend in PhenoCULT®-MEL. Gently triturate until a single cell solution is achieved.
9. Count cells and plate on fibronectin-coated culture surface at a density of 20,000-25,000 cells/cm².
10. 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.
11. Replace medium every other day (add 3 mL/10 cm² culture surface for week-ends).

Important Note:

- We recommend using PhenoCULT®-MEL with fibronectine coating for best PCi-MEL expansion. PCi-MEL will also nicely grow in other culture systems. Note that PCi-MEL morphology will vary depending on the culture medium and extra-cellular matrix used and upon maturation, without impairing functions.
- Because of its high content in growth factors involved in melanogenesis (such as α -MSH) we DO NOT recommend to use PhenoCULT®-MEL for pigmentation/depigmentation studies.