Phenocell
Stem Cells for better Drugs

Human induced pluripotent stem cells PCi

User's guide

PRODUCT INFORMATION

Product Ref. PCi-AFR1 / PCi-AFR2 / PCi-CAU1 / PCi-CAU2 / PCi-ASI1 / PCi-ASI2
Thank you for purchasing PCi, Phenocell's human iPS cells. After receiving a batch of PCi, you may follow this guide for successful culture.
PCi are provided in 0.5 or 1 million-cell format frozen in cryopreservation medium and are shipped in dry ice.

Product	Catalog No.	Quantity	Donor
Human induced pluripotent stem cells	PCi_AFR1_0.5M	$5 * 10^5$ cells/vial	Female
Human induced pluripotent stem cells	PCi_AFR1_1M	$1 * 10^6$ cells/vial	Female
Human induced pluripotent stem cells	PCi_AFR2_0.5M	$5 * 10^5$ cells/vial	Female
Human induced pluripotent stem cells	PCi_AFR2_1M	$1 * 10^6$ cells/vial	Female
Human induced pluripotent stem cells	PCi_CAU1_0.5M	$5 * 10^5$ cells/vial	Male
Human induced pluripotent stem cells	PCi_CAU1_1M	$1 * 10^6$ cells/vial	Male
Human induced pluripotent stem cells	PCi_ASI1_0.5M	$5 * 10^5$ cells/vial	Female
Human induced pluripotent stem cells	PCi_ASI1_1M	$1 * 10^6$ cells/vial	Female
Human induced pluripotent stem cells	PCi_ASI2_0.5M	$5 * 10^5$ cells/vial	Female
Human induced pluripotent stem cells	PCi_ASI2_1M	$1 * 10^6$ cells/vial	Female
Human induced pluripotent stem cells	PCi_CAU2_0.5M	$5 * 10^5$ cells/vial	Male
Human induced pluripotent stem cells	PCi_CAU2_1M	$1 * 10^6$ cells/vial	Male

Tissue origin : dermal fibroblasts or peripheral blood

- Each lot is tested 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 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 are provided in CryoStor® CS10 cryopreservation medium (StemCell Technologies, e.g. #07959). CS10 contains 10% DMSO.

PRODUCT USE

PCi 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 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.
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.

THAWING

Prior freezing, PCi were cultured in feeder-free conditions. Although PCi will easily adapt to culture on feeders, we recommend thawing the cells in feeder-free conditions.

Reagents and medium

- CellAdhere™ Laminin-521 (StemCell Technologies, Cat #77003)-coated tissue culture plates
- mTeSR™1 Kit (StemCell Technologies, Cat #85850)

Procedure

1. Pre-warm complete mTeSR™1 (medium with supplements) culture medium.
2. Quickly thaw PCi cells in 37°C water bath until a small piece of ice remains. DO NOT vortex cells.
3. Wipe out the outside of the vial of cells with 70% ethanol to sterilize.
4. Transfer the cells to a conical tube with 2 mL of complete mTeSR™1 culture medium.
5. Centrifuge cell suspension at 290 g for 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 1mL of complete mTeSR™1 culture medium to resuspend the cell pellet. Dissociate the pellet by gentle pipetting until there is no clumps visible.
8. Count cells and plate on Laminin-521-coated tissue culture surface at a density of 5,000-10,000 cells/cm2. Use 2 mL complete mTeSR™1 culture medium for each 10 cm2 of culture surface.
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. After 48h, PCi will have formed colonies. Replace the medium with complete mTeSR™1. Change medium everyday thereafter.

PCi growth following plating as single cells :

Cells are usually ready for passage after 1 week in culture.

PASSAGING

Reagents and medium

- CellAdhere™ Laminin-521 (StemCell Technologies, Cat #77003) coated tissue culture plates
- Dulbecco's phosphate-buffered saline with Ca++ and Mg++
- mTeSR™1 Kit (StemCell Technologies, Cat #85850)
- Accutase® (Sigma, Cat #A6964)

Procedure

1. Coat culture plate with Laminin-521 (follow instructions from distributor).
2. For PCi maintenance, passage should be performed when colonies are the size of a 10x field of view through a binocular microscope, or when they start touching each other.
3. Pre-warm complete mTeSR™1 (basal medium with supplements) culture medium.
4. Pre-warm Accutase®.
5. Discard culture medium from culture plates, wash once with complete mTeSR™1 medium.
6. Add Accutase® (600 µL for a 35 mm dish) and incubate at 37°C for 5-10 min. Regularly check cell digestion: when individualized cells are visible within each PCi colony, use the medium present in the dish to detach the cells. Gently triturate by pipetting until there is no more clumps visible.
7. Transfer to a 15 mL tube pre-loaded with 2 mL of complete mTeSR™1 culture medium.
8. Centrifuge at room temperature, at 290 g for 3 min.
9. Eliminate supernatant and re-suspend in complete mTeSR™1 medium. Gently triturate until a single cell solution is obtained.

10. Count cells and plate on a Laminin-521-coated tissue culture surface at a density of 5,000-10,000 cells/cm2.

11. 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.

12. After 48h, PCi will have formed colonies. Replace the medium with complete mTeSR™1. Change medium everyday thereafter.

13. For week-ends, change medium for 3 mL/10 cm2 of fresh complete mTeSR™1 on Friday afternoon, and resume to normal culture conditions on Monday morning.