phenocell

stem cells for better drugs

Human iPSC-derived neural progenitor cell
PCi-NPC

User's guide

PRODUCT INFORMATION

Product Ref. PCi-NPC
Additional Ref.: PhenoCULT®-NPC culture medium.
Thank you for purchasing PCi-NPC (human iPSC-derived neural progenitor cells). After receiving your product, you may follow this guide for successful culture of frozen PCi-NPC. Refer to the PCi-NPC Product Sheet for more details on the product.

Product	Catalog No.	Quantity
Human iPSC-derived Neural Progenitor Cells	PCi-NPC_1M	$1 * 10^6$ 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-NPC 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-NPC are provided in CryoStor® CS10 cryopreservation medium (StemCell Technologies, e.g. #07959). CS10 contains 10% DMSO.

PRODUCT USE

PCi-NPC 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-NPC 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-NPC.
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 appropriate handling procedures. PCi-NPC are human cells and, as such, should be handled with required ethical and safety rules.

THAWING

Reagents and medium

- Poly-Ornithine 0.01% Solution (Sigma cat. P4957 - 50 mL) Not included
- Laminin (Sigma cat. L2020-1mg) Not included
- PhenoCULT®-NPC basal (See online)
- Supplement A 10 000X (See online)
- Supplement B 1000X (See online)
- Supplement C 2000X (See online)

PREPARE TISSUE CULTURE PLATES COATED WITH POLYORNITHIN (PO) AND LAMININ (LAM).

1. Dilute 1 volume of 0.01% PO in 5 volumes of PBS:

Final Volume	120 mL	60 mL	30 mL	12 mL
0,01% PO	20mL	10mL	5mL	2mL
PBS 1X	100mL	50mL	25mL	10mL

2. Mix well.
3. Add the PO/PBS solution to culture plates: 1 mL solution per 10 cm2 of culture surface.
4. Transfer to a humidified incubator at 37°C, 5% CO2 for 6 to 24h.
5. PO-coated plates can be stored in these conditions for a maximum of 1 week.

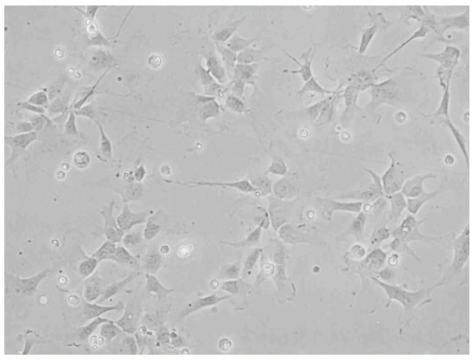
6. Before proceeding to Laminin coating, aspirate the PO solution and rinse 3 times with PBS (use at least 2 mL PBS for each 10 cm2 of surface).
7. Thaw 1 mg/mL LAM solution at room temperature.
8. Dilute 1 volume of LAM solution in 500 volumes of PBS.
9. Mix well.
10. Add the LAM/PBS solution to the PO-coated plates using 1 mL for each 10 cm2 of culture surface.
11. Transfer to a humidified incubator at 37°C, 5% CO2 for 6 to 24h.
12. Remove the LAM solution before NPC seeding. No dish washing is required before PCi-NPC transfer.

THAW PCI-NPC

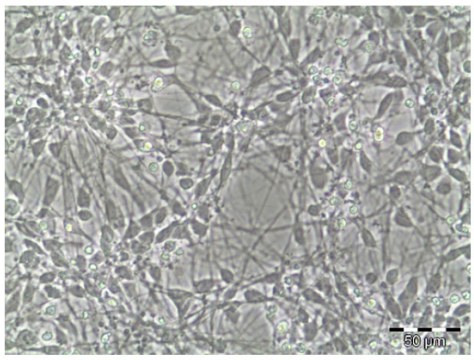
1. Add supplements to basal medium and homogenize.
2. Pre-warm a sufficient volume of NPC Complete medium at 37°C (2 mL for 10 cm2 culture surface).
3. To thaw the cells, transfer the vial of cells from storage by transporting the vial in dry ice. Remove the vial from dry ice and transfer it to a 37°C water bath. Do not submerge the vial. Remove the vial before the last bit of ice has melted (1-2 minutes). Do not vortex.
4. Wipe out the outside of the vial of cells with 70% ethanol and transfer to a biological safety cabinet.
5. Transfer the cells to 2 mL of NPC Complete medium in a 15 mL tube.
6. Centrifuge at 250 g for 3 minutes at room temperature.
7. Carefully remove the supernatant, leaving a small amount of medium to ensure the cell pellet is not disturbed.
8. Gently add 1 mL of NPC Complete medium to resuspend the cell pellet. Dissociate the pellet by gentle pipetting until there is no more clumps visible. Add again 1-2 mL of NPC complete medium and gently homogenize the cell solution.
9. Perform a cell count to determine the number of viable cells and ensure proper seeding.
10. Remove the LAM solution from the culture plate and directly plate PCi-NPC at a density of 40 000 cells/cm2 in NPC complete medium. Use 2 mL of NPC complete medium for each 10 cm2 of culture surface.

11. Place the plate into a humidified incubator (37°C, 5% CO2). To ensure an even plating, gently rock the culture plate back and forth and side-to-side twice.
12. Change medium every other day using 2 mL/10 cm2 culture surface (add 3 mL/10 cm2 culture surface for the week-end).

Morphology of PCi-NPC after thawing

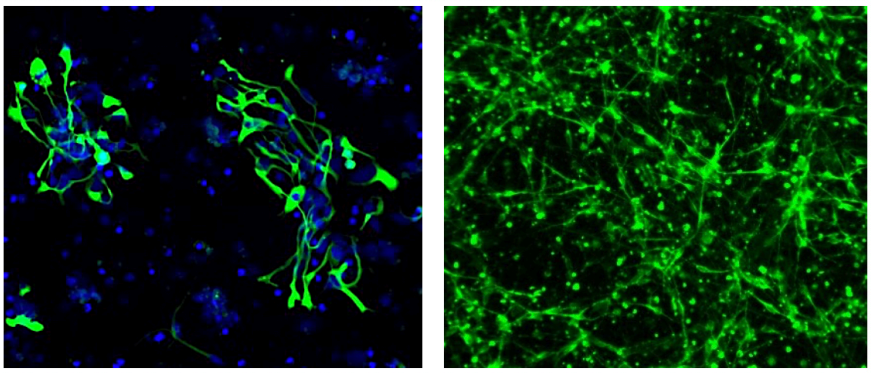


2 days after seeding, PCi-NPC organize into characteristic neural rosettes.



A conspicuous network of neurites will develop within 2 weeks, following mature neuron induction.

Expression of specific markers



Expression of Nestin (2 days post-thaw, left) and β-tubulin III (Tuj1, 2 weeks post-seeding, right), following mature neuron induction.

PASSAGING PCI-NPC

1. PCi-NPC passage is performed when cells reach about 80-90% confluence (usually every 4-5 days with an initial plating density of 40 000 cells/cm2).
2. Prepare PO/LAM-coated plates as indicated previously.
3. Pre-warm NPC Complete medium, and TrypLE™ Express.
4. Discard culture medium from culture plates and briefly wash once with 1x PBS.
5. Add 1 mL TrypLE™ Express per 10 cm2 of culture surface.
6. Incubate at 37°C for 5-10 min. Regularly check the cells, when all the cells look rounded, detach them by gently flushing the culture medium present in the plate.
7. Pipette up and down gently 3-5 times with 1 mL tip to take off all the cells and dissociate cell clumps.
8. Transfer to a 15 mL sterile conical tube containing NPC complete medium (at least a 1/3 dilution ratio is necessary to stop TrypLE™ Express action).
9. Gently centrifuge the cell suspension at 250 g for 3 min at room temperature. PCi-NPC should form a visible pellet after centrifugation.
10. Carefully remove the supernatant and re-suspend the cell pellet in 3 mL NPC Complete medium.
11. Perform a cell count to determine the number of viable cells and ensure optimal seeding density.
12. Seed the cells on PO/LAM-coated tissue culture surface at a density of 40 000 cells/cm2 in NPC Complete medium. Use 2 mL of NPC complete medium for each 10 cm2 of culture surface.
13. Change medium every other day using 2 mL/10 cm2 culture surface (add 3 mL/10 cm2 culture surface for the week-end).