hiPSC derived Sebocytes: PCi-SEB

Description

Product Ref. PCi-SEB

Thank you for purchasing PCi-SEB, human iPSC-derived Sebocytes. After receiving a batch of PCi-SEB, you may follow this guide for successful culture of your frozen cells. This protocol gives access to sebocytes at two different stages, either with basal lipid accumulation (d3-d4 after thawing), or with prominent lipid droplet accumulation (d5 after thawing). If your experiment request a large number of PCi-SEB (>10M cells), bulk prices are available (please send request to contact@phenocell.com).

Storage

PCi-SEB should be kept at liquid nitrogen temperature (-135°C); long-term storage at -80°C is not recommended. PCi-SEB are provided in CryoStor® CS10 cryopreservation medium (StemCell Technologies). CS10 contains 10% DMSO.

Product use

PCi-SEB 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.

Before you start

If this is the first time you perform sebocyte culture or if you need additional details, our technical support staff will be happy to help. We can be reached at *contact@phenocell.com*, by phone (number below) or online. Do not hesitate to contact us to get personalized help and achieve groundbreaking discoveries with your PCi-SEB.

Culture

All steps should be performed in a sterile culture environment using adequate handling procedures. PCi-SEB are human cells and, as such, should be handled with required ethical and safety rules.

IMPORTANT:

PCi-SEB are provided as vials of frozen KRT7$^+$ sebocyte progenitors. Supplement A is required to further differentiate the progenitors into mature and functional MUC1$^+$ sebocytes with low lipid levels. Supplement M can be used as an optional treatment to further mature the sebocytes to their terminal stage, with high lipid content and holocrine secretion. We suggest to follow the chart on page 2 for optimum performance with PCi-SEB.

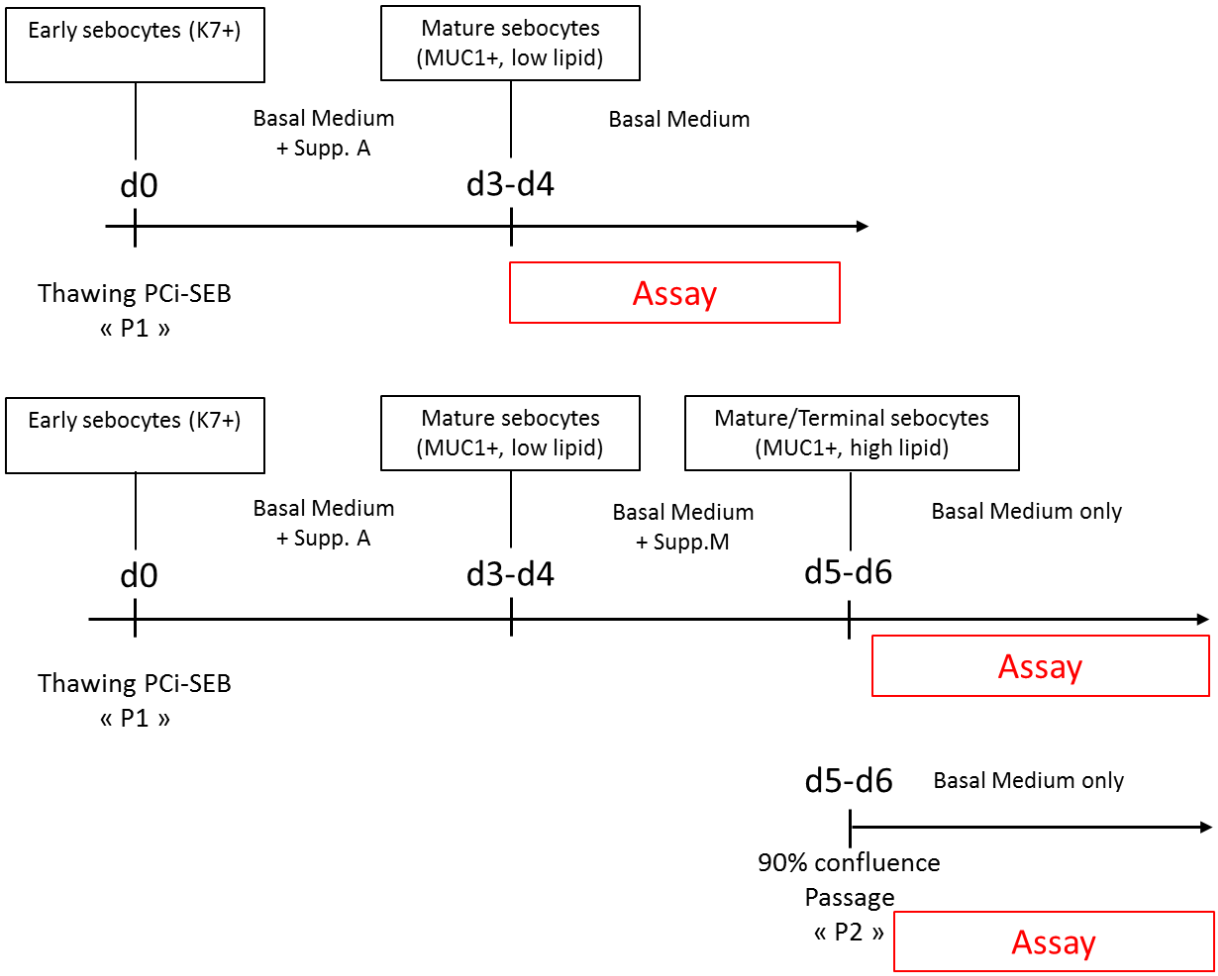

Figure 1 Assay window suggestions during PCi-SEB culture

1. Thawing:

Reagents and media:

- **PhenoCULT-SEB Basal medium** (store at -20°C, after thawing keep at 4°C for up to one month).
- The composition of Sebocyte basal medium is provided below:
  - 3:1 (vol:vol) DMEM-GlutaMAX™ (Thermo Fischer Sci. Cat. 61965-026) : Ham's F-12 (Thermo Fischer Sci. Cat. 21765-029)
  - 2.5% Fetal Bovine Serum (Sigma-Aldrich Cat. F7524)
  - 10 ng/mL Human insulin (Sigma-Aldrich Cat. I2643)
  - 3 ng/mL EGF recombinant Human (Thermo Fischer Sci. Cat. PHG0311L)
  - 45.2 ng/mL Hydrocortisone (Sigma-Aldrich Cat. H0888)
  - 10^{-10}M Cholera toxin B subunit (Sigma-Aldrich Cat. C8052)
  - 24 µg/mL Adenine (Merck Millipore Cat. 1152)
- **Supplement A (Amplification, green cap tube) and M (Maturation, yellow cap tube)**. Store supplements at -20°C upon receipt. After thawing, supplements can be aliquoted and frozen/thawed once; alternatively they can be maintained at room temperature for a maximum of 5 days.
- Fibronectin (Sigma-Aldrich Cat. F1141)

Procedure:

1. Coat tissue culture plates with fibronectin diluted to 1/100 in PBS. Incubate for at least 2 h in 37°C incubator (use 0.1 mL per cm^2 cell culture surface).
2. Pre-warm sebocyte complete medium.
3. Quickly thaw PCi-SEB vial in 37°C water bath until a small frozen cell pellet remains. **Do not vortex cells**.
4. Wipe out the outside of the vial of cells with 70% ethanol.
5. Transfer the cells to 6 mL of sebocyte basal medium.
6. Centrifuge at 250 g for 3 min, discard supernatant and resuspend in 2mL of Sebocyte basal medium supplemented with **1:1000 supplement A.**
7. Count cells; remove fibronectin solution from the culture plates and directly plate on fibronectin-coated surface at a density of 25,000 cells/cm^2. Use 2 mL sebocyte medium with **1:1000 supplement A** per 10 cm^2 of culture surface.
8. Place the plate into the incubator (37°C, 5% CO_2). To evenly distribute the cells, alternatively move the plate forward to backward and side-to-side, in quick motions. Please be aware that PCi-SEB tend to attach rapidly to the culture surface: do not wait too long after plating to homogenize cell distribution.
9. Use Sebocyte basal medium supplemented with **1:1000 supplement A** for at least 3 days after plating, then switch to Sebocyte basal medium supplemented with **1:1000 supplement M** for the next 2 days until cells reach 90% confluence and can be further passaged. Change medium every other day using 2 mL/10cm^2 culture surface (add 3 mL/10cm^2 culture surface for week-ends). Follow chart on page 1 for best results.
   Note: One day after thawing, a significant amount of floating cells might be observed: this corresponds to normal cell death caused by our cell purification process (see figure page 3). At d5 of culture, PCi-SEB lipid droplets accumulate in the cell cytoplasms, while lipid filled cells detach from the culture plate and start the holocrine secretion process (see yellow arrows, figure below).

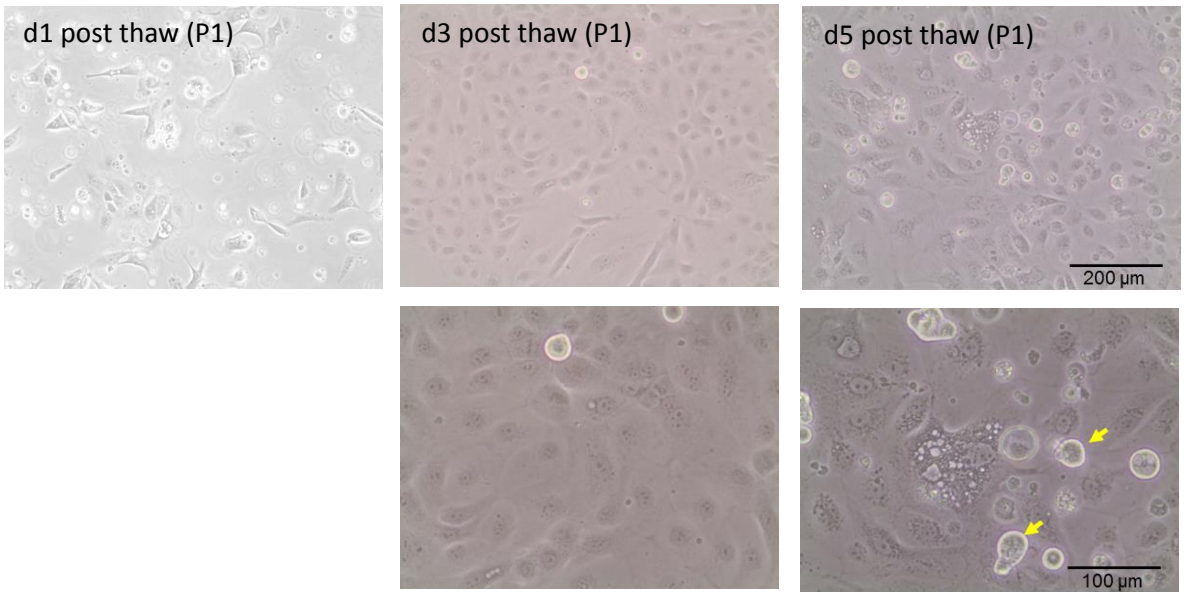

Figure 2 Microscopic phase contrast images showing PCi-SEB morphology changes during in vitro culture

2. Passaging PCi-SEB

Reagents:

- PhenoCULT-SEB: Sebocyte basal medium.
- TrypLE™ Express (Thermo Fischer Sci. Cat.12605)
- Fibronectin (Sigma-Aldrich Cat. F1141)

Protocol:

1. For PCi-SEB maintenance, passage is usually performed when cells reach 90% confluence (usually within 5-6 days after plating). One can expect a yield of about 50,000 cells/cm^2. Do not overgrow PCi-SEB, it might impair survival after passage.
2. Coat tissue culture plate with fibronectin diluted 1/100 in PBS (use 0.1mL per cm^2 culture surface). Incubate for at least 2 h in 37°C incubator. Before use, remove fibronectin solution.
3. Pre-warm sebocyte complete medium and TrypLE™ Express.
4. Discard culture medium, briefly wash once with PBS.
5. Add 1 mL TrypLE™ Express per 10 cm^2 of culture surface, and incubate at 37°C for 5-10 min. Regularly check cell digestion: when sebocytes are rounding up, detach them by gently flushing the culture surface with the TrypLE™ Express contained in the plate.
6. Transfer to a 15 mL tube pre-loaded with sebocytes basal medium (at least a 1/3 dilution ratio is necessary to stop TrypLE™ Express action).
7. Gently centrifuge at RT, 250 g, 3 min. PCi-SEB should form a visible pellet after centrifugation.
8. Eliminate supernatant and re-suspend in Sebocyte basal medium. Gently triturate until a single cell solution is achieved.
9. Count cells and plate on fibronectin-coated culture surface at a density of 25,000 cells/cm^2 in sebocyte basal medium.
10. Place the plate into the incubator. To evenly distribute the cells, alternatively move the plate forward to backward and side-to-side, in quick motions. Change medium every other day using 2 mL/10cm^2 culture surface (add 3 mL/10cm^2 culture surface for week-ends).
11. Cells can be assayed as early as the day following passage.