Human white preadipocyte differentiation

PromoCell®

Application note

Background

Adipogenesis, the process by which preadipocytes differentiate into mature adipocytes, plays a crucial role in metabolism and energy homeostasis [1]. Preadipocytes are progenitor cells derived from mesenchymal stem cells that undergo differentiation to form mature adipocytes [2]. These progenitor cells are characterized by their fibroblast-like morphology, capacity to accumulate lipid droplets, and expression of adipocyte-specific genes [3].

White adipose tissue (WAT) serves as a major energy reservoir and is involved in the regulation of various metabolic functions, including insulin sensitivity and glucose metabolism [4]. The formation of WAT through white preadipocyte differentiation is a tightly regulated, multi-step process. It begins with growth arrest, followed by clonal expansion, early differentiation, and terminal differentiation [5]. This process is orchestrated by a complex network of transcription factors, including PPARγ and C/EBP, which activate adipocyte-specific genes responsible for lipid metabolism, insulin sensitivity, and adipokine production [6].

The role of white adipocytes, the end-product of preadipocyte differentiation, extends beyond energy storage. They are active endocrine organs that secrete various adipokines, influencing systemic metabolism and contributing to the pathophysiology of metabolic diseases such as obesity, type 2 diabetes, and insulin resistance [7]. Dysregulation of adipogenesis can lead to an imbalance in the number and function of adipocytes. This results in adipose tissue hypertrophy and fibrosis, which are closely linked to metabolic disorders [8, 9]. Furthermore, the accumulation of dysfunctional adipocytes can exacerbate inflammation and insulin resistance [10], highlighting the importance of understanding the mechanisms governing preadipocyte differentiation.

Given the rising prevalence of obesity and its associated metabolic diseases, there is a pressing need to investigate the mechanisms underlying adipogenesis and human white preadipocyte differentiation [11]. Such research is essential not only for gaining a molecular understanding of metabolic diseases, but also for identifying potential therapeutic targets for drug development aimed at improving metabolic health. In this application note, we provide a detailed protocol for studying human white preadipocyte differentiation, which is essential for advancing research in adipose tissue biology and its clinical applications.

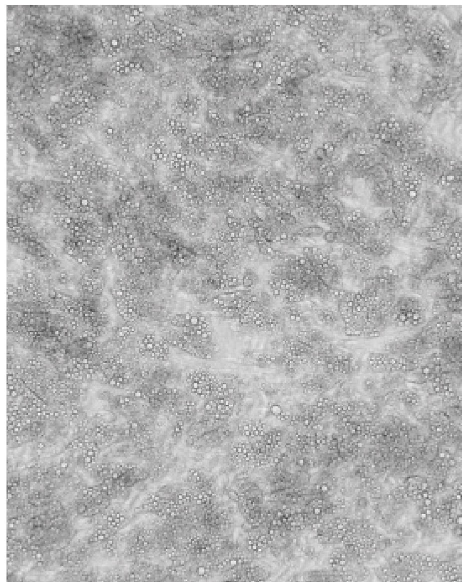
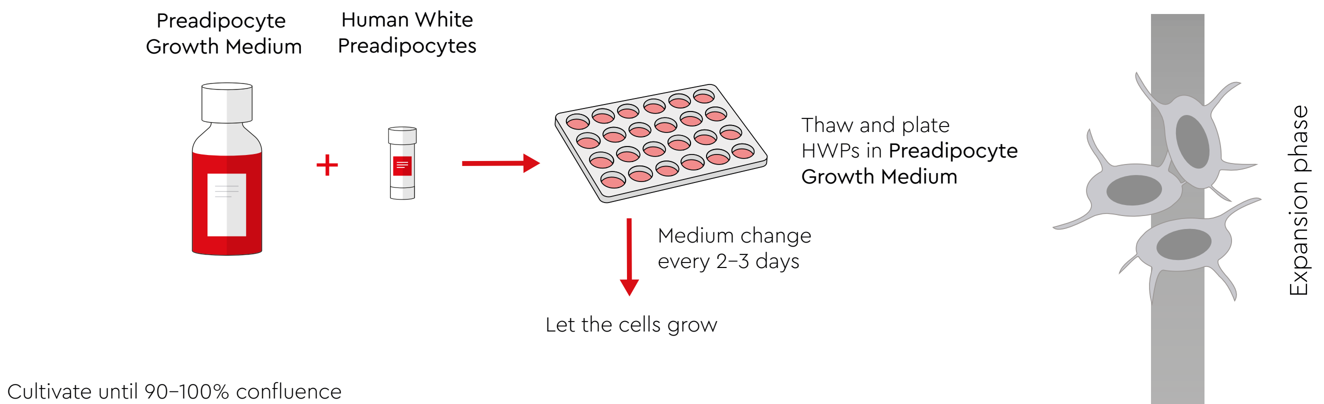


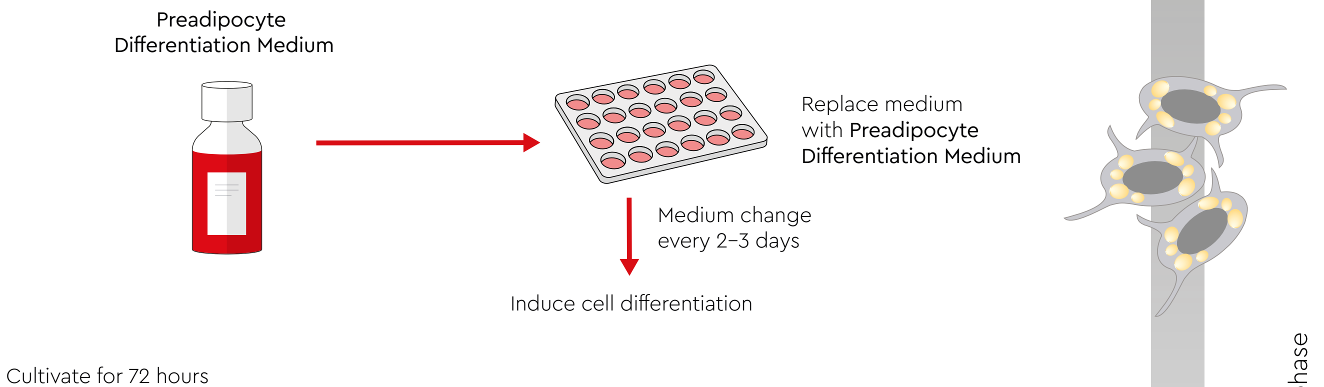
Fig. 1: Day 8 culture of differentiated human white preadipocytes in our Adipocyte Nutrition Medium. *Bright-field microscopy shows the intracellular lipid vacuole formation by mature adipocytes. The differentiation process to mature adipocytes is completed after 14 days.*

A Preadipocyte seeding



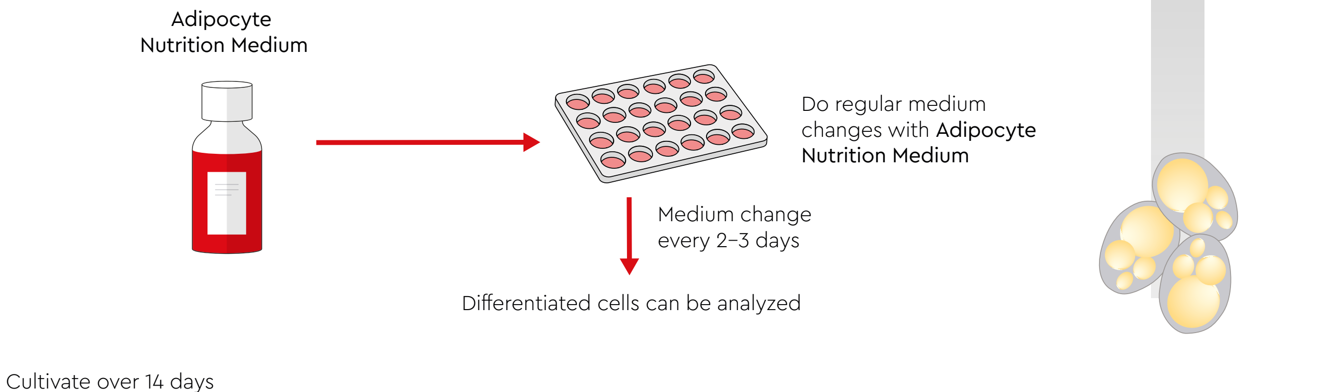
Cultivate until 90–100% confluence

B Induce cell differentiation



Cultivate for 72 hours

C Complete cell differentiation



Cultivate over 14 days

Fig. 2: Schematic overview of culture workflow for human white preadipocyte differentiation. **A:** *Expansion of the Human White Preadipocytes (HWP) in our Preadipocyte Growth Medium till the cells achieve 90–100% confluence.* **B:** *Induce the adipocyte differentiation of HWP cells by replacing the Preadipocytes Growth Media with our Preadipocyte Differentiation Media for 72 hours.* **C:** *Complete the differentiation by replacing the Preadipocyte Differentiation Media with Adipocyte Nutrition Medium. The formation of mature adipocytes takes 14 days in Adipocyte Nutrition Medium. For the detailed steps, see the following protocol.*

Protocol for differentiation of preadipocytes into mature adipocytes

I. Differentiation protocol

Materials

- Human White Preadipocytes (HWP) from subcutaneous or visceral adipose tissue (C-12735/C-12732)
- Preadipocyte Growth Medium (C-27410/C-27417)
- Preadipocyte Differentiation Medium (C-27437)
- Adipocyte Nutrition Medium (C-27438/C-27439)
- Cell culture vessels (24-well plate)

Use aseptic techniques and a laminar flow bench.

To prepare the medium, thaw the SupplementMix or SupplementPack at 15–25°C. Aseptically mix the supplement by carefully pipetting it up and down. Transfer all components to the 500 ml bottle of Basal Medium. Close the bottle and swirl gently until a homogenous mixture is formed. Store the complete growth medium at 2–8°C. For use, pre-warm only an aliquot of the complete medium and keep the remaining medium refrigerated at 4–8°C.

1

Thaw the preadipocytes

Thaw the cells according to the instruction manual, and culture the cells in Preadipocyte Growth Medium.

Note: It is recommended to use a 24-well plates with a seeding density of 16,000 cells per cm^2. Work in duplicates and use 1 well with cells as negative control.

2

Culture the cells

Let the cells grow until they reach 90–100% confluency. Change the medium every 2–3 days (e.g., Mon-Wed-Fri).

3

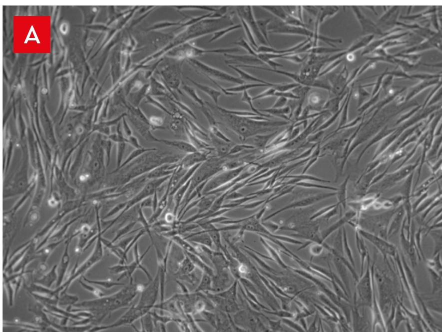
Induce cell differentiation

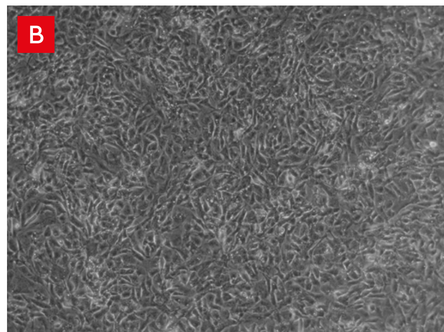
Once the cells have reached 90–100% confluence, you can start with the differentiation process. Replace the Growth Medium with Preadipocyte Differentiation Medium. Culture the cells for an additional 72 hours at 37°C and 5% CO_2.

4

Complete the differentiation process

After 72 hours, replace the Differentiation Medium with Adipocyte Nutrition Medium. Feed the cells every 2–3 days with fresh Adipocyte Nutrition Medium. The differentiation process to mature adipocytes is completed after 14 days.





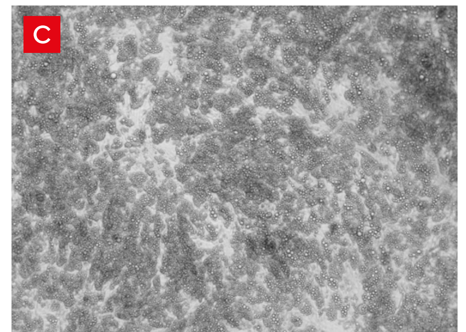


Fig. 3: **Differentiation of preadipocytes into mature lipid vesicle-containing adipocytes.** *Human white preadipocytes (HWP) cultured in Preadipocyte Growth Medium* ***(A)****. After 72 hours in the Preadipocyte Differentiation Medium, the cells undergo significant metabolic changes when shifting from a proliferative state to a state characterized by lipid synthesis and storage* ***(B)****. After 8 days, the differentiated culture exhibits extensive intracellular lipid vacuole formation typical of mature adipocytes* ***(C)****.*

II. Adipogenesis: vesicle staining with Sudan III

Materials

- Saccomanno Fixation Solution
- Sudan III Solution (2.5 g/l)
- Isopropanol 60%
- Dulbecco's phosphate-buffered saline (PBS) without Ca^{++}/ Mg^{++} (C-40232)
- Syringe filter, 0.22 µm
- Distilled water

Mature adipocytes contain fat droplets, and their primary function is to store energy in the form of lipids. Sudan III staining is a technique used to detect lipids in biological tissues, particularly in adipocytes. Sudan III is a fat-soluble dye that binds to lipid molecules, giving them an orange-red color. This method is commonly used in histology to visualize fat droplets within cells and tissues.

1

Wash the cells

Remove the cells from the incubator and carefully aspirate the medium. Gently wash the cells twice with Dulbecco's phosphate-buffered saline (PBS) without Ca^{++}/Mg^{++} (C-40232).

Note: Do not disrupt the cell monolayer.

2

Fix the cells

Carefully aspirate the PBS and add enough Saccomanno Fixation Solution to cover the cell monolayer. Incubate at room temperature for at least 60 minutes.

3

Dilute the staining solution

During fixation, dilute 10 ml Sudan III Solution with 1.5 ml distilled water and pass through a 0.22 µm syringe filter. Use the diluted staining solution within 60 minutes.

4

Wash the cells

Carefully aspirate the fixation solution and wash the cell monolayer with distilled water twice. Gently aspirate the water and add enough 60% isopropanol to cover the cell monolayer. Incubate at room temperature for 3–5 minutes.

5

Add the staining solution

Carefully aspirate the 60% isopropanol and add enough diluted Sudan III staining solution to cover the cell monolayer. Incubate at room temperature for 10–15 minutes.

6

Wash the cells

Carefully aspirate the staining solution and wash the cell monolayer several times with distilled water until the water is clear. Blot the vessel containing the stained cells upside down on a paper towel to remove as much water as possible.

7

Analyze the cells

Cover with PBS and analyze the stained samples promptly, as the dye tends to fade upon prolonged light exposure. Intracellular lipid vesicles in mature adipocytes will appear bright red (see Fig. 4).

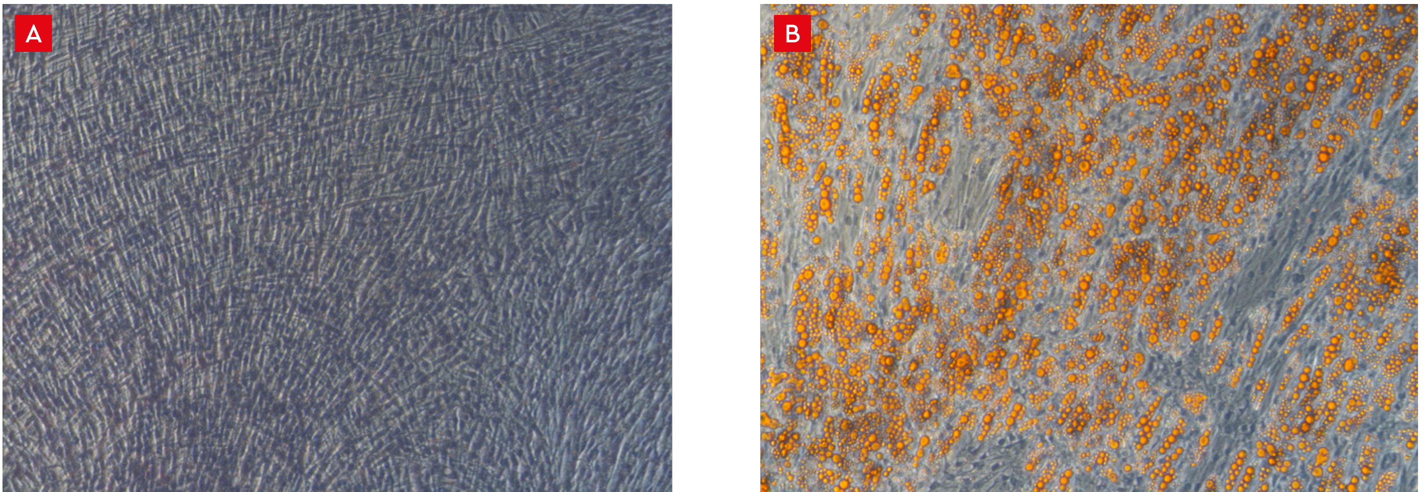


Fig. 4: Sudan III staining of intracellular lipids in mature adipocytes after 14 days. A: *Negative control of HWP in our Growth Medium is showing no sign of differentiation.* ***B:*** *The mature adipocytes differentiated from HWP show accumulation of lipid droplets displayed in a vibrant orange-red color.*

Related products

Product	Size	Catalog number
Human White Preadipocytes (HWP) subcutaneous	1,000,000 cryopreserved cells	C-12735
Human White Preadipocytes (HWP) visceral	500,000 cryopreserved cells	C-12732
Preadipocyte Growth Medium (Ready-to-use)	500 ml	C-27410
Preadipocyte Differentiation Medium	500 ml	C-27437
Adipocyte Nutrition Medium (Ready-to-use)	500 ml	C-27438
Dulbeccos PBS, without Ca^{++}/ Mg^{++}	500 ml	C-40232
DetachKit	30 ml	C-41200
	125 ml	C-41210
	250 m	C-41220
Freezing Medium Cryo-SFM Plus	30 ml	C-29920
	125 ml	C-29922

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