



# Rat Primary Pre-adipocytes Culture Kit

**Primary Cells from rat mesenteric, epididymal, and subcutaneous adipose tissues.**

Catalog # PMC-VAC01-COS, PMC-EAC01-COS,  
PMC-SAC01-COS

For research use only

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## Introduction

Fat distribution in the body is associated with distinct risks for metabolic diseases like diabetes, atherosclerosis, and hypertension. The metabolic function of fat tissue is different depending on the location in the body that the tissue is located. The regional location of the fat tissue probably contributes to the risk of diseases. Fat cells from rat depots vary in physical and functional characteristics, for example, size, fatty acid incorporation, response to insulin and lipolytic agents (1).

Our primary pre-adipocytes are isolated from healthy male Sprague-Dawley rats. Mesenteric (PMC-VAC01-COS) and epididymal (PMC-EAC01-COS) adipocytes are visceral adipocytes. Mesenteric pre-adipocytes are isolated from the mesentery and epididymal adipocytes from epididymal adipose tissue in the abdominal cavity of these rats.

The cells are cultured in Adipocyte Culture Medium, which contains no synthetic compounds or adipogenesis inhibitors, to induce differentiation of precursor cells into mature adipocytes. The culture medium has the unique feature of serving as growth, differentiation, and maintenance medium for mature adipocytes.

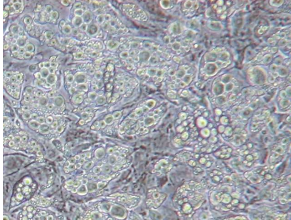
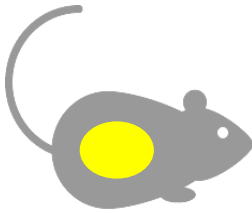
1. Adipocyte Culture Medium does not include differentiation inducers, such as indomethacin, dexamethasone, PPAR- $\alpha$  agonist.
2. Proprietary natural compounds are used to induce differentiation (Patent Pending)
3. The activity of inhibitor for differentiation (VAI) is removed from serum supplemented in the culture medium by a proprietary procedure (Patent Pending)
4. Over 80% of our visceral pre-adipocytes (VAC) differentiate into adipocytes.

The kit provides a convenient system for studying the mechanism of adipogenesis as well as for screening drugs that prevent metabolic diseases.

Our Adipocytes Culture Kits can be used with our cell-based assays – GPDH Activity Assay and Lipid Assay Kit – and our ELISA Kits – Adiponectin, Leptin, and Resistin.

## Adipocytes

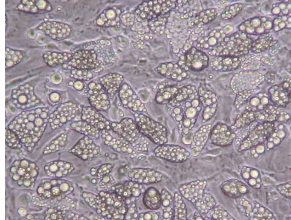
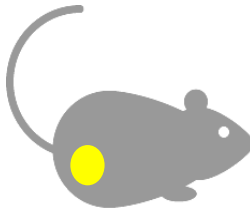
### Mesenteric



Mesenteric adipocytes (Day 9)

Catalog# PMC-VAC01-COS

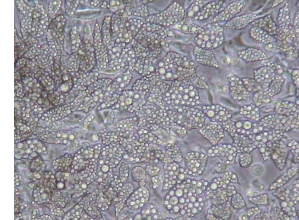
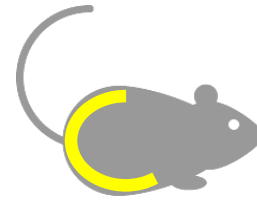
### Epididymal



Epididymal adipocytes (Day 9)

Catalog #PMC-EAC01-COS

### Subcutaneous



Subcutaneous adipocytes (Day 9)

Catalog # PMC-SAC01-COS

## Components

### Components

| Components                       | Size                                  | Quantity |
|----------------------------------|---------------------------------------|----------|
| Pre-adipocytes, Rat, frozen      | Vial containing $3 \times 10^6$ cells | 1        |
| Adipocyte Culture Medium, frozen | 250 ml                                | 1        |

### Storage

| Components                  | Storage Conditions | Shelf Life |
|-----------------------------|--------------------|------------|
| Pre-adipocytes, Rat, frozen | -80°C Freezer      | 1 year     |
|                             | Liquid Nitrogen    | 1 year     |
| Adipocyte Culture Medium    | -20°C Freezer      | 6 months   |
|                             | -80°C Freezer      | 1 year     |

## Additional Materials and equipment may be required

- Pipettes
- 24-well, flat bottom culture plate
- Tubes
- Refrigerated centrifuge
- Water bath

## Precautions

1. Read the instructions carefully before beginning the culture.
2. This kit is for research use only, not for human or diagnostic use.

## Protocol

1. Thaw the Adipocyte Culture Medium in a 37°C water bath with gentle shaking.
2. Quickly thaw the Pre-adipocytes vial in a 37°C water bath.
3. Transfer the thawed cells to a 15 ml centrifuge tube containing 10 ml of Adipocyte Culture Medium. Mix gently then centrifuge 1000 rpm (170 x g) for 5 minutes at 4°C.
4. Remove the supernatant then resuspend the cells in 10 ml of the Adipocyte Culture Medium. Centrifuge 1000 rpm (170 x g) for 5 minutes at 4°C.
5. Resuspend the cell pellet in Adipocyte Culture Medium. **For Epididymal Adipocytes, use 6.2 mL of medium. For Mesenteric and Subcutaneous Adipocytes, use 12.5 mL of medium.**
6. Dispense 0.5 ml of cell suspension to each well of a 24-well plate.
7. Incubate the plate at 37°C under 5% CO<sub>2</sub>, 100% humidity.
8. After 1 day in culture, gently add 0.5 ml of Adipocyte Culture Medium into each well.
9. Change the medium every 2 days. Be careful not to disturb the cell layer.

### **Mesenteric Adipocytes (PMC- VAC01-COS):**

- i. Approximately 3 days in culture, the pre-adipocyte culture will become confluent.
- ii. Approximately 7 days in culture, the cells become mature adipocytes.
- iii. Approximately 8 days in culture, the cells become hypertrophic and start detaching from the well.

### **Epididymal Adipocytes (PMC-EAC01-COS):**

- i. Approximately 3-4 days in culture, the pre-adipocyte culture will become confluent.
- ii. Approximately 5 days in culture, the cells become mature adipocytes.
- iii. Approximately 8 days in culture, the cells become hypertrophic and start detaching from the well.

### **Subcutaneous Adipocytes (PMC-SAC01-COS):**

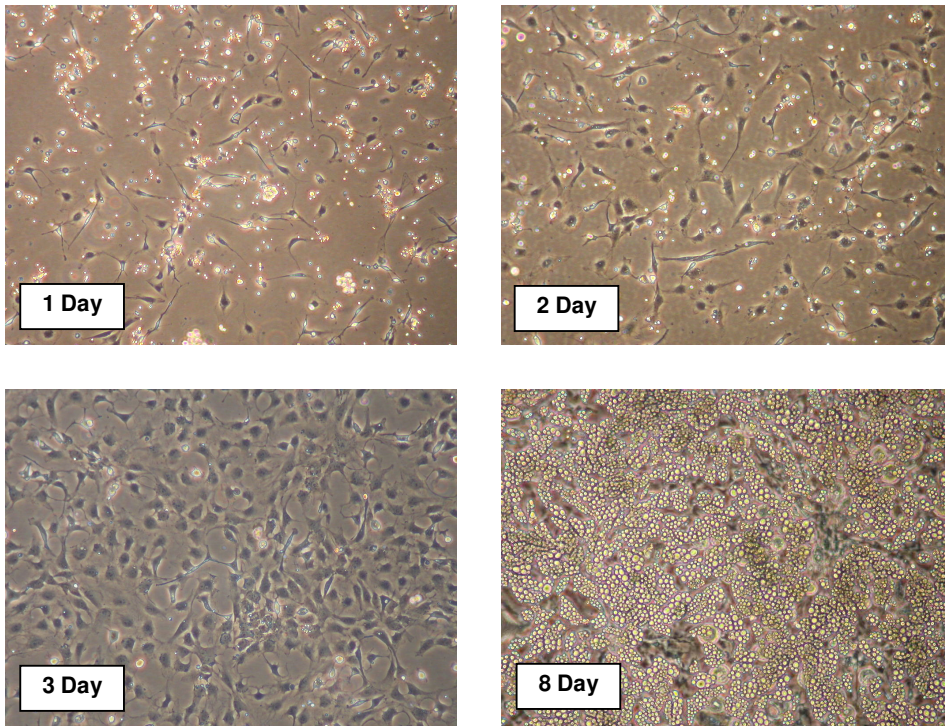
- i. Approximately 3 days in culture, the pre-adipocyte culture will become confluent.
- ii. Approximately 4-5 days in culture, the cells become mature adipocytes.
- iii. Approximately 7 days in culture, the cells become hypertrophic and
- iv. Approximately 8 days in culture, the cells start detaching from the well.

To study adipogenesis control factors, add the reagent to the medium at various stages of adipogenesis.

## Examples

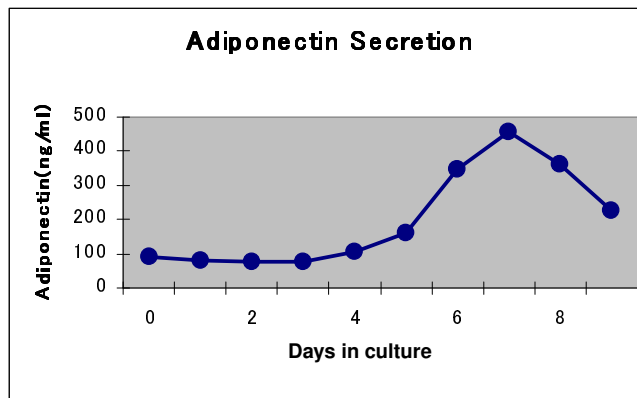
### 1. Adipocyte maturation

Over 80% of the primary pre-adipocytes converted into mature visceral adipocytes.



### 2. Adiponectin secretion

Visceral primary pre-adipocytes were seeded in a 24-well culture plate with 1 ml of culture medium. The culture medium was changed everyday and the conditioned media were stored at -80°C until tested. The condition media were thawed, and adiponectin was measured using B-Bridge's Rat/Mouse Adiponectin ELISA Kit (cat. # K1002-1)



## References

1. Caserta, F., Tchkonja, T., Cibelek, V.N., et al. Fat depot origin affects fatty acid handling in cultured rat and human preadipocytes. *Am J Physiol Endocrinol Metab* 2001; 280:E238-E247.
2. Shimizu, K., Sakai, M., Ando, M., et al. Newly developed primary culture of rat visceral adipocytes and their in vitro characteristics. *Cell Bio Intl* 2006; 30:381-388.

## Companion Assays

### Cell-based assays for adipocytes

1. GPDH Activity Assay, catalog # PMC-AK01-COS  
For the measurement of glycerol-3-phosphate dehydrogenase in pre-adipocytes
2. Lipid Assay Kit, catalog # PMC-AK09F-COS  
Staining and quantification of lipids in adipocytes

### ELISA kits for adipocytes

1. Rat/Mouse Adiponectin, catalog # K1002-1
2. Rat/Mouse Leptin, catalog # K1006-1
3. Rat Resistin, catalog # K1014-1

For questions please contact

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