KAMIYA BIOMEDICAL COMPANY

Rat Adipocyte Culture Kit

For the culture of rat adipocytes from precursor cells.

Cat. No.:
KT-793, Epididymal
KT-794, Subcutaneous
KT-795, Mesenteric

For Research Use Only.

PRODUCT INFORMATION

Rat Adipocyte Culture Kit

Cat. No. KT-793, Epididymal
KT-794, Subcutaneous
KT-795, Mesenteric

PRINCIPLE

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.

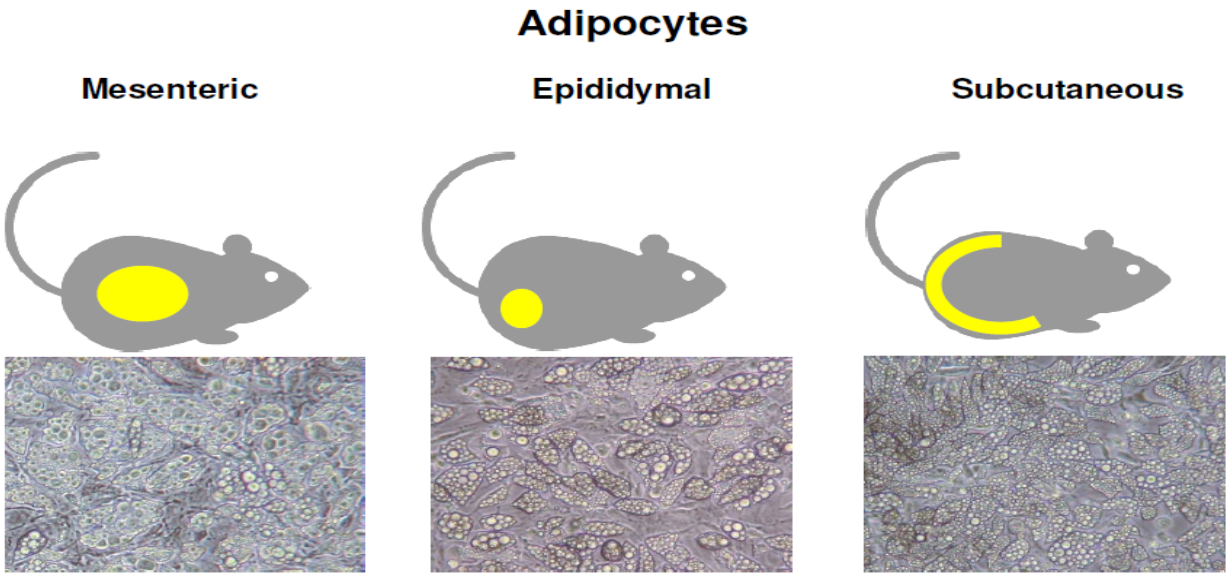
Our primary pre-adipocytes are isolated from healthy male Sprague-Dawley rats. Mesenteric (KT-795) and epididymal (KT-793) adipocytes are visceral adipocytes. Mesenteric pre-adipocytes are isolated from the mesentery and epididymal adipocytes from epididymal adipose tissue in the abdominal cavity of 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-r agonist.
2. Proprietary natural compounds are used to induce differentiation.
3. The activity of inhibitor for differentiation (VAI) is removed from serum supplemented in the culture medium by a proprietary procedure.
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 Adipocyte Culture Kits can be used with our cell-based assays – GPDH Activity Assay (KT-010) and Lipid Assay Kit (KT-101).



Mesenteric adipocytes (Day 9) **KT-795**

Epididymal adipocytes (Day 9) **KT-793**

Subcutaneous adipocytes (Day 9) **KT-794**

COMPONENTS

Components	Size	Quantity
Rat Pre-adipocytes	Vial containing 3 x 10^6 cells	1
Adipocyte Culture Medium	250 mL	1

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

Materials required but not provided

- 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℃ water bath with gentle shaking.

2. Quickly thaw the Pre-adipocytes vial in a 37℃ water bath.

3. Transfer the thawed cells to a 15 mL centrifuge tube containing 10 mL of Adipocyte Culture Medium. Mix gently then centrifuge 1,000 rpm (170 x g) for 5 minutes at 4℃.

4. Remove the supernatant then resuspend the cells in 10 mL of the Adipocyte Culture Medium. Centrifuge 1,000 rpm (170 x g) for 5 minutes at 4℃.

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℃ under 5% CO_2, 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 (KT-795):

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 (KT-793):

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.

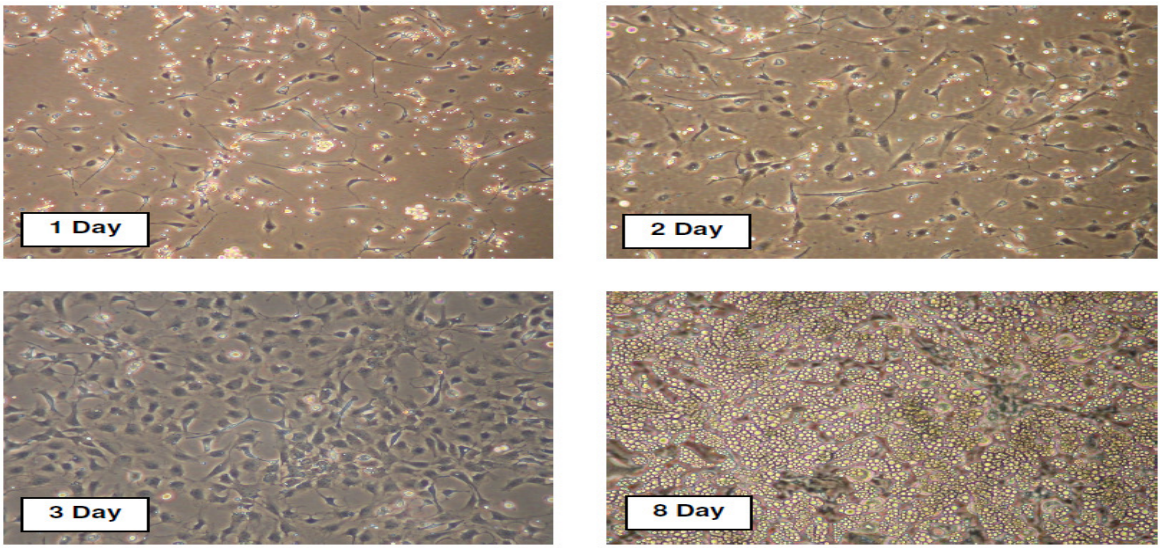
Subcutaneous Adipocytes (KT-794):

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.
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.

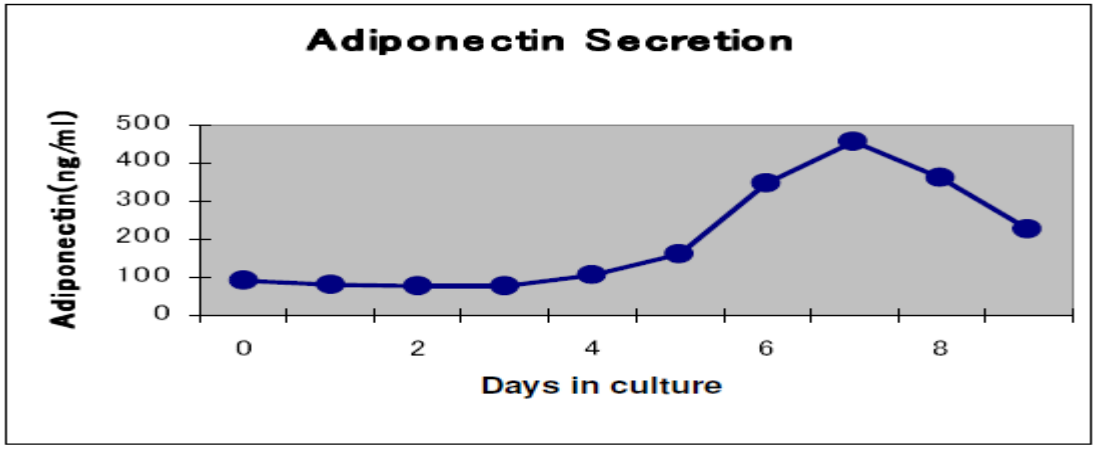
EXAMPLES

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℃ until tested. The condition media were thawed, and adiponectin was measured.



FOR RESEARCH USE ONLY
KAMIYA BIOMEDICAL COMPANY
12779 Gateway Drive, Seattle WA 98168
Tel: (206) 575-8068 Fax: (206) 575-8094
Email: LifeScience@k-assay.com
www.k-assay.com