

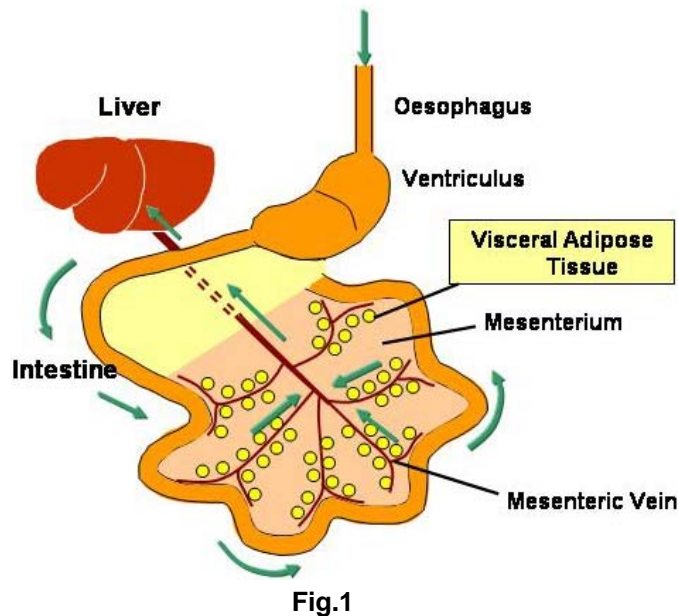


Visceral Adipocyte Culture Kit H-2 ver.2

(Rat Cryopreserved Cell and culture medium)

Principle

Mesenteric adipocytes, a type of visceral adipocytes, are located along the portal vein that transports nutrients absorbed from the intestinal tract to the liver (Fig. 1). Evidence has shown that excess fat accumulation in the visceral adipose tissue contributes to the pathogenesis of Type II diabetes, hypertension and atherosclerosis. The Rat Visceral Adipocyte Culture Kit contains preadipocytes isolated from rat mesentery and culture medium that induces differentiation of precursor cells into mature adipocytes, finally causes hypertrophy (Fig. 2). The kit provides a convenient system for studying the mechanism of adipogenesis as well as for screening drugs that prevent metabolic syndrome such as obesity, diabetes and hypertension by blocking the processes of adipogenesis. The medium contains physiological concentration of insulin. This kit is useful for studying insulin resistance.



Components

Components	Size	Quantity	Storage Conditions	Shelf Life
Visceral Preadipocytes, Rat	1.5 x 10 ⁶ cells / vial	2	Liquid Nitrogen	1 year
Visceral Adipocyte Culture Medium ver.2, (Code No. PMC-VACM2-COS)	250 ml	1	-20°C Freezer	6 months

Components of Medium:

VACM2 is a complete medium designed for differentiation of precursor cells into mature adipocytes in vitro and doesn't contain IBMX, indomethacin, dexamethasone nor PPAR-gamma agonists. It is a sterile, liquid basal medium (DE/F-12) which contains essential and non-essential amino acids, vitamins, other organic compounds, trace minerals, inorganic salts, growth factors, hormones, newborn calf serum, and antibiotics. VACM2 contains growth factors near the physiological concentration



Visceral Adipocyte Culture Medium ver.1 (VACMR) and ver.2 (VACM2)

VACM2 contains more physiological concentration of insulin than VACMR. These medium components are shown in the following.

Components	VACMR	VACM2
Inducers such as indomethacin, dexamethason, PPAR- γ agonists	—	—
Fatty acid	+	+
serum	+	+
insulin	10,000ng/mL	1ng/mL
Other growth hormones	—	+ physiological concentration

Materials required but not provided

- Variable volume pipettes
- Culture plate, 24-well, flat bottom

Precautions

- Read the instructions carefully before beginning the culture.
- This kit is for research use only, not for human or diagnostic use.
- Always wear gloves and lab coat when handling the cell culture.



Protocols

1. Thaw the Visceral Adipocyte Culture Medium in a 37°C water bath with gentle shaking .
2. Quickly thaw the Preadipocytes vial in a 37°C water bath.
3. Transfer thawed cells into a 15 ml centrifuge tube containing 5 ml of Visceral Adipocyte Culture Medium. Mix gently. Centrifuge for 5 minutes at 4°C at 1000 rpm (170 g).
4. After removing the supernatant, resuspend cells in 5 ml of the medium. Centrifuge for 5 minutes at 4°C at 1000 rpm (170 g).
5. After removing the supernatant, resuspend cells in 6.3 ml of the medium.
6. Dispense 0.5 ml of cell suspension to each well of 24-well plate.
7. Incubate the plate at 37°C under 5% CO₂, 100% humidity.
8. After 1 Day culture, add 0.5 ml of Visceral Adipocyte Culture Medium gently to each well of 24-well plate.
9. Change the medium every 2 days. Be gentle not to disturb the cell layer.
 - i. Approximately 3 days into culture, preadipocyte culture becomes confluent.
 - ii. Approximately 7 days into culture, cells become mature adipocytes.
 - iii. Approximately 8 days into culture, cells become hypertrophic and start detaching from the bottom of the well.

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

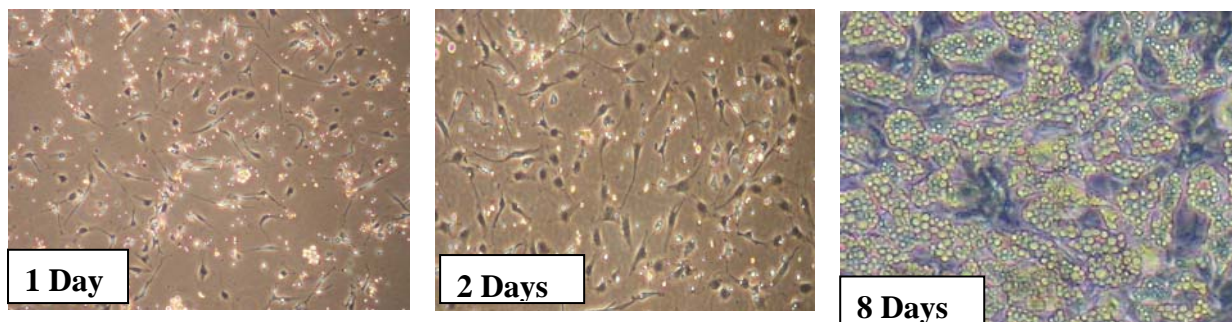


Fig. 2 Over 80 % of the cells converted into visceral adipocytes.

References

- (1) Mineo H, Oda C, Chiji H, Kawada T, Shimizu K, Taira T. Thiazolidinediones exhibit different effects on preadipocytes isolated from rat mesenteric fat tissue and cell line 3T3-L1 cells derived from mice. *Cell Biol Int.* 2007 Jul;31(7):703-10. Epub 2007 Jan 14.
- (2) Shimizu K, Sakai M, Ando M, Chiji H, Kawada T, Mineo H, Taira T. Newly developed primary culture of rat visceral adipocytes and their in vitro characteristics. *Cell Biol Int.* 2006 Apr;30(4):381-8. Epub 2006 Mar 10.

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