Visceral Adipocyte Culture Kit V-1
(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 causing 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.

Components

<table>
<thead>
<tr>
<th>Components</th>
<th>Size</th>
<th>Quantity</th>
<th>Storage Conditions</th>
<th>Shelf Life</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visceral Preadipocytes, Rat</td>
<td>$3 \times 10^6$ cells/vial</td>
<td>1</td>
<td>Liquid Nitrogen</td>
<td>1 year</td>
</tr>
<tr>
<td>Visceral Adipocyte Culture Medium ver.1,</td>
<td>250 ml</td>
<td>1</td>
<td>-20°C Freezer</td>
<td>6 months</td>
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<tr>
<td>(Code No. PMC-VACMR-COS)</td>
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Components of Medium:
VACMR is a complete medium designed for optimal culture of visceral preadipocytes in vitro. It is a sterile, liquid basal medium (DE/F-12) which contain essential and non-essential amino acids, vitamins, other organic compounds, trace minerals, inorganic salts, growth factors, hormones, newborn calf serum, and antibiotics.

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 10 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 10 ml of the medium. Centrifuge for 5 minutes at 4°C at 1000 rpm (170 g).
5. After removing the supernatant, resuspend cells in 12.5 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
