Brown Adipocyte Culture Kit D-i

(Rat Cryopreserved Cell and culture media for Growth, Differentiation and Maintenance)

Principle

BAT10 is preadipocyte isolated from rat brown adipose tissue. Adipose tissue plays an important role in mammalian energy equilibrium not only as a lipid-dissipating. White adipose tissue mainly has energy-storing function, but brown adipose tissue has very different function as energy-dissipating due to a unique mitochondrial uncoupling protein (UCP).

Brown adipose tissue is especially abundant in newborns and in hibernating mammals. Its primary function is to generate body heat in animals or newborns that do not shiver. In contrast to white adipocyte, which contain a single lipid droplet, brown adipocyte contain numerous smaller droplets and a much higher number of mitochondria, which contain iron and make it brown. Brown fat also contains more capillaries than white fat, since it has a greater need for oxygen than most tissues.

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>Brown Preadipocytes, Rat (Adult, 5-8weeks old)</td>
<td>$1 \times 10^6$ cells/vial</td>
<td>1</td>
<td>Liquid Nitrogen</td>
<td>1 year</td>
</tr>
<tr>
<td>Brown Adipocyte Growth Medium</td>
<td>125 ml</td>
<td>1</td>
<td>-20°C Freezer</td>
<td>6 months</td>
</tr>
<tr>
<td>Brown Adipocyte Differentiation Medium</td>
<td>100 ml</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brown Adipocyte Maintenance Medium</td>
<td>125 ml</td>
<td>1</td>
<td></td>
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</tbody>
</table>

Components of Media:

BATGM, BATDM and BATMM are a complete media designed for optimal culture of rat brown preadipocytes in vitro. These are sterile, liquid basal medium (D-MEM, high glucose) which contain essential and non-essential amino acids, vitamins, other organic compounds, trace minerals, inorganic salts, growth factors, hormones, calf serum, and antibiotics. In addition, BATDM contains insulin and dexamethasone, and BATMM contains insulin. To differentiate preadipocytes to mature adipocytes, use BATDM and BATMM.

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

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1. Thaw the Brown Adipocyte Growth 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 Brown Adipocyte Growth Medium (Code: BATGM) and mix gently. Centrifuge for 5 minutes at 4°C at 200 g.
4. After removing the supernatant, resuspend cells in 12.5 ml of the medium.
5. Dispense 0.5 ml of cell suspension to each well of 24-well plate.
6. Incubate the plate at 37°C under 5% CO2, 100% humidity.
7. Next day (after 1 day), add 0.5 ml of Brown Adipocyte Growth Medium gently to each well.
8. After 2 days, change the medium and continue the cell culture with changing the medium at every other day until 80-100% confluent. Preadipocyte culture becomes confluent within 3-4 days. Do not remove all medium, because it is a cause of cell detach.
9. At 80-100% confluent, change to Brown Adipocyte Differentiation Medium (Code: BATDM) and culture.
10. After Differentiation, typically within 2 days cultures in the Differentiation Medium change to Brown Adipocyte Maintenance Medium (Code: BATMM).
11. Culture with changing the medium at every other day.
12. For study of adipogenesis control factors, dose at various stages of adipogenesis. 1uM of NE is effective to induce UCP-1 gene. UCP-1 gene expression is increase 10 fold or more after 6 hours culture with NE.

Fig. 1 Over 80% of the cells converted into brown adipocytes

References
(4) Yasutake Shimizu and Takashi Shimazu, Effects of wortmannin on increased glucose transport by insulin and norepinephrine in primary culture of brown adipocytes. B.B.R.C., (1994) 202 No.2 July 29 660-665

For research use only. Not for clinical diagnosis.