COSMO BIO CO., LTD.
Inspiration for Life Science

For research use only. Not for clinical diagnosis.

Catalog No.PMC-BAT10-COS

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

Components	Size	Quantity	Storage Conditions	Shelf Life
Brown Preadipocytes, Rat (Adult, 5-8weeks old)	1 x 10^6 cells/vial	1	Liquid Nitrogen	1 year
Brown Adipocyte Growth Medium	125 ml	1	-20°C Freezer	6 months
Brown Adipocyte Differentiation Medium	100 ml	1		
Brown Adipocyte Maintenance Medium	125 ml	1		

Shipping: dry ice

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

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 37oC 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 2days culture 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 6hr culture with NE.

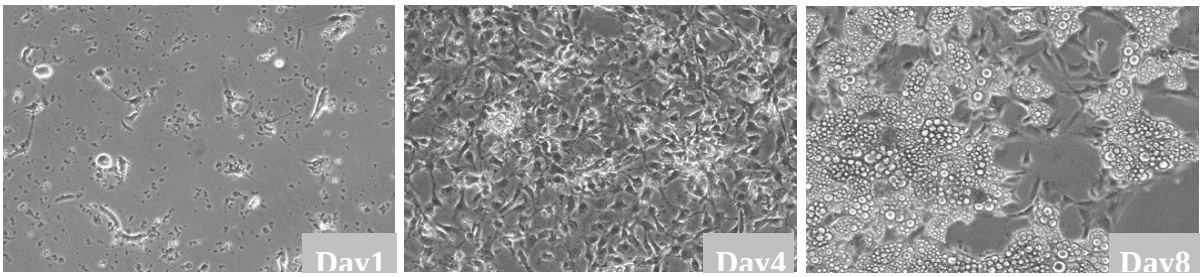

Fig. 1 Over 80% of the cells converted into brown adipocytes

References

(1) Rehnmark S., Kopecky J., Jacobsson A., Nechad M., Herron D., et al. Brown adipocytes differentated in vitro can express the gene for the uncoupling protein thermogenin effects of hypothyroidism and norepinephrine. Exp. Cell Res., (1989) 182:75-83
(2) G.Ailhaud, P. Grimaldi, and R. Negrel, Cellular and molecular aspects of adipose tissue development. Annu. Rev. Nutr., (1992) 12:207-233
(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
(5) Yasutake Shimizu, Danuta Kielar, Yasuhiko Minokoshi and Takashi Shimazu, Noradrenaline increases glucose transport into brown adipocytes in culture by a mechanism different from that of insulin. Biochem. J., (1996) 314:485-490
(6) Hideki Nikami, Yasutake Shimizu, Michihiro Sumida, Yasuhiko Minokoshi, Toshihide Yosida, Masayuki Saito, and Takashi Shimazu. Expression of β3-adrenoceptor and stimulation of glucose transport by β3-agonists in brown adipocyte primary culture. J. Biochem. (1996) 119:120-125

For research use only. Not for clinical diagnosis.

COSMO BIO CO., LTD.
Inspiration for Life Science
TOYO 2CHOME, KOTO-KU, TOKYO, 135-0016, JAPAN
URL: http://www.cosmobio.com e-mail: export@cosmobio.co.jp
[Outside Japan] Phone : +81-3-5632-9617 [国内連絡先] Phone : +81-3-5632-9610
FAX : +81-3-5632-9618 FAX : +81-3-5632-9619