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Inspiration for Life Science

For research use only. Not for clinical diagnosis.

Catalog No. PMC-OBC02-COS

Osteoblast Culture Kit (Rat)

(Rat Cryopreserved Cell and culture medium)

Principle

Bone metabolism is composed of balanced bone formation of osteoblasts and bone resorption of osteoclasts. Osteoblast Culture Kit (Rat) (PMC-OBC02-COS) contains frozen osteoblasts isolated from rat calvariae and culture medium. Osteoblast Culture Kit (Rat) (PMC-OBC02-COS) is a useful to study osteoblast and osteogenesis.

Components

Product Name / Code No.	Size	Quantity	Storage Conditions	Shelf Life
Osteoblast, Rat	1 x 10^6 cells/vial	1	Liquid Nitrogen	1 year
Culture Medium (Code: PMC-OBCM-COS)	500 ml	1	-20°C Freezer	6 months

Shipping: dry ice

Components of Media:

PMC-OBCM-COS is complete medium optimized for in vitro culture of rat osteoblast. This liquid basal medium (α-MEM) which contains 10 % FBS, 10 units/ml penicillin and 10 µg/ml streptomycin.

PMC-OBCM-COS dose not contain β-Glycerophosphate.

Materials required but not provided

- Variable volume pipettes
- Culture flasks

Precautions

1. Read the instructions carefully before beginning the culture.
2. This kit is for research use only, not for human or diagnostic use.
3. Immediately transfer cryovial from dry ice to liquid nitrogen upon receiving and keep the cells in liquid nitrogen until cell culture needed for experiments. It is recommended to use osteoblast vial immediately upon receiving.

Protocols

<1-1. Cultured with the 25 cm^2 flask>

1. Thaw the culture media in a 37°C water bath with gentle shaking.
2. Quickly place osteoblast vial in a 37°C water bath until the contents are thawed.
3. Transfer thawed cells into a 15 ml centrifuge tube containing 10 ml of culture medium and centrifuge for 5 minutes at 4°C at 600 x g for 5 minutes.
4. Remove the supernatant, re-suspend cells in 10 ml of culture medium and centrifuge at 4°C at 600 x g for 5 minutes.
5. Remove the supernatant, and re-suspend the cell pellet in approximately 5 ml of culture medium.
6. Transfer the cell suspension to 25 cm^2 flask and incubate the flask at 37°C under 5% CO_2 and 100% humidity.
7. The next day, change the medium.

   * Approximately 2-3 days of culture, cells become confluent. For subculture, please refer to the protocol below. Subculture of the cells can be performed up to passage 2.

<1-2. Subculture >

1. Subculture the cells when they are confluent.
2. Prepare sterile washing buffer (Hank's BSS or PBS(-)), and trypsin/EDTA solution. Warm washing buffer in a 37°C water bath prior to use.
3. Rinse the cells with 5 ml of washing buffer twice.
4. Remove washing buffer and then add 3 ml of trypsin/EDTA solution into flask (25 cm^2 flask).
5. Gently rock the flask to make sure that the cells are covered by trypsin/EDTA solution and then immediately remove trypsin/EDTA solution.
6. Incubate the flask in a 37°C incubator until cells are completely rounded up (monitored with inverted microscope). Approximately it takes 2 to 3 minutes.
7. Add culture medium to the flask and transfer detached cells to centrifuge tube, and then centrifuge the centrifuge tube at 4°C at 600 x g for 5 minutes.
8. After removing the supernatant, re-suspend cells in culture medium and centrifuge for 5 minutes at 4°C at 600 x g for 5 minutes.
9. Remove the supernatant, and re-suspend cells in culture medium. Count cells and plate cells in a new plate or flask (Adjust cell density to the desired experiment).

   * Approximately 2-3 days of culture, cells become confluent when seeding density is 30,000 cells/ cm2

References

(1) Hisa, I., Inoue, Y., Hendy, G. N., Canaff, L., Kitazawa, R., Kitazawa, S., Komori, T., Sugimoto, T., Seino, S., Kaji, H. Parathyroid Hormone-responsive Smad3-related Factor, Tmem119, Promotes Osteoblast Differentiation and Interacts with The Bone Morphogenetic Protein-Runx2 Pathway. J. Biol. Chem. 286, 9787-9796 (2011)

(2) Itoh, T., Takeda, S., Akao, Y. MicroRNA-208 Modulates BMP-2-stimulated Mouse Preosteoblast Differentiation by Directly Targeting V-ets Erythroblastosis Virus E26 Oncogene Homolog 1. J. Biol. Chem. 285, 27745-27752 (2010)

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