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Inspiration for Life Science

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Catalog No. PMC- OGC11-COS

Osteogenesis Culture kit (Mouse)

(Mouse Cryopreserved Cell and media)

Principle

There are hematopoietic stem cells and bone marrow stromal cells in bone marrow. Bone marrow stromal cells contain undifferentiated mesenchymal stem cells that can differentiate into a variety of cell types such as osteoblasts chondrocytes adipocytes and so on. Osteogenesis Culture Kit (Mouse) (PMC-OGC11-COS) contains cryopreserved cells isolated from mouse bone marrow and two types of culture medium. The cells in this product can be grown using Growth Medium (Code No. PMC- OGCMG-COS), and then can be differentiated into mature osteoblasts, which form calcified nodules, using Culture Medium (Mouse) (Code No. PMC- OGCMO-COS).

Components

Product Name / Code No.	Size	Quantity	Storage Conditions	Shelf Life
Bone marrow stromal cell, Mouse	1.0 x10^6 cells	1	Liquid Nitrogen	1 year
Growth medium (Mouse) (Code No. PMC- OGCMG-COS)	125 mL	1	-20°C Freezer	6 months
			4°C	3 months
Culture medium (Mouse) (Code No. PMC- OGCMO-COS)	250 mL	1	-20°C Freezer	6 months
			4°C	3 months

Shipping: dry ice

Components of Media:

Growth medium for Osteogenesis Culture kit (Mouse) is sterile, liquid basal medium (α-MEM) which contain contains 10 % FBS, 10 units/ml penicillin and 10 µg/ml streptomycin.

Osteogenesis Culture for Osteogenesis Culture kit (Mouse) (Code No. PMC- OGCMO-COS) is sterile, liquid basal medium (α-MEM) which contain contains 10 % FBS, 10 units/ml penicillin, 10 µg/ml streptomycin, dexamethasone, ascorbic acid and β-Glycerophosphate.

Materials required but not provided

- Variable volume pipettes
- Fibronectin-coated 24-well culture plate (flat bottom)

Precautions

1. Read the instructions carefully before beginning the culture.
2. This kit is for research use only, not for human or diagnostic use.

Protocols
< Cultured with the 24well-plate >

1. Thaw the Growth medium in a 37°C water bath with gentle shaking.
2. Quickly place Osteogenesis Cell vial in a 37°C water bath until the contents are thawed.
   * Immediately transfer cryovials 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 cell vial immediately upon receiving.
3. Transfer thawed cells into a 15 mL centrifuge tube containing 10 mL of Growth medium and mix gently.
4. Centrifuge at 4 C at 600 x g for 5 minutes.
5. After removing the supernatant, re-suspend cells in 10 mL of Growth medium and centrifuge for 5 minutes at 4 C at 600 x g for 5 minutes.
6. After removing the supernatant, re-suspend the cell pellet in approximately 5 mL of Growth medium.
7. Dispense 0.5 mL of cell suspension to each well of 24-well plate.
   * In the case of using other types of culture plate, adjust cell density to 2.5×10^4 cells/ cm^2.
8. Change the medium in the following day, and every other day thereafter until the cells become confluent.
   * Cells become confluent within 3-4 days .
9. Once cells become confluent, replace the medium with "Osteogenesis Culture Medium to promote the formation of calcified nodules.
10. Change the medium every 2 or 3 days.

    * Approximately 4-7 days after medium is changed into "Osteogenesis Culture for Osteogenesis Culture kit", cells whiten. Adipocytes appear from a part of cells.

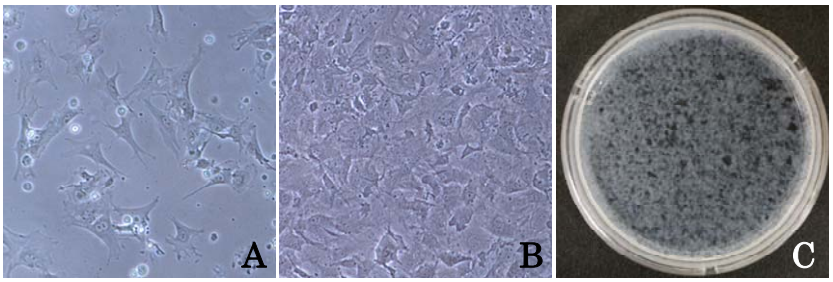


Fig. 1 Morphology of cultured cells

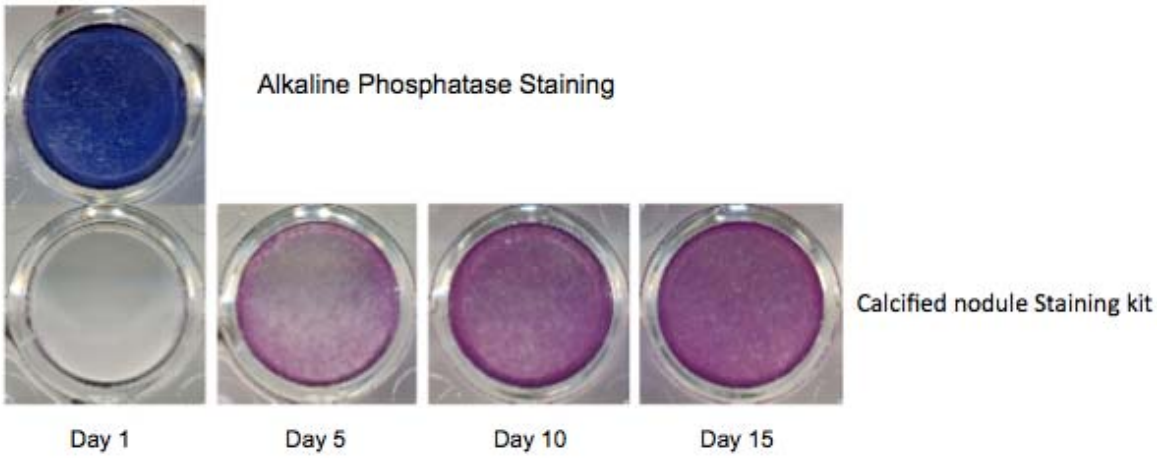
A:Day 0

B: confluent

C: 3 weeks after medium is changed into “Osteogenesis Culture Medium for Osteogenesis Culture kit” (35 mm dish)

Application example

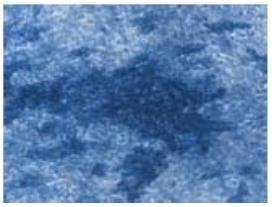
Cells are cultured according to protocol and subjected to activity staining of alkaline phosphatase or calcified nodules. The activity of alkaline phosphatase is detected by Alkaline Phosphatase Staining kit (Code: PMC-AK20-COS) and the calcium deposit is detected by Calcified nodule Staining kit (Code: PMC-AK21-COS).



Introduction of optional kit

1. Alkaline Phosphatase Staining Kit (Cat.No.PMC-AK20-COS)

The Alkaline Phosphatase Staining Kit (Cat.No. PMC-AK20-COS) is used for determining Alkaline Phosphatase activity with ease. The activity of alkaline phosphatase is used as a marker for osteoblasts.



Rat osteoblasts (Cat.No. PMC-OBC02-COS) were cultured in Osteogenesis Culture for Osteogenesis Culture kit and the activity alkaline phosphatase was detected by Alkaline Phosphatase Staining kit (Code: PMC-AK20-COS).

Components

Component	Quantity	Storage
Substrate-containing Buffer	50mL	4ºC
Chromogenic Substrate	10vials	4ºC

One kit contains regents for 10 × 12-well plates

2.Calcified nodule Staining kit (Cat.No.PMC-AK21-COS)

The Calcified nodule Staining Kit (Cat.No. PMC-AK21-COS) is used for the staining of calcified nodules with ease.

3T3-E1 cells were cultured and Calcified nodule were stained with Calcified nodule Staining kit (Code: PMC-AK21-COS).

Components

Component	Quantity	Storage
Substrate-containing Buffer	60 mL	4ºC
Chromogenic Substrate	10 vials	4ºC

One kit contains regents for 10 × 24-well plates

References

(1) SL Cheng et al., Differentiation of human bone marrow osteogenic stromal cells in vitro: induction of the osteoblast phenotype by dexamethasone. Endocrinology, 134(1) p277-286 (1994)

(2) Ohgushi et al., In vitro bone formation by rat marrow cell culture. Journal of Biomedical Materials Research Part A, 32(3) p333-340 (1996)

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Manufactured by **Primary Cell Co., Ltd**.

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