COSMO BIO CO., LTD.
Inspiration for Life Science

Osteoclast Culture Kit (Human, Osteo Assay Plate)

(Human Cryopreserved Cell, Media and Osteo Assay Plate, Code No. PMC-OSC35-COS)

For research use only

Description

Bone metabolism is composed of balanced osteogenesis and bone resorption. Research studies have shown that bone marrow cells can be differentiated into osteoclasts using M-CSF (Macrophage Colony Stimulating Factor) and RANKL (Receptor Activator of NF kappa B Ligand).

Osteoclast Culture Kit (Human) (PMC-OSC35-COS) consists of frozen osteoclast precursors isolated from human bone marrow, differentiation medium including M-CSF and RANKL, and Osteo Assay Plate (consist of wells coated with a thin inorganic 3-dimensional crystalline material) for bone cell growth, functional assay and pit-formation assay. Osteoclast Culture Kit (Human) is useful to evaluate osteoclast formation and activation.

Components/ Storage

Product Name/Code No.	PMC-OSC35-COS	Storage Conditions	Stability
Osteoclast precursor, Human	1.5 x 10^6 cells x 1 vials	Liquid Nitrogen	1 year
Wash Medium (Code No. PMC-OSCMW-COS)	50 ml	-20°C Freezer	6 months
Culture Medium (Code No. PMC-OSCMH-COS)	30 ml	-20°C Freezer	6 months
Osteo Assay Plate (96-well)	1 piece	Room temperature	6 months

※ Shipping: dry ice

Components of Media:

PMC-OSCMW-COS and PMC-OSCMH-COS are complete media formulated for optimal culture of human osteoclast in vitro. These are sterile, liquid basal media (α-MEM) which contain essential and non-essential amino acids, vitamins, other organic compounds, trace minerals, inorganic salts, growth factors, hormones, fetal bovine serum, and antibiotics. In addition, PMC-OSCMH-COS, which is used to differentiate preosteoclast to mature osteoclast, contains 50 ng/mL M-CSF and 100 ng/mL RANKL.

Materials required but not provided

- Variable volume pipettes

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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. Always wear gloves and lab coat when handling the cell culture.

Protocol

1. Thaw the Wash and Culture Media in a 37°C water bath with gentle shaking .
2. Quickly thaw the Osteoclast precursor in a 37°C water bath.
3. Transfer thawed cells into a 15 ml centrifuge tube containing 10 ml of Wash Medium. Mix gently. Centrifuge at 4°C at 200 xg for 5 minutes.
4. After removing the supernatant, resuspend cells in 10 ml of Wash Medium. Centrifuge at 4°C at 200 xg for 5 minutes.
5. After removing the supernatant, resuspend cells in 5 ml of Culture Medium containing M-CSF and RANKL.
6. Dispense 100 μl of cell suspension to each well of Osteo Assay Plate.
7. Incubate the plate at 37°C under 5% CO_2 and 100% humidity.
8. Replace the 100 μl of Culture Medium in each well on day 3.
9. Change the medium every 3-4 days. Osteoclasts will begin to fuse and form osteoclasts after 4 or 5 days of incubation.

- ◆ It is recommended to assay until day 10 in culture, not to be dissolved completely the coating of the Osteo Assay Plate.
- ◆ For study of osteogenesis, add the reagent to the medium at various stages of osteogenesis.
- ◆ To visualize the osteogenesis, the optional TRAP Staining Kit (#AK04F) is useful for staining of tartrate-resistant acid phosphatase.

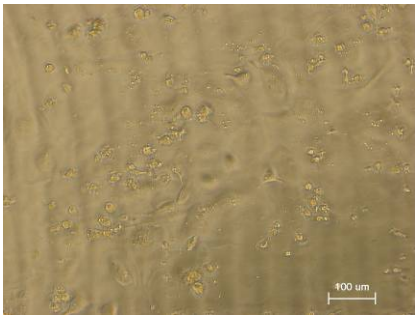

Fig. 1

Phase contrast microscopy of differentiated osteoclasts on the surface of Osteo Assay Plate.

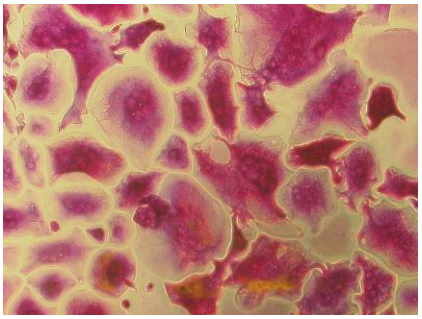

Fig. 2

TRAP staining of Osteoclasts on the surface of Osteo Assay Plate. TRAP staining kit(Cat No. AK04F) was used.

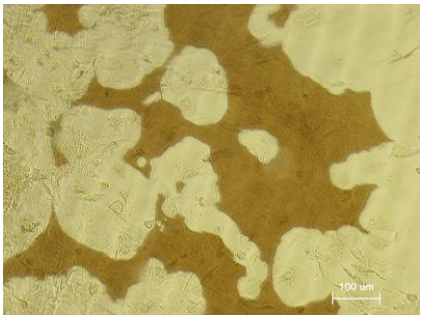

Fig. 3

Pit on the surface of Osteo Assay Plate (Kossa staining)

References

1. Takeshita et al.(2000) Identification and Characterization of the New Osteoclast Progenitor with acrophage Phenotypes Being Able to Differentiate into Mature Osteoclasts. J. Bone Miner. Res. 15, 1477-1488.

2. Scheven et al. (1998) A sequential culture approach to study osteoclast differentiation from nonadherent porcine bone marrow cells. In Vitro Cell Dev Biol Anim 34, 568–577.

3. Martha et al. (1995) Enhanced Expression of αV Integrin Subunit and Osteopontin during Differentiation of HL-60 Cells along the Monocytic Pathway. Exp. Cell Res. 216, 335-341.

4. Itonaga et al.(1999) 1,25-Dihydroxyvitamin D3 and Prostaglandin E2 Act Directly on Circulating Human Osteoclast Precursors. Biochem. Biophys. Res. Commun. 242, 703-709.

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