B-Bridge International, Inc.

Primary Precursor Osteoclasts Culture Kit
Mouse

Catalog # PMC-OSC13-COS and PMC-OSC14-COS

For research use only

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Introduction

In aging societies, osteoporosis and other age-related bone metabolism disorders are a rapidly increasing problem. The amount of bone in an organism is controlled by a balance of osteoblasts (bone-forming cell) and osteoclasts (bone-destroying cell) activities. A method to induce osteoclasts formation from bone marrow cells using M-CSF(macrophage-colony stimulating factor) and RANKL (receptor activator of NF-κB ligand) has been established in recent years.

This kit includes cryopreserved primary precursor osteoclasts from either rat or mouse bone marrow and Culture Medium containing M-CSF and RANKL.

Components

PMC-OSC13-COS (Mouse)

Primary Precursor Osteoclasts	2×10^6 cells/vial, frozen	2 vials
Wash Medium*	50 ml	1 bottle
Culture Medium (containing M-CSF 50 ng/ml, RANKL 25 ng/ml)	25 ml	1 bottle

PMC-OSC14-COS (Mouse)

Primary Precursor Osteoclasts	2×10^6 cells/vial, frozen	1 vial
Wash Medium*	25 ml	1 bottle
Culture Medium (containing M-CSF 50 ng/ml, RANKL 25 ng/ml)	12.5 ml	1 bottle

*Wash medium is culture medium without M-CSF and RANKL. The wash medium can be used as a negative control.

Materials required but not provided

- Pipettes
- 96-well, flat bottom culture plate
- Tubes
- Refrigerated centrifuge
- Water bath

Precautions

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

Protocol

Primary precursor osteoclasts are shipped on dry ice. If not used immediately, store in liquid nitrogen.

1. Thaw the Wash and Culture Medium in a 37°C water bath with gentle shaking.
2. Thaw a vial of primary precursor osteoclasts in a 37°C water bath.
3. After thawing, transfer the cells to a 15 ml centrifuge tube, add 10 ml of Wash Medium and mix briefly. Centrifuge 1000 rpm for 5 minutes at 4°C.
4. Remove supernatant and add 10 ml of Wash Medium and mix briefly. Centrifuge 1000 rpm for 5 minutes at 4°C.
5. Remove supernatant and resuspend the cells in 2.5 – 5 ml of Culture Medium. To study factors that effect osteoclasts formation, add the factors to the Culture Medium.
6. Transfer 100 µl of cell suspension into each well of a 96-well plate. If the cells are resuspended in 5 ml of Culture Medium, there will be enough cell suspension for about 50 wells. To quickly observe osteoclasts formation, culture the cells at a higher density.
7. Incubate the plates at 37°C, 5% CO_2, 100% humidity.
8. Precursor cells are sometimes sticky forming clumps of cells containing cell debris. DO NOT throw the clumps out as they contain viable cells. Replace culture medium on day 2 or 3 with fresh medium. If first medium change is later than day 2 or 3, fewer osteoclasts may develop.
9. Change medium every day. Cells will begin to fuse and form osteoclasts around day 4 (fig 1). Feeding the cells with fresh medium on a frequent basis will maintain the osteoclasts.
10. Count the osteoclasts by staining with tartrate-resistant acid phosphatase (TRAP staining, Catalog # PMC-AK04F-COS).

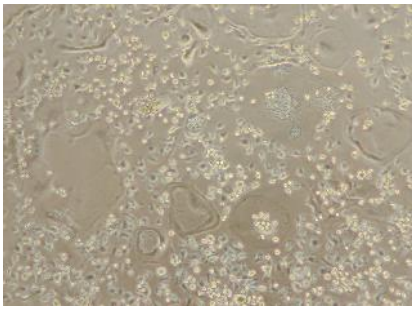

Figure 1
Osteoclasts differentiation

Examples

1. TRAP staining (Catalog #PMC-AK04F-COS) Osteoclasts were fixed then stained with 5 ml of a mixture containing chromogenic substrate and tartrate-containing buffer.

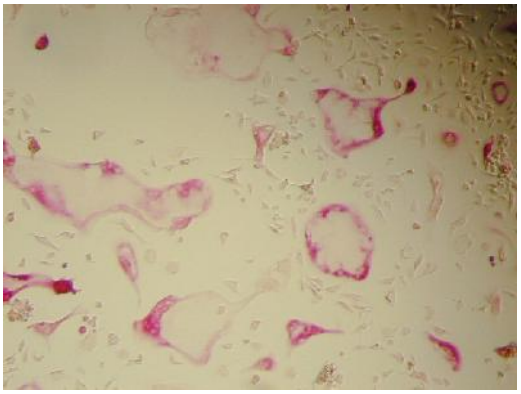

Figure 2: TRAP staining

2. TRAP analysis of culture supernatant is qualitative (Catalog # PMC-AK04F-COS)
Thirty microliters of culture supernatant was incubated for 3 hours in the presence of chromogenic substrate/tartrate-containing buffer. The samples were read at wavelength 540 nm.

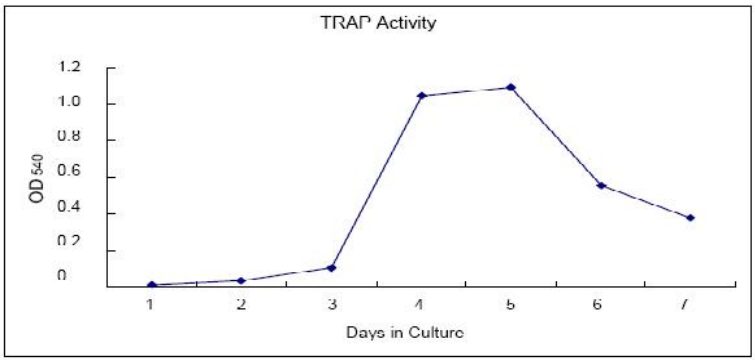

Figure 3: Measurement of TRAP in Osteoclasts culture supernatant

3. Pit Assay
Primary precursor osteoclasts cultured on ivory for 7-14 days. The section was sonicated in 5 ml of 1M ammonia solution to disrupt the cells. The ivory section was stain with Mayer's hematoxylin solution for 1 minute then washed and dried.

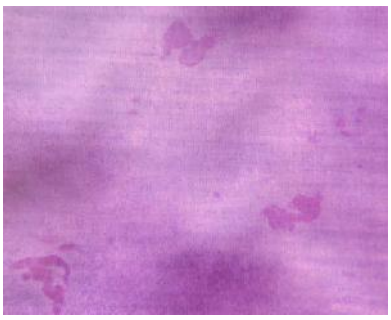

Figure 2: Resorption pits on ivory section (HE staining)

4. Scanning electron microscopy (SEM)
SEM of the ivory section used in the Pit assay.

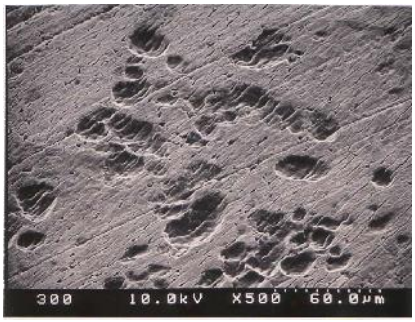

Figure 3: Resorption pits on ivory section

References

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2. Bena A. Scheven, Jones Milne, Simon P. Robins. A sequential culture approach to study osteoclast differentiation from nonadherent porcine bone marrow cells. In Vitro Cell. Dev. Biol. July-August (1998).Animal 34:568-577.

3. Martha J. Somerman, Janice E. Berry, Zhila Khalkhali-Ellis, Philip Osdoby, Robert U. Simpson. Enhanced expression of α_v integrin subunit and osteopontin during differentiation of HL-60 cells along monocytic pathway. Exp Cell Res 216, 335-341(1995).

4. Ichiro Itonaga, Afsie Sabokbar, Susan D. Neale, Nicholas A. Athanasou. 1, 25-Dihydroxyvitamin D_3 and prostaglandin E_2 act directly on circulating human osteoclast pecursors. Biochem and Biophys Res Comm 264, 590-595(1999).

Companion Assays

Cell-based assays for adipocytes

1. TRAP Staining Kit, Catalog # PMC-AK04F-COS

For tartrate-resistant acid phosphatase staining in osteoclasts

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