Mouse Primary Precursor Osteoclasts Culture Kit with Osteoplates

Catalog # OSC123 and OSC124

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 mouse bone marrow and Culture Medium containing M-CSF and RANKL.

Components

Components	OSC123	OSC124
Primary Precursor Osteoclasts	$2x10^6$ cells vial (2 vials)	$2x10^6$ cells vial (1 vial)
Wash Medium*	50 ml	50 ml
Culture Medium (M-CSF 50 ng/ml, RANKL 25 ng/ml)	25 ml	25 ml
Osteoplate	2 plates	2 plates

*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
- Tubes
- Refrigerated centrifuge
- Water bath
- Von Kossa or toluidine blue stains for pit image analysis

Precautions

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

Cell Culture 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. Quickly thaw a vial of primary precursor osteoclasts in a 37°C water bath.
3. Transfer thawed cells to a 15 ml centrifuge tube, add 10 ml of Wash Medium and mix gently.
4. Centrifuge 1000 rpm (170xg) for 5 minutes at 4°C.
5. Remove supernatant and add 10 ml of Wash Medium and mix gently.
6. Centrifuge 1000 rpm for 5 minutes at 4°C.
7. 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.
   a. If the cells are resuspended in 5 ml of Culture Medium, there will be enough cell suspension for about 50 wells.
   b. To quickly observe osteoclasts formation, culture the cells at a higher density.
8. Transfer 100 µl of cell suspension into each well of a 96-well osteoplate.
9. Incubate at 37°C, 5% CO_2, 100% humidity.
10. Precursor cells are sometimes sticky forming clumps of cells containing cell debris. DO NOT throw the clumps out as they contain viable cells. Feed the cells with 100 µl of Culture Medium on day 2 or 3. If first medium change is later than day 2 or 3, fewer osteoclasts may develop.
11. 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.
12. Count the osteoclasts by staining with tartrate-resistant acid phosphatase (TRAP staining, Catalog # AK04) or Pit image analysis.

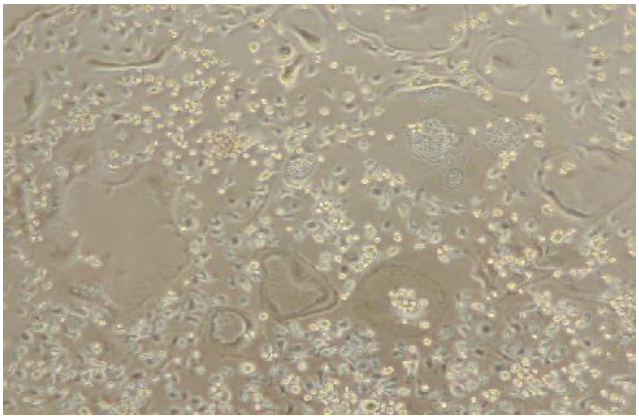
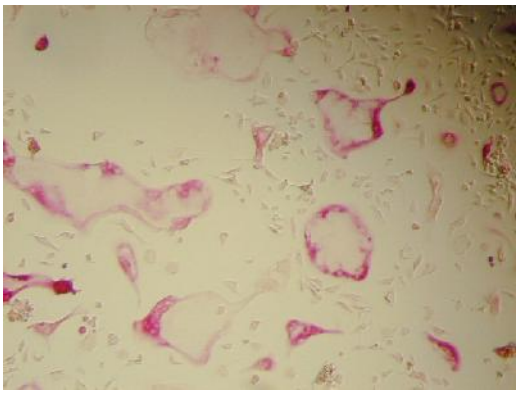


Figure 1
Osteoclasts differentiation

Osteoclasts Observation

1. TRAP staining (Catalog # PMC-AK04F-COS)

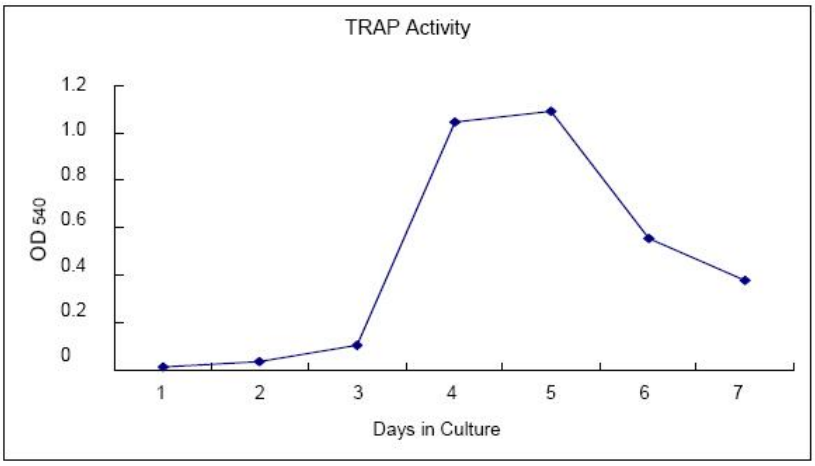
Osteoclasts were fixed then stained with 5 ml of a mixture containing chromogenic substrate and tartrate-containing buffer.



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.



Qualitative analysis of TRAP in Osteoclasts culture supernatant

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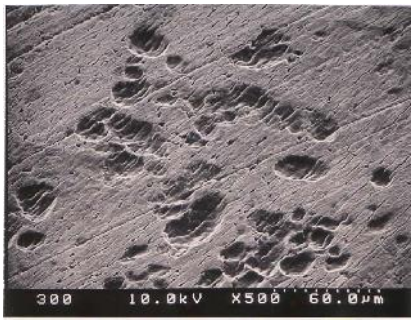
3. Pit Image Analysis

Pits should be observed prior to 10 days in culture and before the cells dissolve the osteoplate coating.

A. Aspirate the medium completely from the wells. Add 100 ul/well of a 10% bleach solution.
B. Incubate in the bleach solution for 5 minutes at room temperature.
C. Aspirate bleach solution and wash each well twice with 150 ul of dH2O.
D. Allow the plate to air dry at room temperature (Recommended time 3 to 5 hours).
E. Observe each well at 100x magnification for the formation of pits.
F. Pits will appear as individual or multiple clusters at the bottom of the well.
G. Analyze data appropriately; recommended methods include:
   - Visual enumeration of pits via a microscope or analysis software.
   - Stain using Von Kossa or toluidine blue stains to increase contrast between pits and surface coating.

4. Scanning electron microscopy (SEM) of Pits



References

1. Sunao Takesita, Keisuke Kaji, Akira Kudo. Identification and characterization of the new osteoclast progenitor with macrophage phenotypes being able to differentiate into mature osteoclasts. J Bone and Mineral Res Volume 15, Number 8(2000) .1477-1488.

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).

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