Primary Precursor Osteoclasts Culture Kit
Rat and Mouse

Rat Catalog # OSC21, OSC22, and OSC25
Mouse Catalog # OSC23 and OSC24

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

**OSC21 (Rat)**
- Primary Precursor Osteoclasts: $2 \times 10^6$ cells/vial, frozen
- Wash Medium: 100 ml
- Culture Medium (containing M-CSF 10 ng/ml, RANKL 10 ng/ml): 50 ml

**OSC22 (Rat) or OSC23 (Mouse)**
- Primary Precursor Osteoclasts: $2 \times 10^6$ cells/vial, frozen
- Wash Medium: 50 ml
- Culture Medium (containing M-CSF 10 ng/ml, RANKL 10 ng/ml): 25 ml

**OSC25 (Rat) or OSC24 (Mouse)**
- Primary Precursor Osteoclasts: $2 \times 10^6$ cells/vial, frozen
- Wash Medium: 25 ml
- Culture Medium (containing M-CSF 10 ng/ml, RANKL 10 ng/ml): 12.5 ml

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 a vial of primary precursor osteoclasts in a 37°C water bath.
2. 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.
3. Remove supernatant and add 10 ml of Wash Medium and mix briefly. Centrifuge 1000 rpm for 5 minutes at 4°C.
4. 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.
5. 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.
6. Feed the cells with 100 μl of Culture Medium every 3-4 days. Cells will begin to fuse and form osteoclasts around day 5 (fig 1).
7. Count the osteoclasts by staining with tartrate-resistant acid phosphatase (TRAP staining, Catalog # AK04).

![Figure 1](image1.png)

Osteoclasts differentiation

**Examples**

1. TRAP staining (Catalog # AK04)
   Osteoclasts were fixed then stained with 5 ml of a mixture containing chromogenic substrate and tartrate-containing buffer.

![Figure 2](image2.png)

TRAP staining
2. **Quantitation of TRAP in culture supernatant (Catalog # AK04)**

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](image)

**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](image)

**Figure 2**
Resorption pits on ivory section (HE staining)
4. Scanning electron microscopy (SEM)
SEM of the ivory section used in the Pit assay.

![SEM of ivory section](image)

Figure 3
Resorption pits on ivory section

References


Companion Assays

Cell-based assays for adipocytes
1. TRAP Staining Kit, catalog # AK04
   For tartrate-resistant acid phosphatase staining in osteoclasts

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