

**KAMIYA BIOMEDICAL COMPANY**

# Osteoclast Culture Kit

**For the culture of Osteoclasts from precursor cells.**

Cat. No.:

KT-646

KT-647

**For Research Use Only.**

**PRODUCT INFORMATION****Osteoclast Culture Kit**

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**PRINCIPLE**

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- $\kappa$ B 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	KT-646	KT-647
Mouse Osteoclast Precursor Cells, frozen	2 vials containing $2 \times 10^6$ cells each	1 vial containing $2 \times 10^6$ cells
Washing Medium	50 mL	50 mL
Culture Medium, M-CSF (10 ng/mL) and RANK Ligand (10 ng/mL)	25 mL	25 mL
Osteoplate	2 plates	2 plates

**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.

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

**PROTOCOL**

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 at 1000 rpm (170xg) for 5 minutes at 4°C.
5. Remove supernatant and add 10 mL of Wash Medium and mix gently.
6. Centrifuge at 1000 rpm (170xg) 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  $\mu$ L of cell suspension into each well of a 96-well plate.
9. Incubate at 37°C, 5% CO<sub>2</sub>, 100% humidity.

10. Replace the medium with fresh culture medium every 3-4 days. Cells will begin to fuse and form osteoclasts after 4 days of incubation (fig 1).
11. Count the osteoclasts by staining with tartrate-resistant acid phosphatase (TRAP Staining Kit, Cat. No. KT-008) or Pit image analysis.

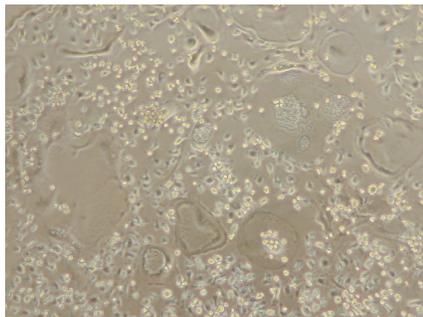
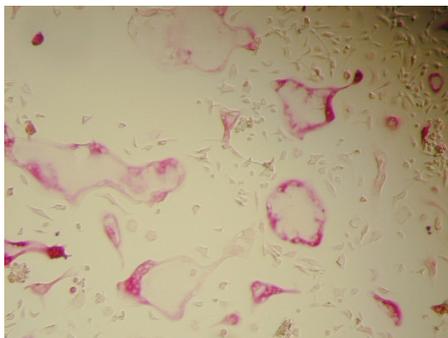


Figure 1: Osteoclasts differentiation

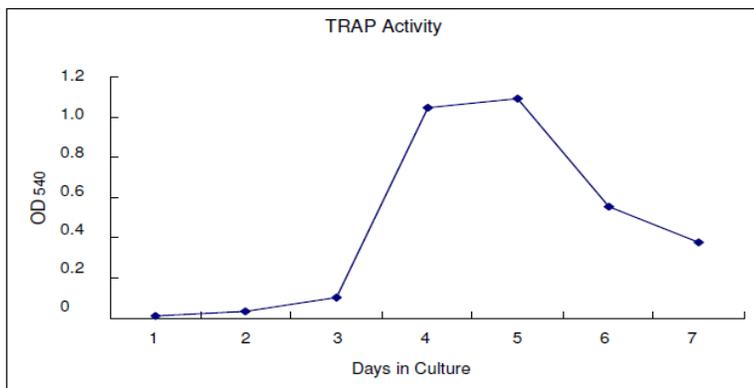
## EXAMPLES

1. TRAP Staining Kit (Cat. No. KT-008):  
Osteoclasts were fixed then stained with 5 mL of a mixture containing chromogenic substrate and tartrate-containing buffer.



TRAP Staining

2. Quantitation of TRAP in culture supernatant (Cat. No. KT-008):  
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.



Measurement of TRAP in Osteoclasts culture supernatant

### 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 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 dH<sub>2</sub>O.
- 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):



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