KAMIYA BIOMEDICAL COMPANY

Osteoclast Culture Kit

For the culture of Osteoclasts from precursor cells.

Cat. No.:
KT-649
KT-650

For Research Use Only.

PRODUCT INFORMATION

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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-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	KT-649	KT-650
Mouse Osteoclast Precursor Cells, frozen	2 vial containing 2 x 10^6 cells	1 vials containing 2 x 10^6 cells each
Washing Medium	50 mL	25 mL
Culture Medium, M-CSF (10 ng/mL) and RANK Ligand (10 ng/mL)	25 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.

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

PROTOCOL

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 1,000 rpm for 5 minutes at 4°C.
3. Remove supernatant and add 10 mL of Wash Medium and mix briefly. Centrifuge 1,000 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 Kit, Cat. No. KT-008).

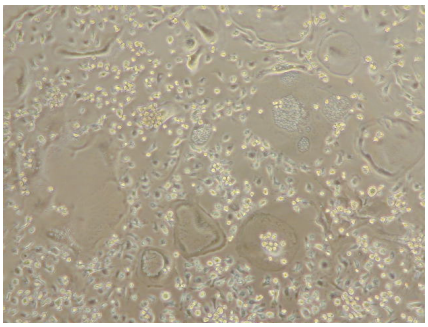
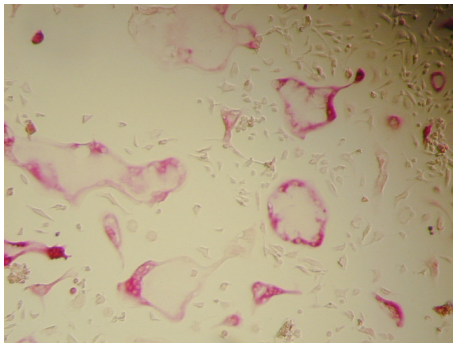


Figure 1: Osteoclasts differentiation

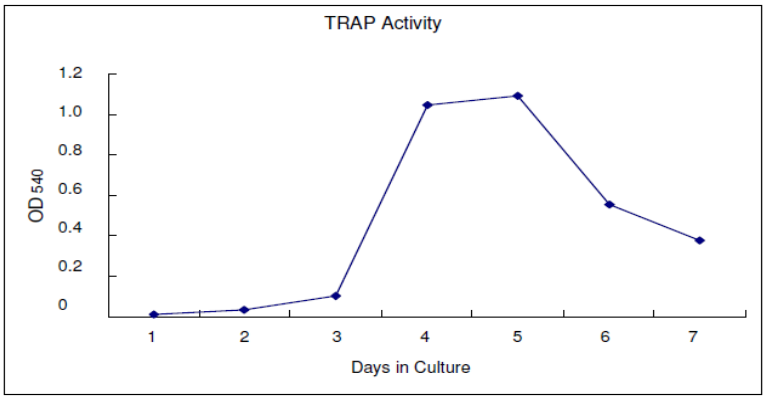
EXAMPLES

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.



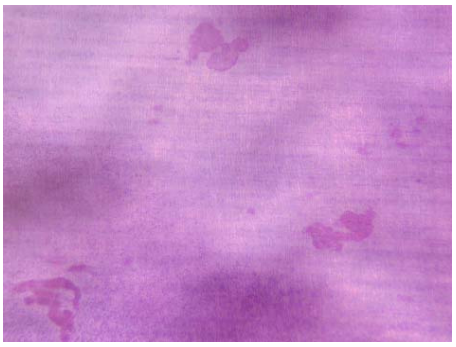
TRAP Staining

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.



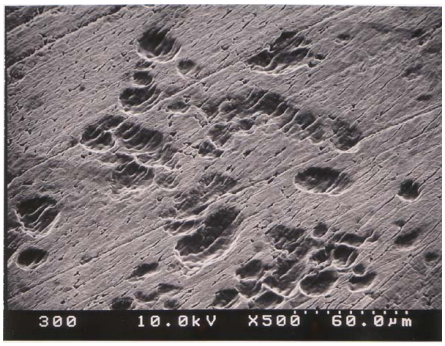
Measurement of TRAP in Osteoclasts culture supernatant

3. Pit 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 stained with Mayer's hematoxylin solution for 1 minute then washed and dried.



Resorption pits on ivory section (HE staining)

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



Readsorption pits on ivory section

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