KAMIYA BIOMEDICAL COMPANY

Rat Osteoclast Culture Kit

For the culture of Osteoclasts from precursor cells.

Cat. No.:
KT-644, KT-645

For Research Use Only.

PRODUCT INFORMATION

Rat 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-κB ligand) has been established in recent years. This kit includes cryopreserved primary precursor osteoclasts from rat bone marrow and Culture Medium containing M-CSF and RANKL.

COMPONENTS

Components	KT-644	KT-645
Rat Osteoclast Precursor Cells, frozen	2x10^6 cells per vial (4 vials)	2x10^6 cells per vial (2 vials)
Washing Medium	100 mL	50 mL
Culture Medium, M-CSF (50 ng/mL) and RANK Ligand (15 ng/mL)	50 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 1,000 rpm (170xg) for 5 minutes at 4°C.
5. Remove supernatant and add 10 mL of Wash Medium and mix gently.
6. Centrifuge at 1,000 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 the 96-well osteoplate included with the kit.
9. Incubate at 37°C, 5% CO_2, 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.

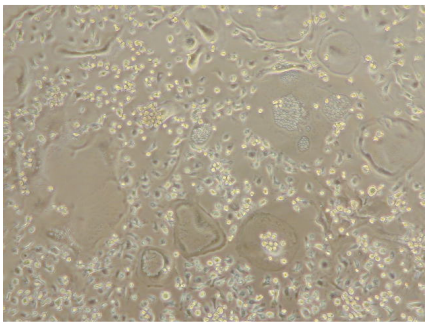
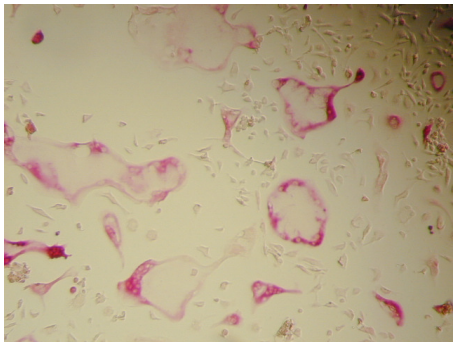


Figure 1: Osteoclasts differentiation

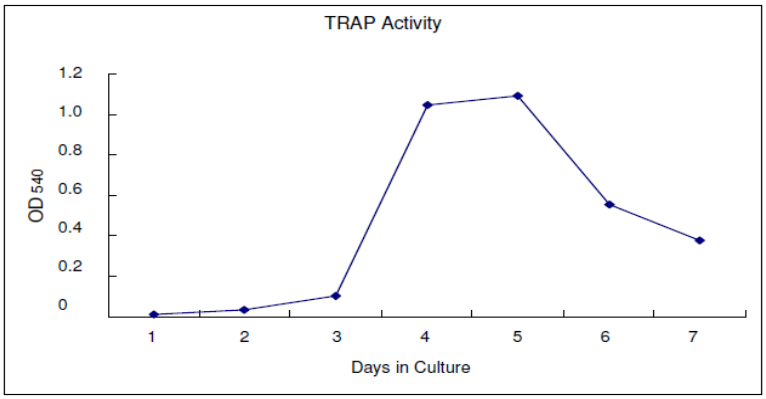
EXAMPLES

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. Qualitative analysis 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.



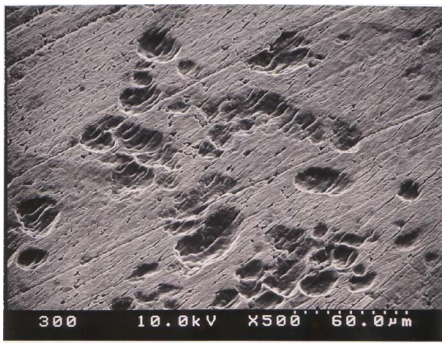
Qualitative Analysis of TRAP in Osteoclasts culture supernatant

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 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):



FOR RESEARCH USE ONLY

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