KAMIYA BIOMEDICAL COMPANY

Rat Osteoclast Culture Kit

For the culture of rat osteoclasts from precursor cells.

Cat. No.: KT-361, KT-703

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

COMPONENTS

Components	KT-361	KT-703
Rat Osteoclast Precursor Cells, frozen	2 vials with 2 x 10^6 cells	4 vials with 2 x 10^6 cells
Wash Medium*	50 mL	100 mL
Culture Medium (containing M-CSF 50 ng/mL, RANKL 15 ng/mL)	25 mL	50 mL

*Wash medium is culture medium without RANKL and M-CSF. Wash medium can be used as a negative control.

Storage

Components	Storage Conditions
Rat Osteoclast Precursor Cells	Liquid Nitrogen (preferred)
Wash Medium	-80°C Freezer
Culture Medium	-80°C Freezer

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

1. Thaw the Wash and Culture Medium in a 37°C water bath with gentle shaking.
2. Thaw a vial of primary precursor osteoclasts in a 37°C water bath.
3. After thawing, transfer the cells to a 15 mL centrifuge tube containing 10 mL of Wash Medium and mix gently. Centrifuge 1,000 rpm for 5 minutes at 4°C.
4. Remove supernatant and resuspend the cells in 10 mL of Wash Medium. Centrifuge 1,000 rpm for 5 minutes at 4°C.
5. Remove supernatant and resuspend the cells in 2.5 - 5 mL of Culture Medium containing M-CSF and RANKL.
6. 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.
7. Incubate the plates at 37℃, 5% CO_2, 100% humidity.
8. Precursor cells are sometimes sticky forming clumps of cells containing cell debris. DO NOT throw the clumps out as they contain viable cells. Replace Culture Medium within 3-4 days. If first medium change is later than day 3 or 4, fewer osteoclasts may develop.

9. After adding fresh medium on day 3 or 4, change the medium every other day. Cells will begin to fuse and form osteoclasts around day 5 (fig 1). Feeding the cells with fresh medium on a frequent basis will maintain the osteoclasts.
10. Count the osteoclasts by staining with tartrate-resistant acid phosphatase (TRAP Staining Kit, Cat. No. KT-008).

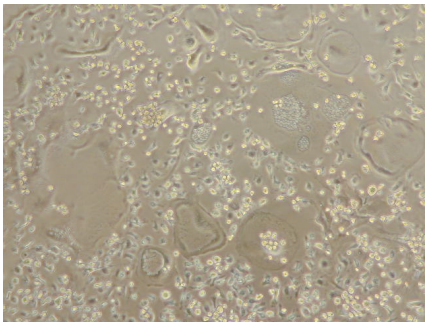
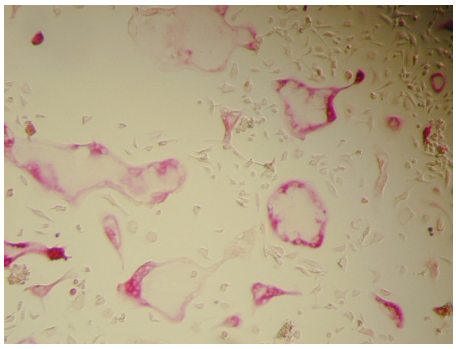


Figure 1: Osteoclasts differentiation

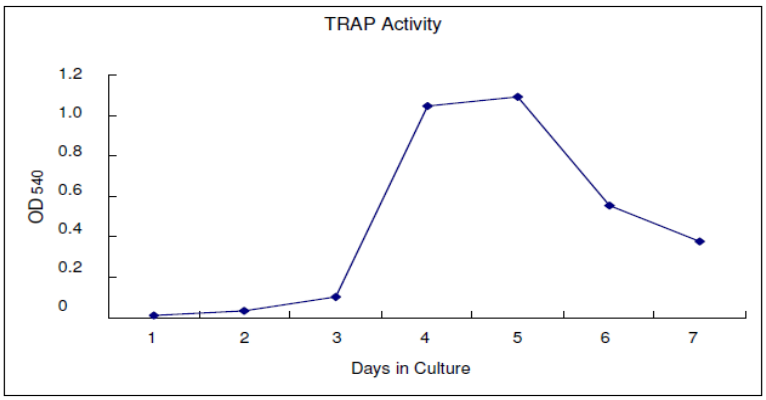
EXAMPLES

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

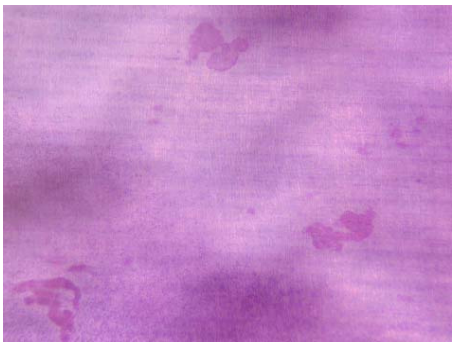
2. TRAP analysis of culture supernatant is qualitative (Cat. No. KT-008):
   Thirty microliters of culture supernatant were incubated for 3 hours in the presence of chromogenic substrate/tartrate-containing buffer. The samples were read at wavelength 540 nm.



Measurement of TRAP in Osteoclasts culture supernatant

3. Pit Assay:

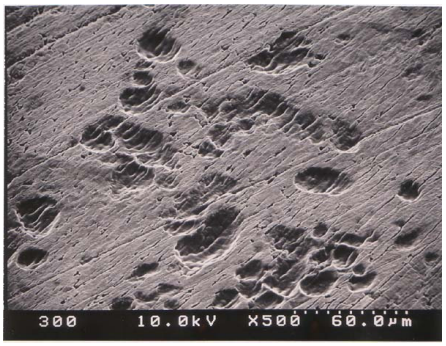
   Primary precursor osteoclasts cultured on ivory for 7 – 14 days. The section was sonicated in 5 mL of 1M ammonia solution to disrupt the cells. The ivory section was stained with Mayer's hematoxylin solution for 1 minute then washed and dried.



Resorption pits on ivory section (HE staining)

4. Scanning electron microscopy (SEM):

   SEM of the ivory section used in the Pit assay.



Reabsorption pits on ivory section

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