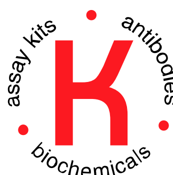


KAMIYA BIOMEDICAL COMPANY

Rat Osteoclast Culture Kit

For the culture of Osteoclasts from precursor cells.

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

For Research Use Only.

PRODUCT INFORMATION

Rat Osteoclast Culture Kit
Cat. No. KT-361, KT-703

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

Rat Osteoclast Culture Kit

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
Washing Medium, alpha-MEM	50 mL	100 mL
Culture Medium, alpha-MEM with M-CSF (50 ng/mL) and RANK Ligand (15 ng/mL)	25 mL	50 mL

Storage

Components	Storage Conditions	Shelf-Life
Rat Osteoclast Precursor Cells	Liquid Nitrogen (preferred)	See expiration date on box
Washing Medium	-20°C Freezer	6 months
	-80°C Freezer	1 year
Culture Medium	-20°C Freezer	6 months
	-80°C Freezer	1 year

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 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).

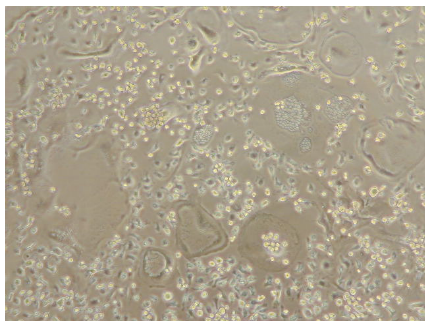
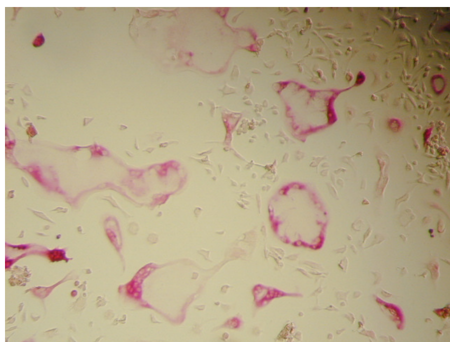


Figure 1: Osteoclasts differentiation

EXAMPLES

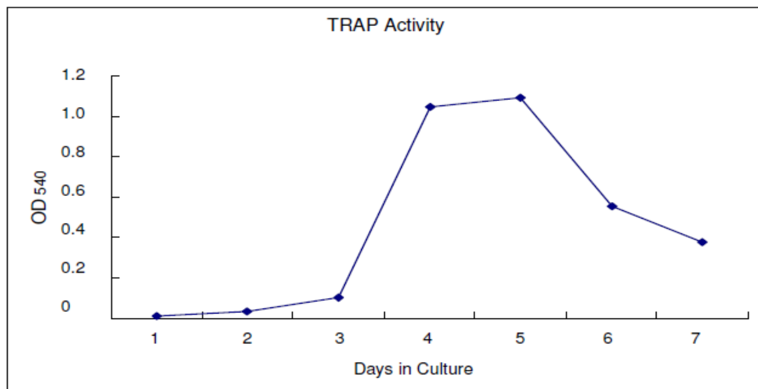
1. TRAP Staining Kit (Cat. No. KT-008):
   Osteoclasts were fixed then stained with 5 mL of a mixture containing chromogenic substrate and tartrate-containing buffer.



TRAP Staining

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

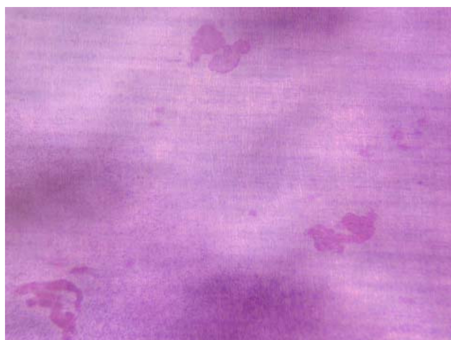
   Thirty microliters of culture supernatant was incubated for 3 hours in the presence of chromogenic substrate/tartrate-containing buffer. The samples were read at wavelength 540 nm.



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

<u>FOR RESEARCH USE ONLY</u>

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