KAMIYA BIOMEDICAL COMPANY

Human Osteoclast Culture Kit

For the culture of human osteoclasts from precursor cells.

Cat. No.: KT-791

For Research Use Only. Not for use in diagnostic procedures.

PRODUCT INFORMATION
Human Osteoclast Culture Kit
Cat. No. KT-791

PRINCIPLE
Bone metabolism is composed of balanced osteogenesis and bone resorption. Research studies have shown that bone marrow cells can be differentiated into osteoclasts using M-CSF (Macrophage Colony Stimulating Factor) and RANKL (Receptor Activator of NF kappa B Ligand).

Human Osteoclast Culture Kit consists of frozen osteoclast precursors isolated from human bone marrow.

Human Osteoclast Culture Kit is useful to evaluate osteoclast formation and activation.

COMPONENTS

Components	Quantity
Human Osteoclast Precursor Cells	1.5 x 10^6 cells
Wash Medium	100 mL
Culture Medium	30 mL

Storage

Components	Storage Conditions
Human Osteoclast Precursor Cells	Liquid Nitrogen (preferred)
Wash Medium	-20°C Freezer
Culture Medium	-20°C Freezer

Components of Media:
Wash Medium and Culture Medium are complete media formulated for optimal culture of human osteoclast in vitro. These are sterile, liquid basal media (α-MEM) which contain essential and non-essential amino acids, vitamins, other organic compounds, trace minerals, inorganic salts, growth factors, hormones, fetal bovine serum, and antibiotics. In addition, culture medium, which is used to differentiate preosteoclast to mature osteoclast, contains M-CSF and RANKL.

Materials required but not provided
- Variable volume pipettes
- Culture vessels
- 15 mL centrifuge tube

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
Cultured with a 96-well culture plate
1. Thaw the Wash and Culture Media at 4°C
2. Carefully remove the cryovial from liquid nitrogen and thaw cells in a water bath at 37℃ with gentle shaking.
3. Transfer thawed cells into a 15 mL centrifuge tube containing 10 mL of Osteoclast Wash Medium and transfer 1 mL of culture medium in the same conical tube back to the cryovial and pour the contents back to 15 mL conical tube.
4. Centrifuge for 5 minutes at 4℃ at 200 x g for 5 minutes.
5. Remove the supernatant, and re-suspend the cell pellet in 2.5 to 5.0 mL of Human Osteoclast Culture Medium.
6. Dispense 100 µL of the cell suspension to each well of 96-well culture plate, and incubate the flask at 37℃ under 5% CO_2 and 100% humidity.
7. Three days later, replace the medium with fresh culture medium.

8. Change the medium every other day.
*Osteoclasts will begin to fuse and form osteoclasts after 4 or 7 days of incubation.

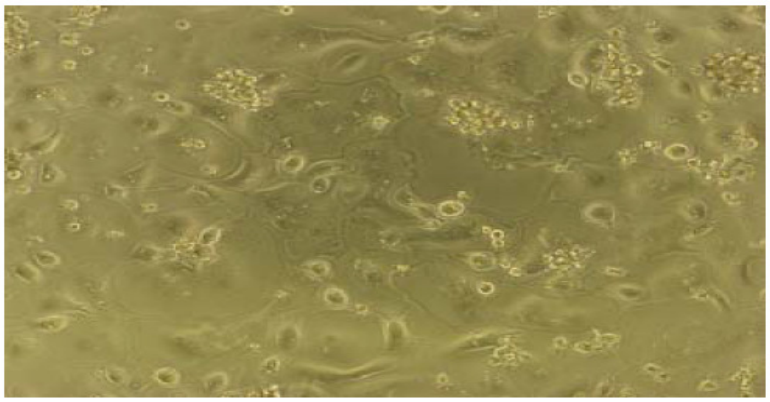

Figure 1: Phase contrast microscopy of differentiated osteoclasts

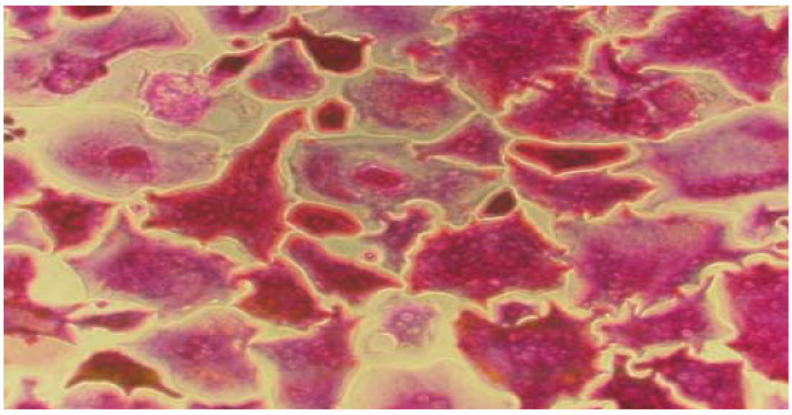

Figure 2: TRAP stained osteoclasts

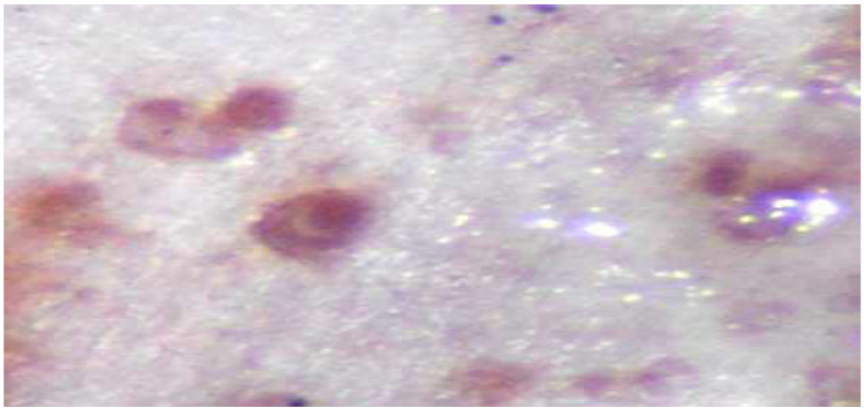

Figure 3: Pit on the slice of ivory (hematoxylin staining)

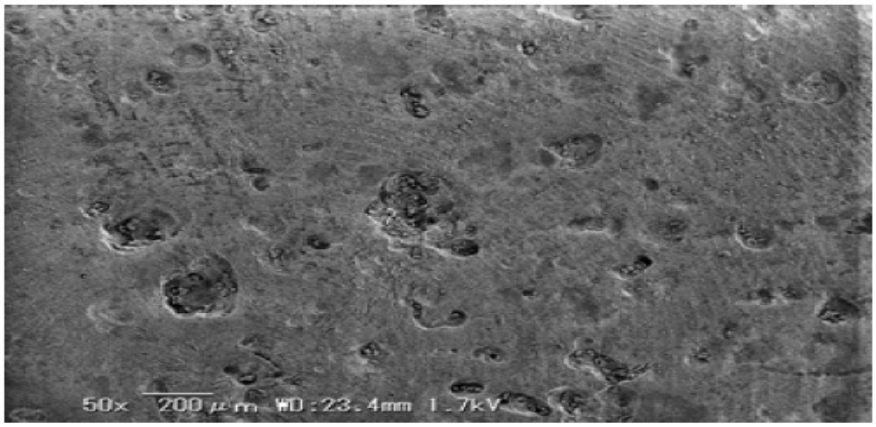

Figure 4: Pit on the slice of ivory (SEM picture)

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