Osteoclast Culture Kit

For the culture of Osteoclasts from precursor cells.

Cat. No.:
KT-361 Rat Osteoclast Culture Kit
KT-362 Mouse Osteoclast Culture Kit
CC-107 Rat Osteoclast Precursor Cells, V-1
CC-109 Mouse Osteoclast Precursor Cells, V-1

For Research Use Only.

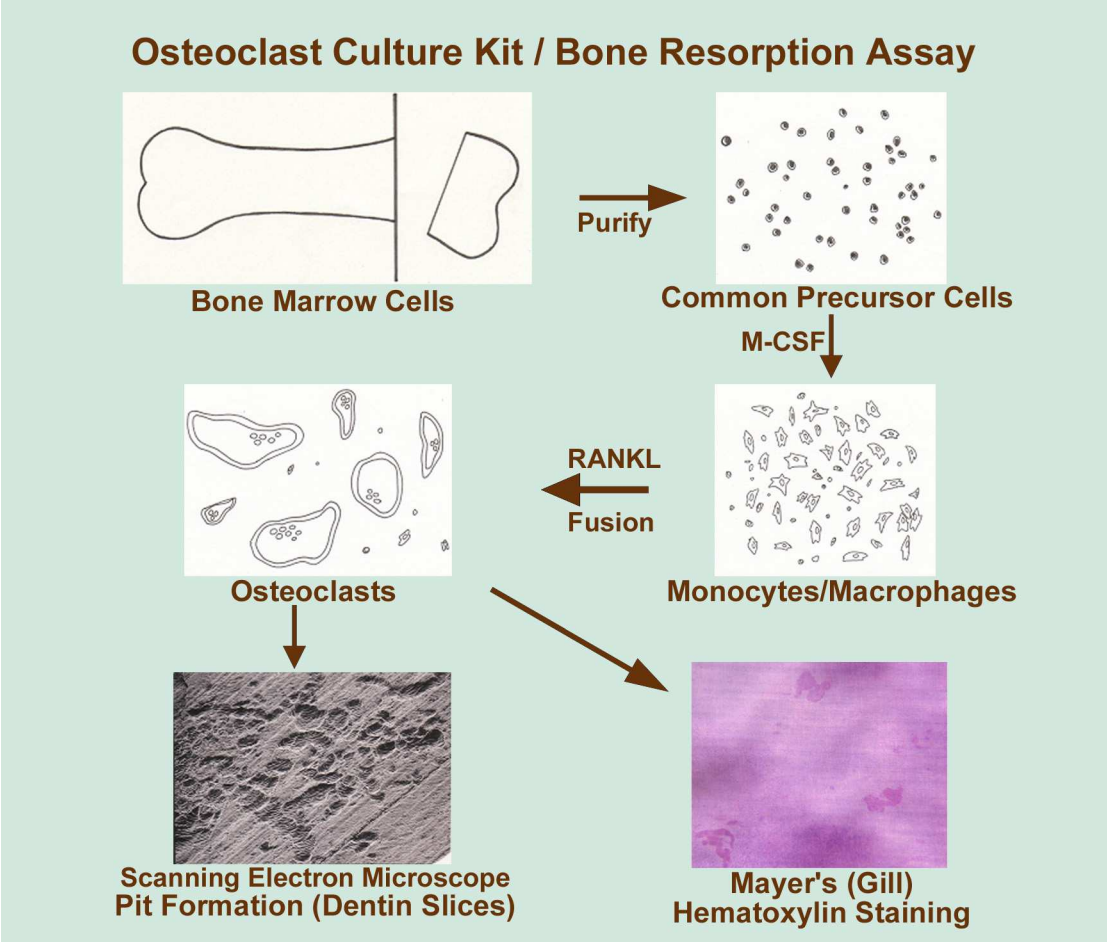
PRODUCT INFORMATION

Osteoclast Culture Kit / Bone Resorption Assay

Cat. No. **KT-361 Rat Osteoclast Culture Kit**
KT-362 Mouse Osteoclast Culture Kit
CC-007 Rat Osteoclast Precursor Cells, V-1
CC-009 Mouse Osteoclast Precursor Cells, V-1

PRINCIPLE

The morphogenesis and remodeling of bone requires the synthesis of bone matrix by osteoblasts and its coordinated resorption by osteoclasts. The ***K-ASSAY***® Osteoclast Culture Kit / Bone Resorption Assay contains frozen mouse or rat osteoclast precursor cells (isolated from bone marrow), culture medium containing M-CSF and RANKL for growing osteoclasts. Tartrate-resistant acid phosphatase can be visualized with the optional ***K-ASSAY***® TRAP Kit (KT-008).



KT-361 Rat Osteoclast Culture Kit

Components	Size	Quantity
Rat Osteoclast Precursor Cells, frozen	Vial containing 2 x 10^6 cells	2
Washing Medium, alpha-MEM	50 mL	1
Culture Medium, alpha-MEM with M-CSF (50 ng/mL) and RANK Ligand (50 ng/mL)	25 mL	1

KT-362 Mouse Osteoclast Culture Kit

Components	Size	Quantity
Mouse (BALB/C) Osteoclast Precursor Cells, frozen	Vial containing 2 x 10^6 cells	1
Washing Medium, alpha-MEM	50 mL	1
Culture Medium, alpha-MEM with M-CSF (50 ng/mL) and RANK Ligand (50 ng/mL)	25 mL	1

CC-107 Rat Osteoclast Precursor Cells, V-1

Components	Size	Quantity
Rat Osteoclast Precursor Cells, frozen	Vial containing 2 x 10^6 cells	1

CC-109 Mouse Osteoclast Precursor Cells, V-1

Components	Size	Quantity
Mouse (BALB/C) Osteoclast Precursor Cells, frozen	Vial containing 2 x 10^6 cells	1

Storage

Components	Storage Conditions	Shelf-Life
Mouse or 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

- Distilled water
- Variable volume pipettes
- Microtiter plate, flat bottom
- Dentin Slices for Pit Formation Assay

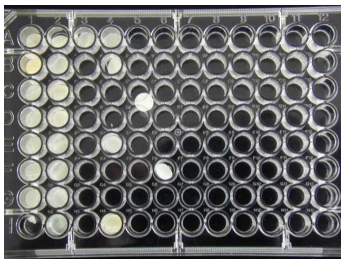
PRECAUTIONS

1. Read the instructions carefully before beginning the assay.
2. This kit is for research use only, not for human or diagnostic use.

PROTOCOLS

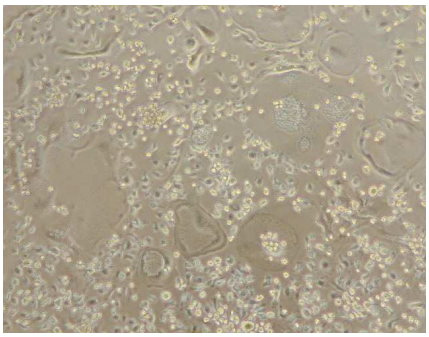
1. Osteoclast Culture Procedure
   a. Quickly thaw osteoclast precursor cell vial in a 37°C water bath.
   b. Transfer thawed cells into a 15 mL centrifuge tube. Add 10 mL of Washing Medium and mix briefly. Centrifuge for 5 minutes at 4°C at 1,000 rpm.
   c. After removing the supernatant, add 10 mL of Washing Medium to the tube and mix briefly. Centrifuge for 5 minutes at 4°C at 1,000 rpm.

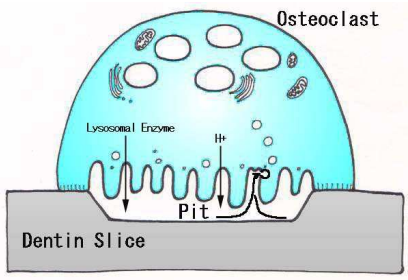
d. After removing the supernatant, add between 2.5 and 5 mL of Culture Medium (containing M-CSF, RANKL) into the tube to prepare a cell suspension. Dispense 100 µL of the suspension into each well of a 96-well plate (if you use 5 mL of the culture medium, half of the plate will be filled).
   i. If you want to see the osteoclast formation in a short period of time, make the cell density higher for the culture.
   ii. When the Pit Formation Assay is run, place a slice of the dentin slices into the well **before starting the assay**.



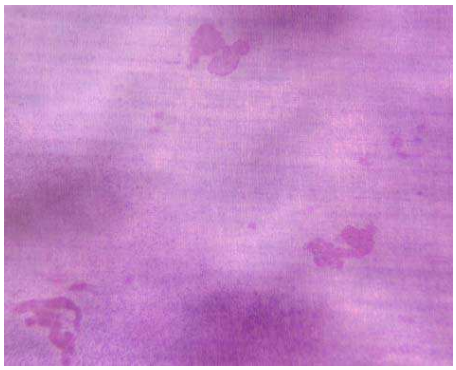
Dentin Slices not included in kit.

e. Change the culture medium (100 µL) every 3 to 4 days.
   i. Approximately 5 days after starting the culture, osteoclasts (formed from the fusion of several cells) will begin to form.
   ii. For an experiment on the control factor of osteoclast formation, add the control factor to the culture medium and culture in the wells for approximately 7 days. Proceed with TRAP staining (tartrate-resistant phosphatase staining) with optional TRAP Staining Kit (Cat. No. KT-008) and count the number of osteoclast cells.





2. Pit Formation Assays (requires Dentin Slices not included in kit)
   a. Pit Formation Assay (Staining Method):
      i. Disrupt cells (after being cultured on the dentin slice for 7 to 14 days) in 5 mL of 1M ammonia by sonication.
      ii. Remove dentin slice from the ammonia and stain with Mayer's (Gill) hematoxylin solution for 1 minute.
      iii. Wash with water and dry.
      iv. Measure the total area of the resorption pit formed by the osteoclast cells.



   b. Pit Formation Assay (SEM (scanning electron microscopy) Method):
      i. Process dentin slice as indicated in step 2.a.
      ii. Observe the dry section surface with scanning electron microscope and measure the number and area of resorption pits. If clear images of the pits are needed, SEM is recommended.