KAMIYA BIOMEDICAL COMPANY

TRAP Staining Kit

For the staining of tartrate-resistant acid phosphatase in osteoclasts

Cat. No. KT-008

For Research Use Only. Not for Use in Diagnostic Procedures.

PRODUCT INFORMATION

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PRINCIPLE
The **K-*ASSAY*** ® TRAP Staining Kit is used for the staining of tartrate-resistant acid phosphatase in osteoclasts. Bone mass is controlled by the balance between the activity of osteoblasts and the activity of osteoclasts. Alkaline phosphatase and tartrate-resistant acid phosphatase are used as markers for osteoblasts and osteoclasts, respectively. For research use only, not for use in diagnostic procedures.

COMPONENTS

Component	Quantity
Fixative, 10% Formalin, neutral buffer (Reagent 1)	1 bottle, 60 mL
Tartrate-containing Buffer, 50 mM, pH 5.0 (Reagent 2)	1 bottle, 50 mL
Chromogenic Substrate (Reagent 3)	10 vials, 3 mg/vial

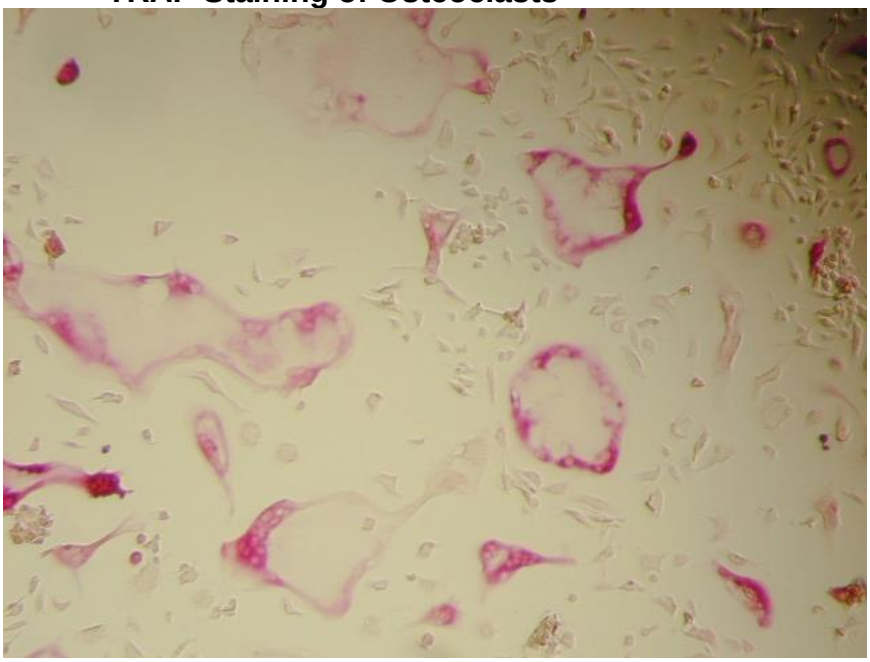
Kit Size: Enough reagents for staining 10 x 96-well plates.

Materials Required But Not Provided
- Pipets
- Microplate reader or spectrophotometer capable of reading absorbance at 540 nm
- Distilled or de-ionized water (dH_2O)
- 37°C incubator

PRECAUTIONS
1. Read the instructions carefully before beginning the assay.
2. This kit is for research use only, not for human or diagnostic use.
3. Great care has been taken to ensure the quality and reliability of this product. However, it is possible that in certain cases, unusual results may be obtained due to high levels of interfering factors.

PROTOCOL
Staining Procedure (96-Well Plate)
1. Remove culture medium. Wash each well once with 100 µL of PBS.
2. Add 50 µL of the Fixative (Reagent 1) to each well and fix for 5 minutes at room temperature.
3. Wash each well 3 times with 250 µL of dH_2O.
4. Dissolve 1 vial of Chromogenic Substrate (Reagent 3) with 5 mL of Tartrate-containing Buffer (Reagent 2).
5. Add 50 µL of Chromogenic Substrate to each well.
6. Incubate at 37°C for 20 to 60 minutes (only osteoclasts are stained as shown in the picture on page 3).
7. Wash with dH_2O water when the best color condition is obtained. (Excess incubation will cause precipitation. Stop the reaction before precipitation starts.)

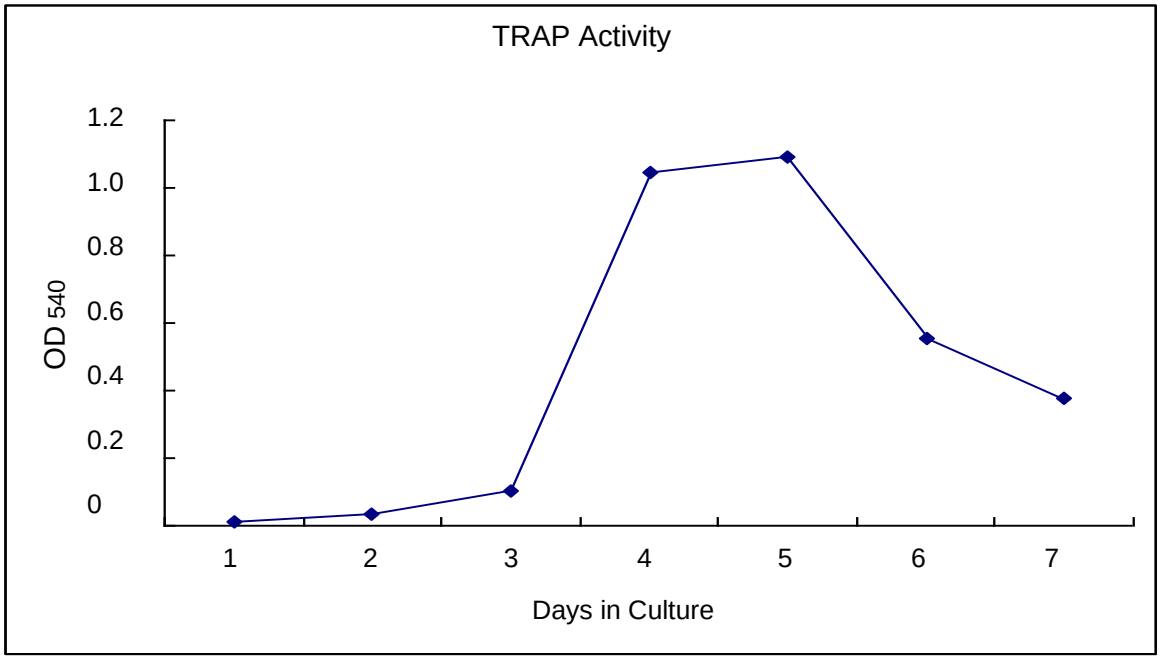
TRAP Staining of Osteoclasts



Qualitative Observation of TRAP in Culture Supernatants

1. Dissolve 1 vial of Chromogenic Substrate (Reagent 3) with 5 mL of Tartrate-containing Buffer (Reagent 2).
2. Dispense 30 μL/well of culture supernatants into 96-well plate and add 170 μL/well of the Chromogenic Substrate/Tartrate-containing buffer prepared above.
3. Incubate at 37°C for 3 hours.
4. Read in a microplate reader at 540 nm.

TRAP Activity Measured in Osteoclast Culture Supernatant



STORAGE

Kit components should be stored at 4°C.

FOR RESEARCH USE ONLY

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