B-Bridge International, Inc.

TRAP Staining Kit

Tartrate-resistant acid phosphatase staining of osteoclasts

Cat. No. AK04

For Research Use Only

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Introduction

The TRAP Staining Kit (cat. #AK04) 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. The TRAP Staining Kit supplements the Bone Reabsorption Assay Kits.

Our TRAP Staining Kit uses the same buffer as our Acid Mucopolysaccharide Assay and our DNA Quantity Assay therefore a single same can be shared among these assays.

List of Components

Store the complete kit at 4ºC

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

1 kit contains reagents for staining 10 x 96-well plates

Additional Materials Required

- Microplate reader or spectrophotometer capable of reading absorbance at 540 nm
- Distilled or deionized water (dH_2O)
- Pipets
- 37°C incubator

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 dH2O.
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 figure 1).
7. Wash with dH_2O water when the best color condition is obtained.

   Note: *Excess incubation will cause precipitation so be sure to stop reaction before precipitation starts.*

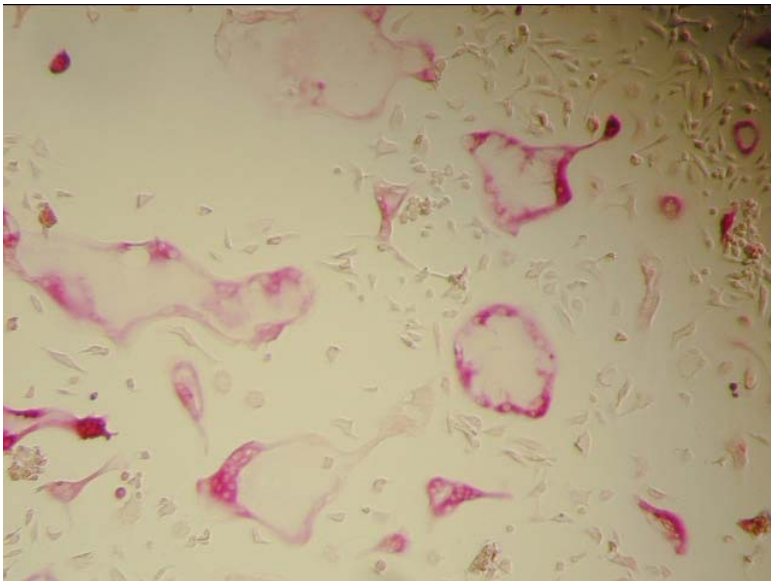

Figure 1
TRAP staining of osteoclasts

Quantitation 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 37ºC for 3 hours.
4. Read in a microplate reader at 540 nm.

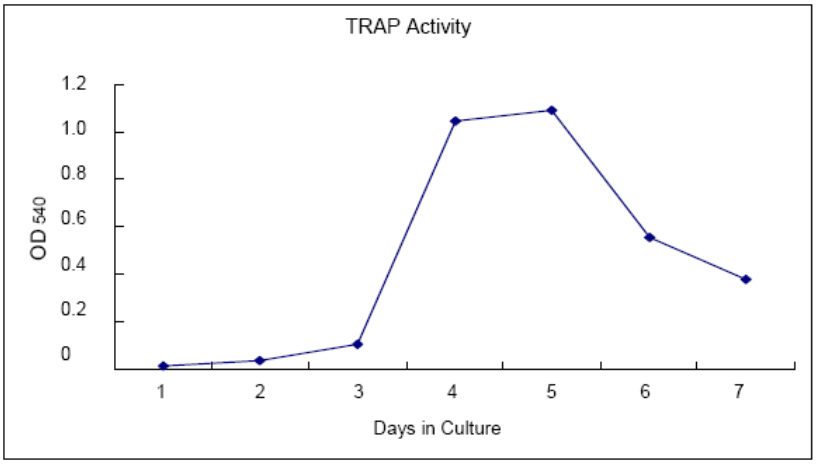

Figure 2
TRAP activity measured in osteoclasts culture supernatant

Companion Kits

Primary Precursor Osteoclasts Culture Kits for Rat and Mouse
Rat Catalog # OSC21, OSC22, and OSC25
Mouse Catalog # OSC23 and OSC24

For questions please contact

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