



# **TRAP Staining Kit**

Tartrate-resistant acid phosphatase staining of osteoclasts

Cat. No. PMC-AK04FN-COS

**For Research Use Only**

Notice to Purchaser

This product is to be used for Research Purposes Only. It is not to be used for Drug or Diagnostic Purposes, nor is it intended for Human Use. As One products may not be resold, modified for resale, or used to manufacture commercial products without the express written consent of As One International, Inc.

EXCEPT AS OTHERWISE EXPRESSLY SET FORTH IN THIS USER MANUAL, AS ONE DOES NOT MAKE ANY REPRESENTATION OR WARRANTIES OR CONDITIONS OF ANY KIND, EITHER EXPRESS OR IMPLIED, WITH RESPECT TO THE PRODUCTS, OR INFORMATION DISCLOSED HEREUNDER, INCLUDING, BUT NOT LIMITED TO, THE IMPLIED WARRANTIES OF MERCHANTABILITY, FIT FOR A PARTICULAR PURPOSE, OR NONINFRINGEMENT OF THE INTELLECTUAL PROPERTY RIGHTS OF THIRD PARTIES.

© 2020, As One International, Inc. All Rights Reserved.

## Introduction

The TRAP Staining Kit (cat. #PMC-AK04FN-COS) 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.

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

## List of Components

### Store the complete kit at 4°C

- 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

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

## Additional Materials and Instruments Required

- Distilled or deionized water (dH<sub>2</sub>O)
- Phosphate buffered saline (PBS)
- 37°C incubator
- Microplate reader or spectrophotometer capable of reading absorbance at 540 nm

## 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<sub>2</sub>O.
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<sub>2</sub>O water to stop the reaction when the best color condition is obtained.

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

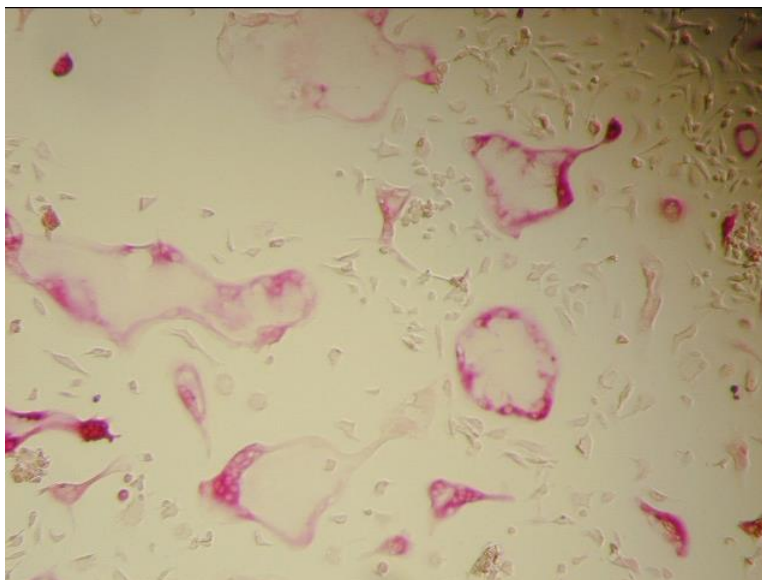


Figure 1  
TRAP staining of osteoclasts

### 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  $\mu$ L/well of culture supernatants into a 96-well plate and add 170  $\mu$ L/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.

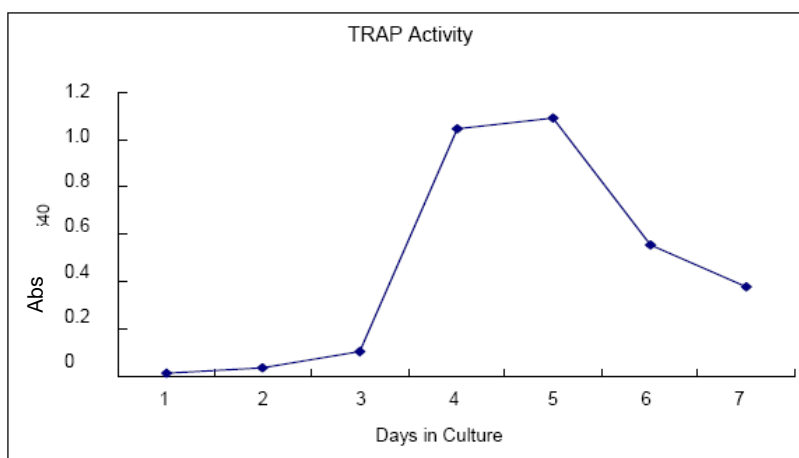


Figure 2  
TRAP activity measured in osteoclasts culture supernatant

## Companion Kits

Primary Precursor Osteoclasts Culture Kits for Rat and Mouse  
Rat Catalog # PMC-OSC11-COS, PMC-OSC12-COS, and PMC-OSC25-COS  
Mouse Catalog # PMC-OSC13-COS and PMC-OSC14-COS

For questions please contact:

**As One International, Inc**

3350 Scott Blvd, #29

Santa Clara, CA 95054 USA

Tel: 408-638-7415 Fax: 408-252-6220

Email: [info@asone-int.com](mailto:info@asone-int.com)

[www.asone-int.com](http://www.asone-int.com)