

BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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Tartrate-resistant Acid Phosphatase (TRAP) Assay Kit (Cat #: E-136)

COMPONENTS: TRAP Assay Solution- 20 ml (for 200 wells); store at -20°C
10x Cell Lysis Solution- 25 ml, store at room temperature (**agitate vial to redissolve contents**)

PRODUCT DESCRIPTION: TRAP is highly conserved throughout mammalian cells, playing a significant role in bone mineralization, ECM remodeling, cytokine production, and immune regulation. The TRAP activity assay kit is based on cleavage of p-nitrophenol phosphate in an acidic buffer. Nitrophenol exhibits an absorption maximum at 405 nm (extinction coefficient = $18 \text{ mM}^{-1}\text{cm}^{-1}$), allowing for measurement of TRAP activity in tissue/cell extracts and biological fluids such as serum and plasma. Assay solution is stable for several years if stored and handled properly.

Preparation of cell/tissue extracts:

1. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with dH₂O. Bring up $\sim 10^5$ washed cells in 50 – 100 μl ice-cold 1x Cell Lysis Solution by pipetting up and down gently. Leave lysate on ice for 5 min with agitation. If lysate is overly turbid, add more 1x Cell Lysis Solution and repeat pipetting. Tissue is homogenized in ice-cold 1x Cell Lysis Solution ($\sim 5 \text{ mg}$ tissue in 0.5 ml).
2. Centrifuge lysate in a cold microfuge at $\sim 14,000 \text{ rpm}$ for 5 min. Supernatant is harvested and stored at -80°C.
3. Use the BCA protein assay method to determine lysate protein concentration. A suggested sample protein concentration range is 0.2 – 3 mg/ml. *Normalization of lysate protein concentration is recommended.*

Enzyme assay for clear sample:

1. Thaw TRAP Assay Solution and keep solution on ice. *Do not over thaw.* Add 20 μl of each sample to a plain (uncoated) 96-well plate. Add 20 μl 1x Cell Lysis Solution or dH₂O to an empty well serving as blank.
2. Reaction is initiated by adding 100 μl TRAP Assay Solution to each well. Mix contents by brief gentle agitation. Cover plate and incubate at 37°C for 60 min. *Do not use CO₂ incubator.*
3. Stop reaction by adding 20 μl 1N NaOH (not included) to each well followed by brief gentle agitation.
4. Measure O.D._{405 nm} using a plate reader. Subtract blank well reading from each sample well reading to generate $\Delta\text{O.D.}$. TRAP activity in IU/L unit = $\mu\text{mol}/(\text{L}\cdot\text{min}) = \Delta\text{O.D.} \times 1000 \times 140 \mu\text{l}/(60 \text{ min} \times 0.6 \text{ cm} \times 18 \times 20 \mu\text{l}) = \Delta\text{O.D.} \times 10.8$. TRAP activity may be presented as units/ μg proteins or normalized by GAPDH activity.

Enzyme assay for colored sample (serum/plasma):

1. Add 10 μl of each sample to duplicate wells of a plain (uncoated) 96-well plate (for control and reaction sets).
2. Add 100 μl ice-cold dH₂O to one set of sample wells (control). Add 100 μl TRAP Assay Solution to the other set of sample wells (reaction). Mix contents by brief gentle agitation. Cover plate and incubate at 37°C for 60 min. *Do not use CO₂ incubator.*
3. Stop reaction by adding 20 μl 1N NaOH (not included) to each control well and reaction well followed by brief agitation.
4. Measure O.D._{405 nm} using a plate reader. Subtract control well reading from reaction well reading to generate $\Delta\text{O.D.}$ for each sample. TRAP enzyme activity in IU/L unit = $\mu\text{mol}/(\text{L}\cdot\text{min}) = \Delta\text{O.D.} \times 1000 \times 130 \mu\text{l}/(60 \text{ min} \times 0.6 \text{ cm} \times 18 \times 10 \mu\text{l}) = \Delta\text{O.D.} \times 20.06$. Enzyme activity may be presented as units/ μg proteins or normalized by GAPDH enzyme activity.

Additional information:

- A solution of 1N NaOH needs to be prepared for reaction termination. Avoid skin contact with NaOH. Please refer to the product webpage or contact us for SDS information.
- 10x Cell Lysis Solution precipitates at cold temperature. Re-dissolve contents by agitation in warm water upon arrival.