Phosphatase Activity Assay Kit (Colorimetric)

Phosphatase Activity Assay Kit (Colorimetric) can be used to measure Phosphatase activity.

Catalog number: ARG82191

Package: 500 tests

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INTRODUCTION

In biochemistry, a phosphatase is an enzyme that uses water to cleave a phosphoric acid monoester into a phosphate ion and an alcohol. Because a phosphatase enzyme catalyzes the hydrolysis of its substrate, it is a subcategory of hydrolases. Phosphatase enzymes are essential to many biological functions, because phosphorylation (E.g., by protein kinases) and dephosphorylation (by phosphatases) serve diverse roles in cellular regulation and signaling. Whereas phosphatases remove phosphate groups from molecules, kinases catalyze the transfer of phosphate groups to molecules from ATP. Together, kinases and phosphatases direct a form of post-translational modification that is essential to the cell's regulatory network.

Phosphatase enzymes are not to be confused with phosphorylase enzymes, which catalyze the transfer of a phosphate group from hydrogen phosphate to an acceptor. Due to their prevalence in cellular regulation, phosphatases are an area of interest for pharmaceutical research. [Provide by Wikipedia: Phosphatase]

PRINCIPLE OF THE ASSAY

This Peroxidase Activity Assay Kit (Colorimetric) is a simple assay that measures the activity of phosphatase. This assay involves simply adding a single reagent to the phosphatase and measuring the product formation. The optical density O.D. 405 nm is a direct measure of the enzyme activity.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped at ambient temperature. Store Reagent at -20°C and other components at 4°C upon receiving. Shelf life: 12 months after receipt.

Component	Quantity	Storage information
Assay Buffer	25 mL	4°C
Reagent	280 µL	-20°C
Stop Solution	25 mL	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 405 nm
- Centrifuge and centrifuge tube
- Clear flat-bottom 96 well microplate
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- Fresh reconstitution of the Reagent is recommended although the reconstituted pNPP Substrate may be stable for up to 4 weeks when stored at-20°C.
- The pH of the Assay Buffer is 7.2 and is compatible with the majority of neutral phosphatases such as protein phosphatases. For an acid phosphatase, we recommend using 100 mM sodium acetate (pH 5.5), 10 mM $MgCl_2$ as Enzyme Buffer.
- For an alkaline phosphatase, we recommend using ARG81296 ALP / Alkaline Phosphatase Assay Kit.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

REAGENT PREPARATION

- **pNPP Substrate:** mix 0.5 µL of Reagent and 50 µL of Assay Buffer.

ASSAY PROCEDURE

Equilibrate all components to room temperature. Briefly centrifuge the tubes before opening.

1. Serial dilute enzyme in a proper enzyme buffer. Prepare enough solution for triplicate assays.
2. Transfer **50 µL** of each **enzyme dilution** to wells of a clear, flat-bottom 96-well plate. In addition, prepare a blank control that contains **50 µL** of **Enzyme Buffe**r only. Initiate the reaction by adding 50 µL pNPP Substrate to each well
3. Incubate for **10-30 minutes** at **room temperature** or at **37°C.**
4. Stop the reaction by adding **50 µL** of **Stop Solution**. Mix by quickly tapping the plate. Alternatively, plates can be shaken for 10 seconds on an orbital plate shaker.
5. Read the absorbance at **O.D. 405 nm**.

CALCULATION OF RESULTS

1. Calculate the average and standard derivations of the triplicate assays and subtract the blank values. Enzyme activity is calculated from Beer-Lambert law as follows,:

   Enzyme Activity (µmoles/min/µg)

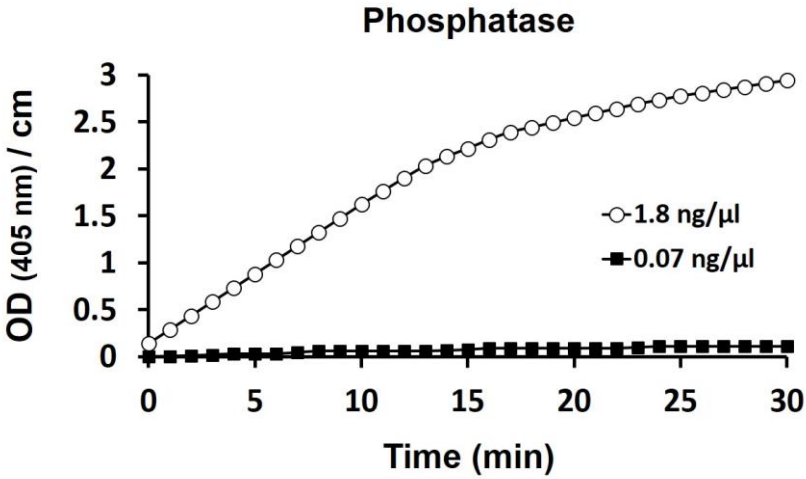
   = [V (µL) x OD_{405nm} (cm^{-1})] / [ε x incubation time (min) x enzyme(µg)]

   Note:

   - ε: the molar extinction coefficient ($M^{-1} \times cm^{-1}$). For *p*-nitrophenol, ε = 1.78 x 10^4 $M^{-1} \times cm^{-1}$.
   - OD_{405nm} (cm^{-1}) is the absorbance at 405 nm divided by the light-path length (cm).
   - V is the final assay volume. (E.g., 150 µL for 96-well plate assay).

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Phosphatase Activity Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

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