CREATIVE BIOMART®
Assay Kit

# Continuous Phosphatase Assay Kit

## Product Information

**Cat**

Kit-0881

**Common Name**

Phosphatase

**Cat.No.** Kit-0881

**Product Overview**

Phosphatase Assay Kit provides a simple one-step spectrophotometric method for the quantitation of inorganic phosphate (Pi) in solution, including Pi released from enzymatic reactions. This kit enables continuous assay of reactions that generate Pi such as those catalyzed by ATPases and GTPases.
The release of inorganic phosphate is measured through coupling to PNP-XDH enzymatic system. In the presence of inorganic phosphate, PNP (purine nucleoside phosphorylase) catalyzes inosine phosphorolysis leading to the formation of hypoxanthine and P-ribose. XDH enzyme catalyzes irreversible oxidation of hypoxanthine to uric acid with simultaneous reduction of NAD to NADH2, measurable by absorbance at 340nm. The formation of NADH2 is continuously monitored spectrophotometrically at 340nm. The assay is linear in 5-300μM range.
For maximal accuracy, the assays with cell lysates are run with and without inosine in parallel. The absorbance rate observed in the absence of inosine is used as blank and is subtracted from the absorbance rate measured in its presence.

**Description**

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 a myriad of biological functions, because phosphorylation and dephosphorylation 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 effect a form of post-translational modification that is essential to the cell's regulatory

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network. Phosphatase enzymes are not to be confused with phosphorylase enzymes, which introduce phosphate groups into organic molecules. Due to their ubiquity in cellular regulation, phosphatases are an area of interest for pharmaceutical research.

## Storage

Once dissolved, the reagents provided in the kit are not stable and should be stored on ice and used the day of preparation.

## Size

1 plate (96 assays)

## Kit Components

A standard Phosphatase Assay Kit contains:
one 2mL-tube "Cofactor 1" (DTT);
one 2mL-tube "Cofactor 2" (NAD);
one 15mL-tube with "Substrate" (inosine);
one vial "PNP Enzyme";
one vial "XDH Enzyme";
one tube with 1mL of "20x Reaction buffer";
one transparent 96-well plate.

## Materials Required but Not Supplied

1) Plate agitator
2) Plate reader fitted with a filter 340nm, Epoch; PerkinElmer.

## Assay Protocol

1. Add 1mL of deionized water to the tube with "Cofactor 1" to prepare 0.1M DTT. Agitate until complete dissolution of the powder. Transfer dissolved "Cofactor 1" to the tube with "XDH enzyme". Leave on ice for 30min for complete dissolution of the powder.
2. Label a clean 50mL tube as "Blank", fill it with 19ml of deionized water, add the content of "20x Reaction Buffer" tube.
3. Quantitatively transfer the content of the tubes with "Cofactor 2" and "PNP enzyme" to "Blank" tube:

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To do so:
-Add 1ml of "Blank" solution to each tube;
- agitate and transfer by pipeting back to "Blank";
- repeat to be sure that all reagent of the small tube are recovered. mix by gently inverting until complete dissolution. Avoid bubbles.
4. Add solubilized "XDH enzyme" to "Blank";
5. Transfer 10 mL of "Blank" to "Substrate" tube containing inosine;
You have thus prepared: 10ml of "Blank" (background non related to phosphate release); 10ml of "Substrate" solution (for measuring phosphate release).
6. Run in parallel the reaction with 200μL of "Blank" and 200μL of "Substrate" solutions per well.
7. Follow phosphate release by measuring absorbance at 340nm as a function of time.
- Program plate reader for kinetics absorbance reading at 340nm.
- Insert the plate into the reader, agitate for 1min and monitor the reaction at 340nm every 30sec.
- Subtract the values obtained for "Blank" well from the corresponding values for "Substrate".