# Alkaline Phosphatase Assay Kit

Catalog Number KA0817

500 assays

Version: 02

Intended for research use only

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## Introduction

### Background

Alkaline phosphatase (ALP) catalyzes the hydrolysis of phosphate esters in alkaline buffer and produces an organic radical and inorganic phosphate. Changes in alkaline phosphatase level and activity are associated with various disease states in the liver and bone. The Alkaline Phosphatase Assay Kit is a highly sensitive, simple, direct and HTS-ready colorimetric assay designed to measure ALP activity in serum and biological samples. It contains 10 substrate tablets providing convenience for multiple usages. The kit uses p-nitrophenyl phosphate (pNPP) as a phosphatase substrate which turns yellow (λmax= 405 nm) when dephosphorylated by ALP. The Kit can detect 10-250 μU ALP in samples.

## General Information

### Materials Supplied

List of component

| Component | Amount |
|---|---|
| ALP Assay Buffer | 100 ml |
| pNPP (10 TAB) | 1 vial |
| ALP Enzyme | 1 vial |
| Stop Solution | 10 ml |

### Storage Instruction

Store the kit at -20°C, protect from light. Allow Assay Buffer to warm to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

## Assay Protocol

### Reagent Preparation

**pNPP Solution:** Dissolve 2 tablets pNPP into 5.4 ml Assay Buffer to make 5 mM work solution. Two tablets are sufficient for 100 assays. NEVER TOUCH THE TABLETS WITH BARE HANDS. The pNPP solution is stable for 12 hours on ice.

**ALP Enzyme:** Reconstitute ALP Enzyme with 1 ml Assay Buffer. DO NOT FREEZE! The enzymes are stable for up to 2 month at 4°C after reconstitution.

***Note:*** *Ensure that the Assay Buffer is at room temperature before use. Keep samples, ALP Enzyme and pNPP solution on ice during the assay.*

### Sample Preparation

Inhibitors of ALP, such as EDTA, oxalate, fluoride, and citrate should be avoided in sample preparation. Serum and plasma shoule be diluted 10 times; cell culture media can be measured directly. To measure intracellular ALP, washed cells ($1\times10^5$) can be homogenized in the Assay Buffer, centrifuge to remove insoluble material at 13,000g for 3 minutes. Add different volume of samples into 96-well plate; bring the total volume to 80 µl with Assay Buffer.

Colored samples may interfere with O.D. 405 nm readings, so use a sample background control. Add the same amount of sample into separate wells, bring volume to 80 µl. Add 20 µl stop solution and mix well to terminate ALP activity in the sample.

### Assay Procedure

1. Add 50 µl of the 5 mM pNPP solution to each well containing the test samples and background controls. Mix well. Incubate the reaction for 60 min at 25°C, protect from light.
2. Standard Curve:
   Dilute 40 µl of the 5 mM pNPP solution with 160 µl Assay Buffer to generate 1 mM pNPP standard. Add 0, 4, 8, 12, 16, 20 µl into 96-well plate in duplicate to generate 0, 4, 8, 12, 16, 20 nmol/well pNPP standard. Bring the final volume to 120 µl with Assay Buffer. Add 10 µl of ALP enzyme solution to each well containing the pNPP standard. Mix well. The ALP enzyme will convert pNPP substrate to an equal amount of colored p-Nitrophenol (pNP). Incubate the reaction for 60 min at 25°C, protect from light.
3. Stop all reactions by adding 20 µl Stop Solution into each standard and sample reaction except the sample background control reaction (since 20 µl Stop Solution has been added to the background control when prepared in step 1), gently shake the plate. Measure O.D. at 405 nm in a micro plate reader.
4. Calculation: Correct background by subtracting the value derived from the 0 standards from all standards, samples and sample background control (The background reading can be significant and must be

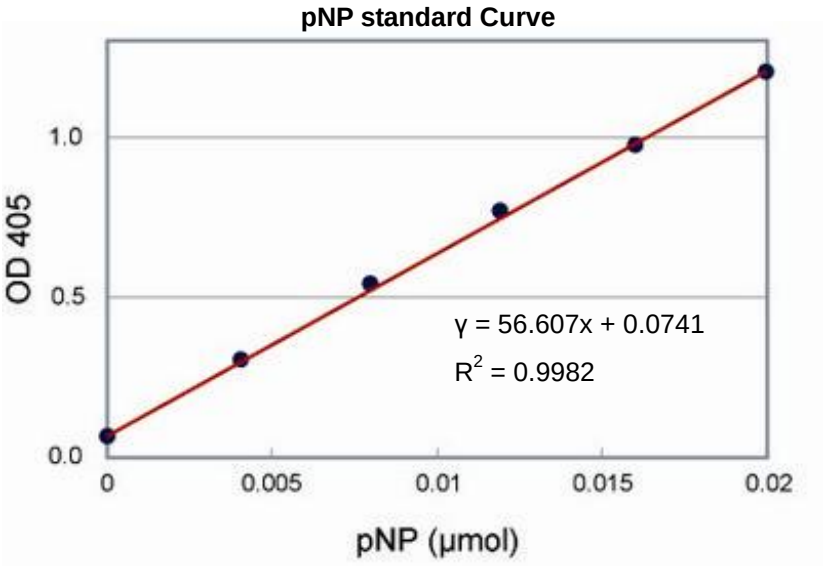
subtracted from sample readings). Plot pNP Standard Curve. Apply sample readings to the standard curve to get the amount of pNP generated by ALP sample. ALP activity of the test samples can then be calculated:

**ALP activity(U/ml) = A/V/T**

Where A is amount of pNP generated by samples (in μmol).

V is volume of sample added in the assay well (in ml).

T is reaction time (in minutes)




- **Unit Definition**

  All the Units mentioned in this protocol are Glycine Units.

  **Glycine Units:** The amount of enzyme causing the hydrolysis of one micromole of pNPP per minute at pH 9.6 and 25°C (glycine buffer).

  **DEA Units:** The amount of enzyme causing the hydrolysis of one micromole of pNPP per minute at pH 9.8 and 37°C (diethanolamine buffer).

  **Unit Conversion:** One Glycine unit as described above is equivalent to approximately three DEA units. This reaction system is in Glycine buffer.