

Alkaline Phosphatase Assay Kit

(Catalog #K412-500; 500 Reactions; Store kit at -20°C)

I. Introduction:

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. BioVision's 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 (*p*NPP) as a phosphatase substrate which turns yellow (λ_{\max} = 405 nm) when dephosphorylated by ALP. The Kit can detect 10-250 μ U ALP in samples.

II. Kit Contents:

Components	K412-500	Cap Code	Part No.
ALP Assay Buffer	100 ml	NM	K412-500-1
<i>p</i> NPP (10 TAB)	1 vial	Red	K412-500-2
ALP Enzyme	1 vial	Green	K412-500-3
Stop Solution	10 ml	WM	K412-500-4

III. Storage and Handling:

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.

IV. Reagent Reconstitution and General Consideration:

***p*NPP Solution:** Dissolve 2 tablets *p*NPP into 5.4 ml Assay Buffer. Two tablets are sufficient for 100 assays. **NEVER TOUCH THE TABLETS WITH BARE HANDS.** The *p*NPP 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 *p*NPP solution on ice during the assay.

V. Alkaline Phosphatase Assay Protocol:

1. Sample Preparations:

Inhibitors of ALP, such as EDTA, oxalate, fluoride, and citrate should be avoided in sample preparation. Serum and plasma should be diluted 10 times; cell culture media can be measured directly. To measure intracellular ALP, washed cells (1×10^5) 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 μ l 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 μ l. Add 20 μ l stop solution and mix well to terminate ALP activity in the sample.

2. Add 50 μ l of *p*NPP 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.

3. Standard Curve:

Dilute 40 μ l of the 5 mM *p*NPP solution with 160 μ l Assay Buffer to generate 1 mM *p*NPP standard. Add 0, 4, 8, 12, 16, 20 μ l into 96-well plate in duplicate to generate 0, 4, 8, 12, 16, 20 nmol/well *p*NPP standard. Bring the final volume to 120 μ l with Assay Buffer.

Add 10 μ l of ALP enzyme solution to each well containing the *p*NPP standard. Mix well. The ALP enzyme will convert *p*NPP substrate to an equal amount of colored *p*-Nitrophenol (*p*NP). Incubate the reaction for 60 min at 25°C, protect from light.

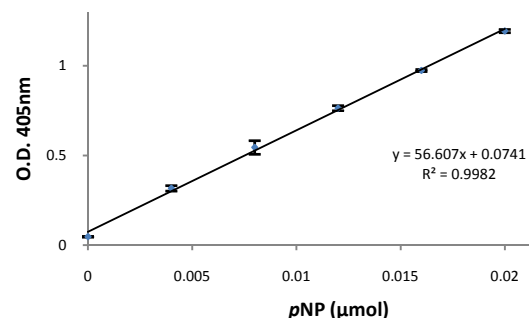
4. Stop all reactions by adding 20 μ l Stop Solution into each standard and sample reaction except the sample background control reaction (since 20 μ l Stop Solution has been added to the background control when prepared in step 1), gently shake the plate. Measure O.D. at 405 nm in a micro plate reader.

5. **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 *p*NP Standard Curve. Apply sample readings to the standard curve to get the amount of *p*NP generated by ALP sample. ALP activity of the test samples can then be calculated:

$$\text{ALP activity(U/ml)} = AV/T$$

Where A is amount of *p*NP generated by samples (in μ mol).
V is volume of sample added in the assay well (in ml).
T is reaction time (in minutes)

***p*NP Standard Curve**



VI. 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 *p*NPP per minute at pH 9.6 and 25°C (glycine buffer).

DEA Units: The amount of enzyme causing the hydrolysis of one micromole of *p*NPP 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.

VII. Related Products:

Acid Phosphatase Assay Kit
Phosphate Fluorescence Assay Kit
NAD/NADH Quantification Kit
Pyruvate Assay Kit
Glutamate Assay Kit
Glucose Assay Kit
Uric Acid Assay Kit
Ascorbic Acid Assay Kit
HDL and LDL/VLDL Assay Kit

ADP/ATP Ratio Assay Kit
Phosphate Colorimetric Assay Kit
NADP/NADPH Quantitation Kit
Lactate Assay Kits
Glycogen Assay Kit
Fatty Acid Assay Kit
Sarcosine Assay Kit
Cholesterol Assay Kits