ALP / Alkaline Phosphatase Assay Kit

ALP / Alkaline Phosphatase Assay Kit is a detection kit for the quantification of ALP / Alkaline Phosphatase activity in serum and plasma.

Catalog number: ARG81296

Package: 250 tests

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INTRODUCTION

Alkaline phosphatase (ALP, ALKP, ALPase, Alk Phos) (EC 3.1.3.1), or basic phosphatase, is a homodimeric protein enzyme of 86 kilodaltons. Each monomer contains five cysteine residues, two zinc atoms and one magnesium atom crucial to its catalytic function, and it is optimally active at alkaline pH environments.

ALP has the physiological role of dephosphorylating compounds. The enzyme is found across a multitude of organisms, prokaryotes and eukaryotes alike, with the same general function but in different structural forms suitable to the environment they function in. Alkaline phosphatase is found in the periplasmic space of E. coli bacteria. This enzyme is heat stable and has its maximum activity at high pH. In humans, it is found in many forms depending on its origin within the body – it plays an integral role in metabolism within the liver and development within the skeleton. Due to its widespread prevalence in these areas, its concentration in the bloodstream is used by diagnosticians as a biomarker in helping determine diagnoses such as hepatitis or osteomalacia. [Provide by Wikipedia: Alkaline phosphatase]

PRINCIPLE OF THE ASSAY

This ALP / Alkaline Phosphatase Assay Kit is a simple colorimetric assay that measures the amount of ALP activity in biological samples (E.g., serum, plasma and other sources). The improved method utilizes p-nitrophenyl phosphate that is hydrolyzed by ALP into a yellow-colored product (maximal absorbance at O.D. 405 nm). The rate of the reaction is directly proportional to the enzyme activity.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped with blue ice. Store pNPP Liquid at -20°C and all other components at 4°C upon receiving. Shelf life: 12 months after receipt.

Component	Quantity	Storage information
Assay Buffer, pH 10.5	50 mL	4°C
Mg Acetate, 0.2 M	1.5 mL	4°C
pNPP Liquid, 1 M	600 µl	-20°C
Standard (Tartrazine)	10 mL	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 405 nm
- Clear flat-bottom 96 well microplate
- Centrifuge and centrifuge tube
- 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.
- EDTA, oxalate, fluoride and citrate are known inhibitors of ALP and should be avoided in sample preparation.
- 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.
- This assay is based on a kinetic reaction. Use of a multi-channel pipettor is recommended. Addition of Working Reagent to samples should be quick and mixing should be brief but thorough. Assays can be executed at room temperature or 37°C.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C. Collection the supernatant for assay. Aliquot & store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma: Collect blood with heparin and centrifuge at 2000 x g for 10 minutes at 4°C. Collection the supernatant for assay. aliquot & store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Cell culture supernatant: Remove particulates by centrifugation for 10 min at 1500 x g at 4°C and aliquot & store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Cell lysate: cell samples ($1 x10^4$) can be washed with PBS and lysed in 0.5 mL of 0.2% Triton X-100 in distilled water by shaking for 20 minutes at room temperature. Centrifugation for 10 min at 14000 x g at 4°C and aliquot & store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note:

- EDTA, oxalate, fluoride and citrate are known inhibitors of ALP and should be avoided in sample preparation.
- Do not use haemolytic serum or plasma sample.
- ALP is stable for 48 hours at 4°C and 2 months at-20°C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **Working Reagent:** Equilibrate reagents to room temperature before using. For each well, mixing **200 µl** of **Assay Buffer**, **5 µl** of **Mg Acetate** stock and **2 µl** of **pNPP Liquid**. The Working Reagent is stable for at least one day at room temperature, however, fresh preparation working Reagent is recommended.

ASSAY PROCEDURE

All components should be equilibrated to room temperature and briefly mix and centrifuge reagent tubes before use. Standards and samples should be assayed in at least duplicates.

1. Add **200 µl** of **distilled water** and **200 µl** of **Standard** into separate wells of the clear flat bottomed 96-well microplate as background control and standard wells.
2. Add **5-50 µl** of **each sample** into the corresponding wells.
3. Add **195-150 µl** of **Sample Working Reagent** into each sample well. The *final reaction volume in the sample wells should be **200 µl***. Tap the plate to mix it well immediately.
4. **Read O.D.** with a microplate reader at **405 nm** immediately **(time zero, ODs_0)**. Incubate the plate at room temperature. And **read O.D. again at 4 min (time 4 min, ODs_4).**

Summary:

	H_2O well	Standard well	Sample well
Standard	-	200 µl	-
Distilled water	200 µl	-	-
Each Sample	-	-	5-50 µl
Working Reagent	-	-	195-150 µl
The sample well final reaction volume should be **200 µl**. Tap plate to mix briefly.			
Read the absorbance at **O.D. 405 nm** at **0** and **4 minutes**.			

CALCULATION OF RESULTS

1. ALP activity of the sample (U/L = µmol/(L·min)) is calculated as follow:

   ALP Activity (U/L)

   = [(OD_{S4} – OD_{S0}) x 1000 x Reaction Vol] / (t x ε x *l* x Sample Vol)

   =35.3 X [(OD_{S4} – OD_{S0}) x Reaction Vol] / [($OD_{Standard}$ – OD_{H2O}) x Sample Vol x t]

   Note:

   OD_{S4} and OD_{S0}: the O.D. 405 nm value of sample at 4 and 0 minute.

   $OD_{Standard}$, $OD_{H2O:}$ the O.D. 405 nm value of standard or Distilled water at 4 minutes.

   The factor 1000 converts mmol/L to µmol/L.

   t: the incubation time (min).

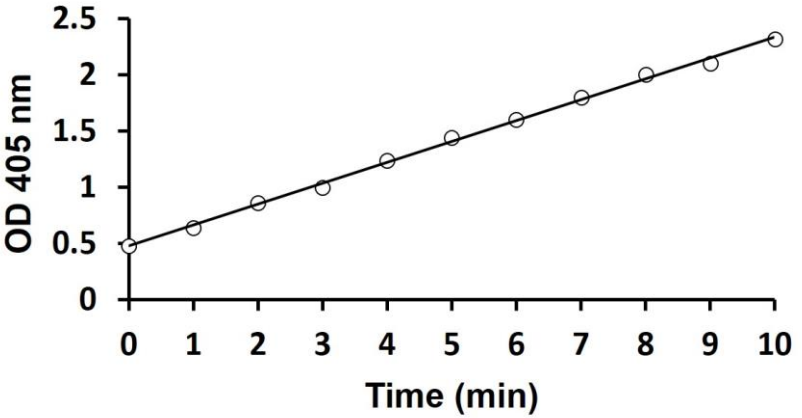
   ε: for *p*-nitrophenol, =18.75 mM^{-1} x cm^{-1}

   l: the light path, = ($OD_{Standard}$ – OD_{H2O}) / (ε x c)

2. If sample ALP activity exceeds 800 U/L, dilute samples in saline and repeat the assay, multiply the result by the dilution factor.
3. Incubation time can be prolonged for samples with low ALP activity.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the ALP / Alkaline Phosphatase Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

2 U/L