

Alkaline Phosphatase (AP) Assay Kit (Colorimetric)

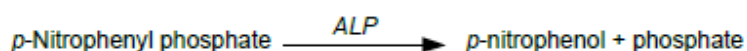
LS-K307-250 (250 Tests) • See Storage Conditions Below



Introduction

Alkaline phosphatase (ALP) catalyzes the hydrolysis of phosphate esters in an alkaline environment, resulting in the formation of an organic radical and inorganic phosphate. In mammals, this enzyme is found mainly in the liver and bones. Marked increase in serum ALP levels, a disease known as hyperalkalinephosphatasemia, has been associated with malignant biliary obstruction, primary biliary cirrhosis, primary sclerosing cholangitis, hepatic lymphoma and sarcoidosis.

Simple, direct and automation-ready procedures for measuring ALP activity in serum are becoming popular in Research and Drug Discovery. This Alkaline Phosphatase Assay Kit is designed to measure ALP activity directly in biological samples without pretreatment. The improved Method utilizes p-nitrophenyl phosphate that is hydrolyzed by ALP into a yellow colored product (maximal absorbance at 405nm). The rate of the reaction is directly proportional to the enzyme activity.



Key Features

- High sensitivity and wide linear range. Use 5 μL serum or plasma sample. The detection limit is 2 U/L, linear up to 800 U/L.
- Homogeneous and simple procedure. Simple "mix-and-measure" procedure allows reliable quantitation of ALP activity within 5 minutes.
- Robust and amenable to HTS. All reagents are compatible with high-throughput liquid handling instruments.

Applications

- Direct Assays: ALP activity in serum, plasma and other sources.
- Characterization and Quality Control for ALP production.
- Drug Discovery: high -throughput screen for ALP inhibitors and evaluation of ALP inhibitors.

Components

| Component | K307-250 |
|-------------------------|-------------------|
| | 250 Tests |
| Assay Buffer (pH 10.5) | 50 mL |
| Mg Acetate (0.2 M) | 1.5 mL |
| pNPP Liquid (1 M) | 600 μL |
| Calibrator (Tartrazine) | 10 mL |

Materials Not Supplied

Pipetting devices and accessories (e.g. multi-channel pipettor).

Procedure using 96-well plate: Clear bottom 96-well plates (e.g. Corning Costar) and plate reader.

Procedure using cuvette: Spectrophotometer and cuvettes for measuring OD 405nm.

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Storage

The kit is shipped at room temperature. Store pNPP Liquid at -20°C and all other components at 4°C. Shelf life of 12 months after receipt.

Assay Procedure

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.

Reagent preparation: equilibrate reagents to room temperature. The Working Solution is prepared by mixing for each 96-well assay, 200 µL Assay Buffer, 5 µL Mg Acetate (final 5 mM) and 2 µL pNPP liquid substrate (10 mM). Fresh reconstitution is recommended, although the Working Solution is stable for at least one day at room temperature.

Sample preparation: ALP is stable for 48 hours at 4°C and 2 months at -20°C. EDTA, oxalate, fluoride, citrate are known inhibitors of ALP and should be avoided in sample preparation. Serum, plasma (no EDTA/citrate, ideally unhemolyzed) and cell culture media can be assayed directly. To measure intracellular ALP, cell lysate can be prepared as follows: 104 cells are washed with PBS and lysed in 0.5 mL 0.2% Triton X-100 in distilled water by shaking for 20 min at room temperature.

Procedure using 96-well plate

1. Transfer 200 µL distilled water (H₂O) and 200 µL Calibrator into separate wells of a clear bottom 96-well plate.
2. Carefully transfer 5 to 50 µL samples into other wells.
3. Pipet 150 to 195 µL Working Solution to sample wells. The final reaction volume in the sample wells should be 200 µL. Tap plate briefly to mix.
4. Read OD_{405nm} (t = 0), and again after 4 min (t = 4 min) on a plate reader.

Calculation

ALP activity of the sample (IU/L = µmol/(L·min)) is

$$\begin{aligned} &= \frac{(OD_{\text{SAMPLE } t} - OD_{\text{SAMPLE } 0}) \cdot 1000 \cdot \text{Reaction Vol}}{t \cdot \epsilon \cdot l \cdot \text{Sample Vol}} \\ &= \frac{(OD_{\text{SAMPLE } t} - OD_{\text{SAMPLE } 0}) \cdot \text{Reaction Vol}}{(OD_{\text{CALIBRATOR}} - OD_{\text{H}_2\text{O}}) \cdot \text{Sample Vol} \cdot t} \times 35.3 \end{aligned}$$

OD_{SAMPLE t} and OD_{SAMPLE 0} are OD_{405nm} values of sample at time t (e.g. 4) and 0 min. The factor 1000 converts mmol/L to µmol/L. t is the incubation time (min). For p-nitrophenol, ε = 18.75 mM⁻¹·cm⁻¹. l (light path, cm) is 1 cm for cuvette, and calculated for 96-well assay from the Calibrator, l = (OD_{CALIBRATOR} - OD_{H2O})/(ε · c).

Procedure using Cuvette

1. Transfer 50 µL samples into 1-cm cuvettes.
2. Pipet 950 µL Working Solution to samples. Mix briefly.
3. Read OD_{405nm} shortly after the mixing, and again after 4 min.

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Calculation

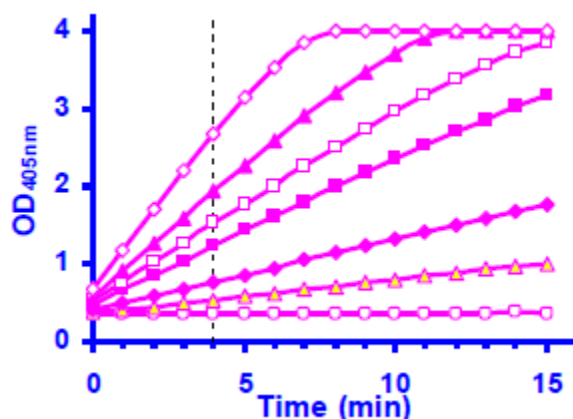
ALP activity of the sample (IU/L) is

$$\begin{aligned} &= \frac{(OD_{\text{SAMPLE } t} - OD_{\text{SAMPLE } 0}) \cdot 1000 \cdot \text{Reaction Vol}}{t \cdot \epsilon \cdot l \cdot \text{Sample Vol}} \\ &= 266.7 \times (OD_{\text{SAMPLE } t} - OD_{\text{SAMPLE } 0}) \end{aligned}$$

Note: (1) if sample ALP activity exceeds 800 IU/L, dilute samples in saline and repeat the assay, multiply the result by the dilution factor. (2) incubation can be prolonged for samples with low ALP activity.

Sample Data

Samples were assayed in duplicate (n = 2) using the 96-well plate protocol. The ALP activity (U/L) was 13.4 ± 0.4 for a human serum, 190.4 ± 1.6 for rat serum and 202.8 ± 4.3 for goat serum.



Kinetics of ALP reaction in 96-well plate assay with increasing ALP concentration

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