

User's Manual and Instructions

Alkaline Phosphatase Assay Kit (Z5030033)

Colorimetric Kinetic Determination of Serum Alkaline Phosphatase Activity

DESCRIPTION

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. Biochain's 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 IU/L, linear up to 800 IU/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.

KIT CONTENTS (250 tests in 96-well plates)

Assay Buffer: 50 mL, pH 10.5 Mg Acetate: 1.5 mL 0.2 M

pNPP Liquid: 600 μL 1 M Calibrator: 10 mL Tartrazine

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

Precautions: 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.

PROCEDURES. 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: 10^4 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 (H_2O) and 200 μL Calibrator into 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 $\text{OD}_{405\text{nm}}$ ($t=0$), and again after 4 min ($t=4$ min) on a plate reader.

5. Calculation: ALP activity of the sample ($\text{IU/L} = \mu\text{mol}/(\text{L}\cdot\text{min})$) is

$$= \frac{(\text{OD}_{\text{SAMPLE } t} - \text{OD}_{\text{SAMPLE } 0}) \cdot 1000 \cdot \text{Reaction Vol}}{t \cdot \epsilon \cdot l \cdot \text{Sample Vol}}$$

$$= \frac{(\text{OD}_{\text{SAMPLE } t} - \text{OD}_{\text{SAMPLE } 0}) \cdot \text{Reaction Vol}}{(\text{OD}_{\text{CALIBRATOR}} - \text{OD}_{\text{H}_2\text{O}}) \cdot \text{Sample Vol} \cdot t} \times 35.3$$

$\text{OD}_{\text{SAMPLE } t}$ and $\text{OD}_{\text{SAMPLE } 0}$ are $\text{OD}_{405\text{nm}}$ values of sample at time t (e.g. 4) and 0 min. The factor 1000 converts mmol/L to $\mu\text{mol/L}$. t is the incubation time (min). For *p*-nitrophenol, $\epsilon = 18.75 \text{ mM}^{-1} \cdot \text{cm}^{-1}$. l (light path, cm) is 1 cm for cuvette, and calculated for 96-well assay from the Calibrator, $l = (\text{OD}_{\text{CALIBRATOR}} - \text{OD}_{\text{H}_2\text{O}}) / (\epsilon \cdot c)$.

Procedure using Cuvette:

1. Transfer 50 μL samples into 1-cm cuvettes.
2. Pipet 950 μL Working Solution to samples. Mix briefly.
3. Read $\text{OD}_{405\text{nm}}$ shortly after the mixing, and again after 4 min.
4. Calculation: ALP activity of the sample (IU/L) is

$$= \frac{(\text{OD}_{\text{SAMPLE } t} - \text{OD}_{\text{SAMPLE } 0}) \cdot 1000 \cdot \text{Reaction Vol}}{t \cdot \epsilon \cdot l \cdot \text{Sample Vol}}$$

$$= 266.7 \times (\text{OD}_{\text{SAMPLE } t} - \text{OD}_{\text{SAMPLE } 0})$$

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.

MATERIALS REQUIRED, BUT NOT PROVIDED

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

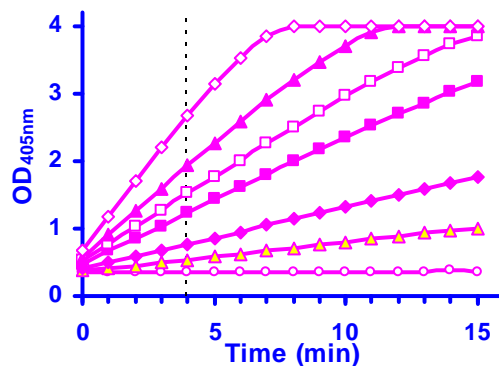
Procedure using 96-well plate:

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

Procedure using cuvette:

Spectrophotometer and cuvetts for measuring $\text{OD}_{405\text{nm}}$.

EXAMPLES. Samples were assayed in duplicate ($n = 2$) using the 96-well plate protocol. The ALP activity (IU/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

LITERATURE

1. Kim, H.J. et al (2006) Glucocorticoids suppress bone formation via the osteoclast. *J. Clin. Invest.* 116:2152–2160.
2. Bhattacharya, A. et al (2006). Effect of fish oil on bone mineral density in aging C57BL/6 female mice. *J. Nutr. Biochem* 18(6):372-379.
3. Wan, Y., Chong, L-W., & Evans, R.M. (2007). PPAR- γ regulates osteoclastogenesis in mice. *Nature Med.* 13(12): 1496-1503.