Technical Bulletin

Alkaline Phosphatase Assay Kit

Catalog Number MAK447

Product 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-suitable procedures for measuring ALP activity in serum are valuable in Research and Drug Discovery. The 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 405 nm). The rate of the reaction measured spectrophotometrically is directly proportional to the enzyme activity. The assay method has a lower detection limit of 2 units/liter (U/L) and has a linear response up to 800 U/L.

The kit is suitable for the quantitative determination of alkaline phosphatase (ALP) activity in serum, plasma etc.

Components

The kit is sufficient for 250 colorimetric assays in 96-well plates.

- Assay Buffer (pH 10.5) 50 mL
  Catalog Number MAK447A
- Mg Acetate (0.2 M) 1.5 mL
  Catalog Number MAK447B
- pNPP Liquid (1 M) 600 µL
  Catalog Number MAK447C
- Calibrator (Tartrazine) 10 mL
  Catalog Number MAK447D

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- 1.5 mL microcentrifuge tubes
- Phosphate Buffered Saline (PBS) (Catalog Number P3813 or equivalent)
- Triton™ X-100 (Catalog Number X100 or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate reagents to room temperature prior to use.

Procedure

All samples and standards should be run in duplicate.

Assays can be executed at room temperature or 37 °C.

Sample Preparation

ALP is stable for 48 hours at 4 °C and 2 months at -20 °C.

EDTA, oxalate, fluoride, and citrate are known inhibitors of ALP and should be avoided in sample preparation.

Serum, Plasma, or Cell Culture Media

Serum, plasma (no EDTA/citrate, ideally unhemolyzed) and cell culture media can be assayed directly.

Cell Lysate for intracellular ALP

1. Wash 10^4 cells with PBS.
2. Lyse the washed cells in 0.5 mL 0.2% Triton X-100 in purified water by shaking for 20 minutes at room temperature.

All Samples

Transfer 5 to 50 µL of Sample into appropriate wells of a clear flat-bottom 96-well plate.

Working Reagent

Mix enough reagents for the number of assays to be performed. For each well, prepare 207 µL of Working Reagent according to Table 1. Fresh preparation is recommended, although the Working Reagent is stable for at least one day at room temperature.

Table 1.
Preparation of Working Reagent

Reagent	**Working Reagent**
Assay Buffer	200 µL
Mg Acetate (0.2 M)	5 µL
pNPP Liquid (1 M)	2 µL

Assay Reaction

Note: 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.

1. Transfer 200 µL of purified water (Blank well) and 200 µL of Calibrator into separate wells of the 96-well plate.
2. Pipet 150 to 195 µL Working Reagent to Sample wells. The final reaction volume in the Sample wells should be 200 µL.
3. Tap plate briefly to mix.

Measurement

Read optical density (OD) at 405 nm immediately (T = 0 minutes), and again after 4 minutes (T = 4 minutes) on a plate reader.

Results

Calculate the Alkaline Phosphatase (ALP) activity of the sample:

$$\text{ALP (IU/L or } \mu\text{mol/(L·min))} = \frac{(OD_{T4} - OD_{T0}) \times 1000 \times RxnVol}{\varepsilon_{PNP} \times L \times SmplVol \times T}$$

$$= \frac{(OD_{T4} - OD_{T0}) \times 1000 \times RxnVol}{18.75 \times 1.51 \times (OD_{Cal} - OD_{Blank}) \times SmplVol \times T}$$

$$= \frac{(OD_{T4} - OD_{T0}) \times RxnVol \times 35.3}{(OD_{Cal} - OD_{Blank}) \times SmplVol \times T}$$

where

OD_{T4} = OD value at 405 nm of Sample at 4 minutes

OD_{T0} = OD value at 405 nm of Sample at 0 minutes

OD_{Cal} = OD value at 405 nm of Calibrator

OD_{Blank} = OD value at 405 nm of Blank

RxnVol = Final reaction volume (200 µL)

T = Reaction time (standard procedure is for 4 minutes. Reaction time may be extended in the case of low ALP activity)

SmplVol = The amount of Sample (µL) used in the reaction

1000 = Conversion from mmol/L to µmol/L.

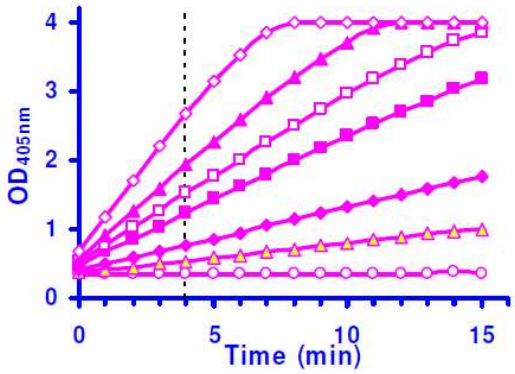
ε_{PNP} = Absorptivity coefficient for p-Nitrophenol (18.75 $mM^{-1} \times cm^{-1}$)

L = Light path in cm. The light path for the 96-well assay is calculated from the corrected Calibrator optical density:

$L = (OD_{Cal} - OD_{Blank})/(\varepsilon \cdot c)$
$= (OD_{Cal} - OD_{Blank})/(16.2 \times 0.0408)$
$= 1.51 \times (OD_{Cal} - OD_{Blank})$

Note: if sample ALP activity exceeds 800 U/L, dilute samples in PBS and repeat the assay, multiplying the result by the dilution factor. Incubation time can be prolonged beyond 4 minutes for samples with low ALP activity.

Figure 1.
Kinetics of ALP reaction in 96-well plate assay with increasing ALP concentration. Samples were assayed in duplicate (n = 2) using the assay 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.



References

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