Technical Bulletin

Alkaline Phosphatase (ALP) Assay Kit

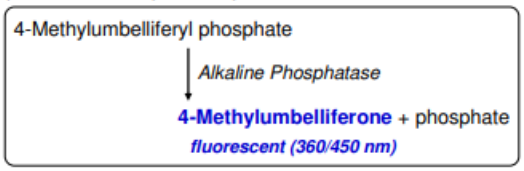
Catalogue number MAK530

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.

Simple, direct, and automation-ready procedures for measuring ALP activity in serum are becoming popular in research and drug discovery. This improved method utilizes 4-methylumbelliferyl phosphate that is hydrolyzed by ALP into a highly fluorescent product 4-methylumbelliferone. The rate of the fluorescence increase is directly proportional to the enzyme activity.

The detection limit of the kit is 0.02 U/L(20 minutes. reaction). The kit is used to detect ALP activity in serum, plasma, and other sources.



Components

The kit is sufficient for 100 fluorometric assays in 96-well plates.

- Reagent (pH 10.5) 14 mL
  Catalogue Number MAK530A
- 100x Standard 120 µL
  Catalogue Number MAK530B

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (for example., multichannel pipettor)
- Fluorescent multiwell plate reader.
- Black flat-bottom 96-well or 384-well plates. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes

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 at room temperature. Store components at -20 °C.

Preparation Instructions

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

Procedure

All Samples and Standards should be run in duplicate.

Note: For low ALP activity Samples (< 1 U/L), it is recommended to prolong the incubation time to for example 60 minutes.

This procedure is written for 96-well plates.

Sample Preparation

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

Note: 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.

Standard Preparation

1. Mix 5 µL of the provided 100x Standard (2 mM 4-methylumbelliferone) with 495 µL purified water to obtain 1x Standard (20 µM 4-methylumbelliferone).
2. Prepare Standards as shown in Table 1.

Table 1.

Preparation of Standards

Well No.	1X Standard (µL)	Purified Water	4-Methylumbelliferone (µM)
1	100	0	20
2	60	40	12
3	30	70	6
4	0	100	0

3. Transfer 10 µL of each Standard and each Sample to separate wells of the plate.

Measurement

1. Add 90 µL Reagent to all Standard and Sample wells.
2. Tap plate to mix and incubate for a desired period (20 minutes) at desired temperature (25 °C).
3. Read fluorescence intensity (λ_{exc}= 360 nm, λ_{em} = 450 nm) on a plate reader.

Results

1. Plot the RFU measured at 20 minutes for each Standard against the 4-methylumbelliferone concentration. Determine the slope using linear regression fitting.
2. ALP activity of the sample is

$$\text{ALP Activity (U/L)} = \frac{F_{Sample}-F_{blank}}{\text{Slope} \times t} \times n$$

Where:

F_{SAMPLE} = Fluorescence intensity values of the Sample

F_{BLANK} = Fluorescence intensity values of the blank

t = Reaction time (20 minutes)

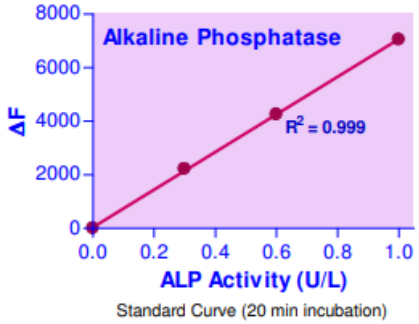
n = Dilution factor

Note: If the calculated value is higher than 1 U/L, use a shorter incubation time or dilute Sample in water and repeat assay. Multiply the result by the dilution factor n.

Unit definition: 1 unit (U) of ALP catalyzes the conversion of 1 µmole of 4-methylumbelliferyl phosphate to 4-methylumbelliferone per minute at pH 10.5 and room temperature (25 °C)

Figure 1.

Typical Standard Curve for Alkaline Phosphatase activity



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