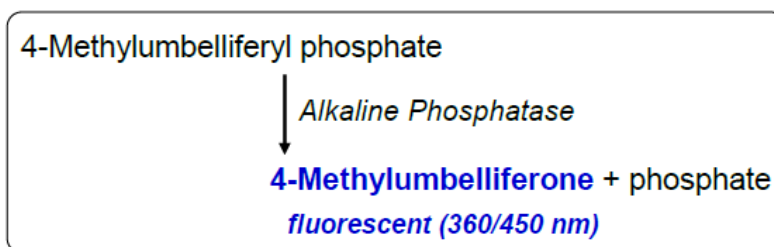


Alkaline Phosphatase (ALP) Assay Kit (Fluorometric)

LS-K301-100 (100 Tests) • Store at -20°C

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 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.



Key Features

- High sensitivity and wide linear range. Use 10 µL sample. Detection limit of 0.02 U/L (20 min reaction).
- Homogeneous and simple procedure. Simple "mix-and-measure" procedure allows reliable quantitation of ALP activity within 20 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 modulators.

Components

Component	K301-100
	100 Tests
Reagent (pH 10.5)	14 mL
100x Standard	120 µL

Materials Not Supplied

Pipetting devices and accessories (e.g. multi-channel pipettor). Black flat-bottom 96-well plates (e.g. Corning Costar) and plate reader.

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Storage

The kit is shipped at room temperature. Store at -20°C. Shelf life of 2 years after receipt.

Assay Procedure

Thaw reagents to room temperature prior to use. This assay is based on a kinetic reaction. Use of a multi-channel pipettor is recommended. Addition of Reagent to samples should be quick and mixing should be brief but thorough. 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, 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.

Procedure Using 96-Well Plate

- Standards. First mix 5 µL of the provided 100x Standard with 495 µL distilled water to obtain 1x Standard. Transfer 0, 3, 6 and 10 µL provided standard into separate wells of a black 96-well plate and add 10, 7, 4 and 0 µL distilled water to each standard well respectively to bring each volume up to 10 µL.
Samples. Transfer 10 µL of each Sample to separate wells of the plate.
- Using a multi-channel pipettor, add 90 µL Reagent to all Standard and Sample wells. Quickly tap plate to mix and incubate for a desired period of time (e.g. 20 min) at desired temperature (e.g. 25°C).
- Read fluorescence intensity ($\lambda_{exc} = 360 \text{ nm}$, $\lambda_{em} = 450 \text{ nm}$) on a plate reader.

Calculations

Plot the RFU measured at 20 min for each Standard against the ALP activity. Determine the slope using linear regression fitting. ALP activity of the sample is

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

where F_{SAMPLE} and F_{BLANK} are the fluorescence intensity values of the Sample and the Blank (i.e. no Standard well) respectively. t is the reaction time (e.g. 20 min). n is the dilution factor. If the calculated value is higher than 10 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).

General Considerations

- For low ALP activity samples (< 1 U/L), it is recommended to prolong the incubation time to for example 60 min.
- The reaction volumes can be scaled down for 384-well assay or up for cuvette assays.

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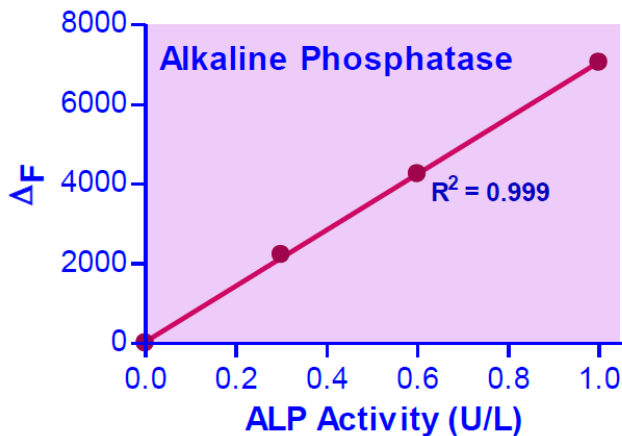
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Sample Data



Standard Curve (20 min incubation)

Version: V.08.09.2018

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