Genorise

For research use only

Alkaline Phosphatase Activity Fluorometric Assay Kit

(Catalog #107016; 200 assays; store at -20°C)

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. The change in alkaline phosphatase level and activity is associated with a lot of diseases in the liver and bones. Alkaline phosphatase is also a popular enzyme conjugated to secondary antibody in ELISA.

Alkaline Phosphatase Assay Kit is designed to measure ALP activity directly in biological samples without pretreatment. ALP cleaves the phosphate group of the non-fluorescent 20minutes phosphate disodium salt (MUP) substrate resulting in an intense fluorescent signal (Ex/Em = 360nm/440nm). The kit is an ultra sensitive, simple, direct and HTS-ready assay designed to measure ALP activity in serum and bio-samples, more sensitive than colorimetric assays.

APPLICATIONS

Direct Assays: ALP activity in serum, plasma, urine, cell lysate and other bio-samples.
Drug Discovery: high-throughput screen for ALP inhibitors.
ELISA: detecting ALP-conjugated Abs levels.

KEY FEATURES

Sensitive and accurate Use 5 µL samples. Detection ranges 0.02-5 U/L in 96-well plate assay.
Simple and high-throughput Simple procedure; takes less than 30 minutes. Kit is designed to be a robust method.

KIT CONTENTS

Assay Buffer 25 mL	Reagent 20 mL
ALP Enzyme 100 µL 50 U/L	Stop Solution 25 mL

STORAGE AND HANDLING

Store kit at -20°C. Shelf life of six months. Protect from light. Allow Reagent to warm to room temperature before use. Briefly centrifuge vials prior to opening.

ASSAY PROTOCOL

Ensure the Reagent is at room temperature before use. Keep samples and ALP enzyme on ice during the assay.

1. Sample Preparations:
Inhibitors of ALP, like tartrate, fluoride, EDTA, oxalate, and citrate, should be avoided in sample preparation. Serum, plasma, urine, semen, and cell culture media can be assayed directly. Cells (1×10^5) or tissue (~10 mg) can be homogenized in 100µL Assay Buffer, centrifuge to remove insoluble material at 15,000g for 2 minutes. Add 5µL test samples directly into 96-well black plate (in duplicate).

2. Standard Curve Preparations:
Dilute 10 µL of the ALP enzyme (50 U/L) with 90µL of Assay Buffer to generate 5 U/L ALP std. Continue 2 fold series dilute 5 U/L ALP with Assay Buffer to prepare 50 µL 0, 0.3125, 0.625, 1.25, 2.5 & 5 U/L ALP std. Transfer 5 µL series diluted ALP std into a 96-well plate.

3. Reaction: Add 95 µL of the Reagent to each well containing the ALP Standard and test samples. Tap plate lightly to mix. Incubate at room temperature for protect from light.

4. Add 100 µL Stop Solution to each well and mix well to terminate ALP activity. Measure fluorescence intensity at Ex/Em 360/440 nm using a fluorescence plate reader.

5. Calculation: Correct background by subtracting the value of the 0 U/L ALP std (blank) from all standard readings. Plot the value against standard concentration. Determine the slope using linear regression fitting.

$ALP = (RFU_{sample} - RFU_{blank})/Slope\ (U/L)$
Where: RFU_{SAMPLE} and RFU_{blank} are optical fluorescence values of the sample and buffer.
If unknown sample results over standard curve range, dilute sample in assay buffer. Repeat the assay; multiply the results by the dilution factor n.

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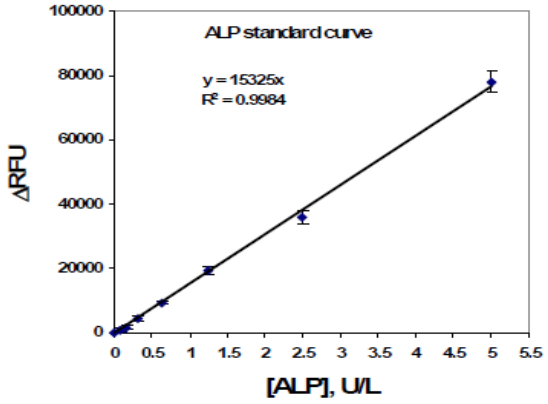

Standard Curve in 96-well plate.

RELATED PRODUCTS:

ALP Activity Colorimetric Assay Kit (Cat107015)
Cell Viability Assay Kits (Cat# 110001)
ATP Colorimetric/Fluorometric Assay Kit (Cat#107002)
ADP Colorimetric/Fluorometric Assay Kit (Cat#107004)
ADP/ATP Ratio Assay Kit (Cat#107003)