Alkaline Phosphatase Activity Colorimetric Assay Kit
Catalog# 107015

This kit is to detect alkaline phosphatase activity and is for 200 applications.
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, hepatic lymphoma and sarcoidosis. Simple, direct and automation-ready procedures for measuring ALP activity in serum are becoming popular in Research and Drug Discovery. Genorise Alkaline Phosphatase Activity Colorimetric Assay Kit 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.

$$\text{p-Nitrophenyl phosphate} \xrightarrow{\text{ALP}} \text{p-nitrophenol} + \text{phosphate}$$

Kit Contents for 200 Assays:

1.Assay buffer 25 mL

2.Substrate 500 µL

3.ALP Enzyme 100 µL 600 U/L

4.Stop Solution 25 mL

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.

Storage:

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

Material Needed But NOT Supplied with the Kit:

96 well tissue culture plates

Microplate reader

Protocol

Note : Warm all kit components to room temperature before starting the experiment.

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 100 µL Assay Buffer, centrifuge to remove insoluble material at 15,000g for 2 minutes. Add 10µL test samples directly into 96-well clear plate.

2. Standard Curve Preparations:

Dilute 20 µL of the ALP enzyme (600 U/L) with 20 µL of Assay Buffer to generate 300 U/L ALP std. Continue 2 fold series dilute 300 U/L ALP with Assay Buffer to prepare 20 µL 0, 4.6875, 9.375, 18.75, 75, 150 & 300 U/L ALP std. Transfer 10 µL series diluted ALP std into a 96-well plate.

3. Working solution: Prepare enough working solution by mix 90 µL Assay Buffer with of 2 µL substrate for each reaction. Transfer 90 µL working solution to each well containing the ALP Standard and test samples. Tap plate lightly to mix. Incubate at room temperature for 20 minutes (or longer if ALP activity in sample is low), protect from light.

4. Terminate Reaction: Add 100 µL Stop Solution to each well and mix well to terminate ALP activity. Measure O.D. at 405nm in a 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.

$ALP = (OD_{sample}-OD_{blank})/Slope\ (U/L)$

Where: OD_{sample} and OD_{blank} are optical absorbance 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.

RELATED PRODUCTS:

Cell Viability Kit(5,000 reactions) (catalog#107015-5)
Cell Viability Kit(10,000 reactions) (catalog#107015-10)
Alkaline Phosphatase Activity Fluorometric Assay Kit(catalog# 107016)
PBS-1x (catalog# 103004)