Alkaline Phosphatase Assay Kit

Catalog No.: abx294066

Size: 100 tests

Detection Range: 0.2 King unit/100 ml – 55.6 King unit/100 ml

Sensitivity: 0.2 King unit/100 ml

Storage: Store all liquid components at 4°C in the dark.

Application: For quantitative detection of alkaline phosphatase activity in serum, plasma, urine, tissue homogenates, cell lysates, cell culture supernatants, and other biological fluids.

Introduction

Alkaline phosphatases (ALPs) are a group of enzymes that catalyze the hydrolysis of phosphate esters at alkaline pH. In mammals, ALP is primarily found in the liver, kidney, and bone. High serum ALP concentrations are associated with primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), sarcoidosis, malignant biliary obstruction, and hepatic lymphoma.

Abbexa's Alkaline Phosphatase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Alkaline phosphatase activity. Alkaline phosphatase decomposes benzene disodium phosphate to produce phenol and phosphoric acid. Phenol reacts with 4-aminopyrline in an alkaline solution, it is then oxidized with potassium ferricyanide to form a red quinone biological pigment. The concentration of the reaction product can then be calculated indirectly by measuring the absorbance at 520 nm.

Kit components

1. Buffer Solution: 60 ml
2. Substrate: 60 ml
3. Chromogenic Reagent: 3 x 60 ml
4. Phenol Standard (0.5 mg/ml): 1.5 ml

Materials required but not provided

1. Microplate reader (520 nm)
2. Double-distilled water
3. Normal saline (0.9 % NaCl)
4. PBS (0.01 M, pH 7.4)
5. Pipette and pipette tips
6. 1.5 ml microcentrifuge tubes
7. Centrifuge
8. Vortex mixer
9. Incubator

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum** and **Plasma:** Serum and plasma samples can be tested directly. If not tested within 24 hours, serum/ plasma samples can be stored for up to 1 month at -80°C.
- **Tissue Homogenates:** Carefully weigh at least 20 mg of tissue, and wash in cold PBS (0.01 M, pH 7.4). Per 20 mg of tissue, add 180 µl of normal saline (0.9 % NaCl) or PBS (0.01 M, pH 7.4) and homogenize manually, using a dounce homogenizer at 4°C. Centrifuge at 10,000 × g for 10 minutes to remove insoluble material. Collect the supernatant, keep on ice and assay immediately.
- **Cell lysates/ Cell culture supernatants:** Harvest at least 1 × 10^6 cells and wash with PBS (0.01 M, pH 7.4). Per 1 × 10^6 cells, add 300 – 500 µl of normal saline (0.9 % NaCl) OR PBS (0.01 M, pH 7.4) and Homogenize with an ultrasonic cell disruptor at 4°C.

Note: To calculate Alkaline phosphatase activity in tissue homogenates and cell samples using the formulae in section **C. Calculation of Results.** The protein content of the supernatant should be determined separately **(abx097193)**.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9 % NaCl) or PBS (0.01 M, pH 7.4), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human serum	1
Human urine	1
Rat serum	1
Cell culture supernatant	1
10% Mouse kidney tissue homogenate	30 – 50
10% Mouse liver tissue homogenate	1
10% Mouse brain tissue homogenate	1
HePG2 cells	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

Allow all reagents to equilibrate to room temperature before use.

Standard solution: To prepare 0.1 mg/ml phenol standard solution, add 40 µl of double-distill water into a tube with 10 µl of 0.5 mg/ml Phenol Standard and mix thoroughly.

B. Assay Procedure

1. Control tube: Add 50 µl of double-distilled water to a 5 ml centrifuge tube.
2. Standard tube: Add 50 µl of 0.1 mg/ml prepared Standard solution to a 5 ml centrifuge tube.
3. Sample tube: Add 50 µl of sample to a 5 ml centrifuge tube.
4. Add 500 µl of Buffer Solution and 500 µl of Substrate to each tube and mix thoroughly with a vortex mixer.
5. Incubate for 15 minutes at 37°C.
6. Add 1500 µl of Chromogenic Reagent immediately and mix thoroughly.
7. Set the spectrophotometer to zero using double-distilled water.
8. Measure the OD of each tube at 520 nm with 0.5 cm optical path cuvette.

C. Calculation of Results

1. Serum (Plasma) samples:

$$\text{ALP activity (King unit/100 mL)} = \text{MF} \times \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{blank}}) \times \text{V}_1}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{blank}}) \times \text{V}}$$

2. Tissue samples:

Alkaline phosphatase activity in tissue samples can be calculated according to total protein concentration (which must be assayed separately).

$$\text{ALP activity (King unit/ g Protein)} = \text{MF} \times \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{blank}}) \times \text{C}_{\text{Protein}} \times \text{V}}$$

where:

OD_{Sample}	OD value of sample
$OD_{Standard}$	OD value of standard
OD_{Blank}	OD value of control
V	Volume of sample (0.05 ml)
V_1	Volume of sample per King unit (100 ml)
$C_{Protein}$	Concentration of protein in sample (g Protein / ml)
M	Phenol Standard content of standard tube, 0.005 mg
F	The dilution factor of sample

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.