Alkaline Phosphatase Detection Assay

Catalog Number: EZ2020

For Research Use Only. Not for use in Diagnostic Procedures.

1. Intended Use

For plate-based colorimetric enzymatic determination of alkaline phosphatase content in cell culture. The kit uses a spectrophotometric assay to detect alkaline phosphatase directly from samples. The unique features of the kit are:

- High sensitivity
- Rapid
- Robust
- High reproducibility
- Flexible format
- **Sensitivity:** Based on an instrument resolution of Abs. = 900 IU/L

2. Kit Contents

Component Name	Volume	Storage
Alkaline Phosphatase Substrate	5mL	Refrigerate
Alkaline Phosphatase Standard (Equivalent to 50IU/L of enzyme activity)	120 µL	Refrigerate
Alkaline Phosphatase Color Development Solution	24mL	Refrigerate
96 well plate	1 each	RT
Manual	1 each	RT

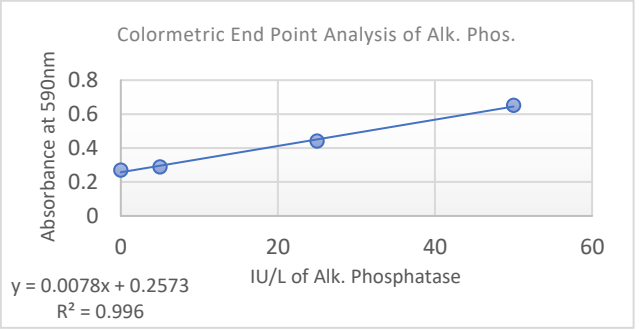
3. Introduction

For plate-based colorimetric enzymatic determination of alkaline phosphatase:

Distributed in almost every tissue of the body, serum alkaline phosphatase (ALP) levels are of interest in the testing for hepatobiliary disorder and bone disease. Most of the ALP in the normal adult serum is from the liver or biliary tract. Normal alkaline phosphatase levels are age-dependent and are elevated during periods of active bone growth. Moderate elevations of ALP (not involving the liver or bone) may be attributed to Hodgkin's disease, congestive heart failure, and abdominal bacterial infections.

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 hyper-alkalinephosphatasemia, has been associated with malignant biliary obstruction, primary biliary cirrhosis, primary sclerosing cholangitis, hepatic lymphoma, and sarcoidosis.

The kit contains sufficient materials to rapidly test 42 samples in duplicate.



4. Procedure Overview

After preparing and diluting the sample, the assay is performed by adding 50 µL of alkaline phosphatase Reagent into each appropriate microplate well. The reagent will then be incubated for 3 minutes at 37°C. From here 5 µL of diluted sample and standards will be added to the wells. After a 10-minute incubation at 37°C, 250 µL of the color development solution will be carefully added to each well. The absorbance of each well is read at 590nm using a plate reader. The concentration of alkaline phosphatase in each sample is then directly determined from the 590nm absorbance.

This kit has the capacity for 96 determinations or testing of 42 samples in duplicate (using 12 wells for standards). Store the kit at 4°C. The shelf life is 6 months after receipt when the kit is properly stored.

5. User Supplied Materials

- Micro-pipettes with disposable plastic tips to pipet 5-20 µL.
- Micro-pipettes with disposable plastic tips to pipet 20-200 µL.
- Micro-pipettes with disposable plastic tips to pipet 200-1000 µL.
- Timer

- Microtiter plate reader (wavelength 590 nm)
- Incubator

6. Precautions

Add user obtained standards to plate only in order from low concentration to high concentration, as this will minimize the risk of compromising the standard curve.

1. Carefully prepare at least 20 µL of sample using standard production procedure (if determinations are performed in singlet, then 5 µL is sufficient). Avoid contamination of reagents. Do not open tubes unnecessarily. Keep container tightly closed.

2. Carefully dilute all samples with an equal volume of water. The diluted samples are now ready for use in the assay.

Note:

Samples with values above 50 IU/L should be further diluted with water and re-tested. Concentration can be determined by multiplying resultant IU/L by the dilution factor.

7. Instructions

Setup: Turn on the plate reader and allow lamp to warm up. Set the wavelength of the plate reader to 590nm and the temperature to 37°C. If your plate reader doesn't have temperature control capabilities, you may substitute this with a conventional incubator.

Reagents should be stored in the closed, tightly secured, bottle at (2 -8° C).

8. Sample Test Procedure

1. Pre-warm Reagents at 37°C for 15 minutes.
2. Add 50µL of AlkPhos substrate to each well.
3. Incubate plate at 37°C for 3 minutes.
4. Add 5µL each diluted standard or diluted sample (in duplicate) to the microplate wells. Use water for dilution and for reagent blank.
5. Incubate at 37°C for 10 minutes.
6. Add 250µL of AlkPhos color development solution to the wells.
7. Measure the absorbance of each well at 590 nm.

8. For each standard or sample, subtract the absorbance of each well from the absorbance of the blank (= A_{blank}, from Tube #1) to obtain the corrected absorbance for each point (= A_{blank}-$A_{standard}$ or = A_{blank}-A_{sample}) for the standards or the samples, respectively.

Concentration Calculation:

Concentration can be determined by taking the Unknown Absorbance divided by the known standards concentration and multiplying this value by 50. If a sample has been diluted multiply IU/L by the dilution factor of your sample.

9. Determination of AlkPhos in Samples

EDTA, oxalate, fluoride, and citrate are known inhibitors of ALP and should be avoided in sample preparation. Serum, Plasma, or Cell Culture Media Serum, plasma (no EDTA/citrate, ideally unhemolyzed) and cell culture media can be assayed directly. Cell Lysate for intracellular ALP 1. Wash 10^4 cells with PBS. 2. Lyse the washed cells in 0.5 mL 0.2% Triton X-100 in purified water by shaking for 20 minutes at room temperature. All Samples Transfer 5 to 15 µL of Sample into appropriate wells of a clear flat-bottom 96-well plate.

Notes on the measurement: The color of the reaction may continue to change after the specified reaction time has elapsed. The rate of the reaction is impacted by the room temperature thus, incubating the plate in a set temperature incubator at 25°C, it can help ensure consistency.

Note: If the test shows the concentration may be higher than a diluted standard. In this case, we recommend carrying out a stepwise dilution of the sample with distilled water, to bring the content into the measuring range of the known concentration positive control. The dilution factor must be considered when calculating the concentration.