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IDTox™ Alkaline Phosphatase (AP) Color Endpoint Assay Kit

SUP6003-C

Colorimetric-Endpoint Assay for the determination of the Alkaline Phosphatase enzyme in serum samples.

PRODUCT DESCRIPTION

Alkaline phosphatase (AP) is a ubiquitously-expressed intracellular enzyme which catalyzes the hydrolysis of organic phosphoesters. AP is a clinically important protein marker in serum because its level changes in response to a number of health-related states. For example, changes in AP serum levels are often caused by liver and liver and bone diseases. Also, the presence of elevated levels of the enzyme in serum after administration of drugs and experimental therapeutic agents is associated with organ toxicity. Therefore, monitoring serum levels of AP enzyme has become a routine method to monitor drug toxicity.

The features of the kit are:

- Rapid and simple method
- Minimal sample prep
- Highly accurate and reproducible
- High sensitivity (3 U/L detection limit)

PROCEDURE OVERVIEW

The ID Labs Alkaline Phosphatase CE Assay Kit uses an enzymatic assay to determine the amount of alkaline phosphatase in serum and other liquid samples. The kit enables biomedical researchers to determine alkaline phosphatase levels in liquid samples such as serum. The kit contains sufficient materials to test 42 samples in duplicate. The ID Labs AP CE Kit measures the concentration of AP using a direct, plate-based, colorimetric reaction. When serum is added to the reaction mix, the AP in the sample converts the thymolphthalein monophosphate substrate into phosphate and thymolphthalein (blue-colored) over a 10 minute reaction time. The production of the latter product is monitored by measuring the absorbance at 590 nm.

The assay utilizes a simple colorimetric (visible) enzymatic assay to specifically detect AP in fluids. The kit provides accurate, proven results even in complex liquid mixtures. The kit is designed to be used with a microplate reader. The kit contains a thymolphthalein (TP) standard to construct a linear calibration curve and verify assay performance.

KIT REAGENTS SUPPLIED

The assay kit has the capacity for 96 determinations or testing of 42 samples in duplicate (using 12 wells for serially-diluted standards). **Upon receipt of the kit, place the dilution buffer at -20°C**. Store the remainder of the kit at 4 °C. The shelf life is noted on the label when the kit is properly stored.

Kit Contents	Amount	Storage
Microtiter Plate	1 x 96-well Plate (8 wells x 12 strips)	Room temp or 2-8 ℃
AP Reagent Solution	8 ml	2-8 ℃
AP Developer	28 ml	2-8 ℃
TP Standard	0.35 ml	2-8 ℃
TP Dilution Buffer	5 ml	**- 20 ℃**

MATERIALS REQUIRED BUT NOT PROVIDED

Microtiter plate reader (with 590 nm absorbance filter).
Distilled or deionized water.
Microcentrifuge.
Microcentrifuge tubes.

For *in vitro* research use. CAUTION: Not for human or animal therapeutic use.
Uses other than the labeled intended use may be a violation of local law.

Multichannel pipet (recommended).

SENSITIVITY (Serum Detection Limit)
3 U/l

WARNINGS AND PRECAUTIONS
It is strongly recommended that you read the following warnings and precautions to ensure your full awareness of the techniques and other details you should pay close attention to when running the assays. Periodically, optimizations and revisions are made to the kit and manual. Therefore, it is important to follow the protocol coming with the kit.
Add standards to plate only in the order from low concentration to high concentration, as this will minimize the risk of compromising the standard curve. When pipetting samples or reagents into an empty microtiter plate, place the pipette tips in the lower corner of the well, making contact with the plastic. Use only distilled or deionized water since water quality is very important. Try to maintain a laboratory temperature of (20–25°C/68–77°F). Avoid running assays under or near air vents, as this may cause excessive cooling, heating and/or evaporation. Also, do not run assays in direct sunlight, as this may cause excessive heat and evaporation. Cold bench tops should also be avoided.

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ASSAY PROCEDURE

PREPARATION OF REAGENTS
Serum

1. Carefully collect whole blood in a 1.5 ml microfuge tube or serum collection tube making sure to avoid hemolysis as it will release erythrocyte AP enzyme into the serum.
2. Incubate the blood sample at 37°C for 10 minutes.
3. Centrifuge sample at 10,000 rpm for 10 minutes.
4. Remove serum layer to a clean tube avoiding the “buffy coat” layer.
5. Store serum samples on ice or at 4°C prior to testing; do not freeze samples. Serum samples can be stored at 4°C for up to one week.

ASSAY PROCEDURE
Set up

1. Turn on the plate reader, allow light source to warm up, and set the absorbance wavelength to 590 nm.
2. Warm up kit reagents to room temperature for 30 minutes.

Preparation Standards for Standard Curve

1. Label six clean microcentrifuge tubes 1, 2, 3, 4, 5 and 6 (Neg).
2. Serially dilute the TP Standard by adding the appropriate volumes of TP Standard and TP Dilution Buffer:

Standard Tube #	Preparation	Equivalent Std Conc.
1	Add 125 µl of TP Standard.	50 U/l
2	Add 75 µl from Standard Tube # 1 + 75 µl of TP Dilution Buffer. Mix thoroughly.	25 U/l
3	Add 75 µl from Standard Tube # 2 + 75 µl of TP Dilution Buffer. Mix thoroughly.	12.5 U/l

4	Add 75 µl from Standard Tube # 3 + 75 µl of TP Dilution Buffer. Mix thoroughly.	6.3 U/l
5	Add 75 µl from Standard Tube # 4 + 75 µl of TP Dilution Buffer. Mix thoroughly.	3.1 U/l
6 (Neg)	Add 100 µl of TP Dilution Buffer only.	NA

Sample Test Procedure

1. Add 5 µl of each sample or standard (in duplicate) to microplate wells.
2. Add 50 µl AP Reagent Solution to the wells. Tap gently to mix.
3. Incubate the plate for 10 min at 37 °C.
4. Carefully add 250 µl AP Developer to the wells. Mix gently.
5. Measure absorbance at 590 nm using a plate reader.

Note: If the absorbance of a serum sample is > 0.8 absorbance units, then dilute the serum 1:1 with saline and retest.

CALCULATION OF RESULTS

Standard Curve Construction

A standard curve can be constructed using the serially-diluted standards by plotting the average absorbance for each TP standard against its concentration in U/l.

Determination of Alkaline Phosphatase Activity in Serum Samples

Calculate the slope and the y-intercept for the line which best fits the standard curve data plot.
The AP concentration in each sample can be described by the equation:

AP concentration = (mean absorbance – y-intercept)/slope

Use the mean absorbance values for each serum sample to determine the corresponding concentration of AP from the standard curve.

Reference

Nwokocha C., et al. “The Effects of Bitter Kola Supplemented Diet on Hepatoxicity of Mercury in Wistar Rats”. JASEM. Vol. 14 (1). pp. 89-95. 2010