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IDTox™ Alkaline Phosphatase (AP) Enzyme Assay Kit

SUP6003

Enzyme Immunoassay 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 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

PROCEDURE OVERVIEW

The ID Labs Alkaline Phosphatase Enzyme Assay Kit uses an enzymatic assay to determine the amount of alkaline phosphatase in rodent 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 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 p-nitrophenol standard to construct a linear calibration curve and verify assay performance.

The ID Labs Alkaline Phosphatase Enzyme Assay 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 p-nitrophenyl phosphate substrate into phosphate and p-nitrophenol. The production of the latter product is directly monitored by measuring the increase in absorbance of the reaction at 405 nm over a 3 minute interval.

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 standard and dilution buffer at -20°C**. Store the remainder of the kit as below. The shelf life of the kit 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
AP Reagent Solution	27 ml	2-8 ℃
pNP Standard	0.2 ml	**- 20 ℃**
pNP Dilution Buffer	1.8 ml	**- 20 ℃**

MATERIALS REQUIRED BUT NOT PROVIDED

Microtiter plate reader (with 405 nm absorbance filter).
Microcentrifuge.
Microcentrifuge tubes.
Multichannel pipet (recommended).

WARNINGS AND PRECAUTIONS

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.

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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.
Do not use the kit past the expiration date. Do not intermix reagents from different kits or different lots. Try to maintain a laboratory temperature of 20-25°C (68-77°F). Make sure you are using only distilled or deionized water since water quality is very important. 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.

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ASSAY PROCEDURE

PREPARATION OF SAMPLES
Serum

1. Carefully prepare at least 20µl of serum using standard production procedure (if determinations are performed in singlet then 10µl is sufficient). Avoid hemolysis as it will release erythrocyte AP enzyme into the serum.
2. Avoid using samples with visible hemolysis since the serum AP would be contaminated with red blood cell AP.
3. Transfer the supernatant (serum) to clean tube. It is best to test the serum immediately; however if the sample is not tested right away (within 8 hours of collection), store the serum samples at 4°C and test no later than 3 days after collection.

ASSAY PROCEDURE
Set up

1. Turn on the plate reader, allow light source to warm up, and set the absorbance wavelength to 405 nm.
2. Warm up kit reagents to room temperature for 30 minutes.

Preparation Standards for Standard Curve - Optional

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

Tube #	Calibrant Source	Volume of Calibrant	Volume of pNP Dilution buffer	Final Equivalent Calibrant Conc
1	vial	150 µl	None	800 U/l
2	Tube 1	75 µl	75 µl	400 U/l
3	Tube 2	75 µl	75 µl	200 U/l
4	Tube 3	75 µl	75 µl	100 U/l
5	Tube 4	75 µl	75 µl	50 U/l
Neg	Not applicable	None	150 µl	0 U/l

Sample Test Procedure

1. The microplate supplied with the kit contains 96 wells which permits the analysis of 42 samples in duplicate (84 wells) and 6 calibrant dilutions in duplicate (12 wells).
2. Add 5 µl of each standard in duplicate to microplate wells.
3. Add 5 µl of serum sample in duplicate to clean microplate wells.
4. Add 250 µl AP Reagent Solution to the wells.

5. Measure absorbance at 405 nm (= initial reading). Wait 3 minutes and read absorbance again (= 3 minute reading). Average the duplicate readings.

Note: If the 3 minute reading of a serum sample is > 0.9 absorbance units, then dilute the serum 1:1 with saline and retest.

CALCULATION OF RESULTS

Standard Curve Construction

A calibration curve to confirm assay linearity can be constructed using the calibration standards supplied with the kit as follows:

1. A standard curve can be constructed from the change decrease in absorbance observed for each pNP concentration tested in the standard curve.
2. For each of the diluted pNP standards, at the 3 minute time point, subtract for the 405 nm absorbance from the Neg tube ("no pNP") 3 minute absorbance. This optional standard curve provides a reference for the linear range of the assay.

Determination of Alkaline Phosphatase Activity in Serum Samples

1. For each sample subtract the initial absorbance from the 3 minute absorbance. Average these values to obtain the average absorbance increase in 3 minutes for each sample.
2. Multiply the average 3 minute absorbance increase by 1209 (conversion factor) to obtain AP activity (IU/l).

For example, if the absorbance of a sample increases by 0.3 over 3 minutes then the alkaline phosphatase activity of the sample is:

0.3 x 1209 = 362.7 IU/l