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IDTox™ Alanine Transaminase (ALT) Enzyme Assay Kit

SUP6001

Enzyme Immunoassay for the determination of the alanine transaminase enzyme in serum samples.

PRODUCT DESCRIPTION

The ID Labs Alanine Transaminase (ALT) Enzymatic Assay Kit is a plate-based colorimetric enzymatic assay for the determination of the alanine transaminase enzyme in serum samples. Alanine transaminase (ALT) (also known as alanine aminotransferase or sGPT) is a metabolic enzyme expressed primarily in the liver. Damage to the liver causes the release of this enzyme into the blood. Elevation of ALT levels is an indication of liver damage and has been associated with liver injury. ALT levels are monitored routinely in patients with liver diseases. ALT is also a very useful tool for preclinical investigation of experimental drug formulations and ALT levels are commonly used to monitor and attenuate the hepatotoxic effects of experimental drugs in rodents.

The kit uses a spectrophotometric, kinetic assay to detect changes in alanine transaminase levels directly from serum samples. The features of the kit are:

- High sensitivity and low detection limit (20 U/L)
- A rapid (5 minutes) and robust enzyme-based assay which does not require expensive instrumentation
- High reproducibility

PROCEDURE OVERVIEW

The Alanine Transaminase (ALT) Enzymatic Assay Kit uses a coupled enzymatic reaction scheme: alanine and α-ketoglutarate are first converted to glutamate and pyruvate which is converted by lactate dehydrogenase to make lactate and NAD^+. The conversion of the NADH chromophore to NAD^+ product, measured at 340 nm, is proportional to the level of ALT enzyme in the sample. The absorbance of each well at 340 nm is measured using a plate reader. The concentration of ALT in each sample is then directly determined from the change in absorbance at 340 nm within 5 minutes time. Dilutions of the Pyruvate Control, included in the kit, can be used to construct a standard curve to calibrate the assay and confirm assay linearity.

KIT REAGENTS SUPPLIED

The Alanine Transaminase (ALT) Enzymatic Assay Kit has the capacity for 96 determinations or testing of 42 samples in duplicate (using 12 wells for standards). The kit also contains enough material to construct four standard curves. Store the kit at 4°C. The shelf life of the kit, when properly stored is noted on the label. Once the Reagent Mix is reconstituted the shelf life of the kit is 3 months when properly stored. For more details, see "Preparation of Reagent Mix"

Kit Contents	Amount	Storage
Microtiter Plate	1 x 96-well Plate (8 wells x 12 strips)	2-8ºC
Reagent Mix	Makes Up to 27 ml	2-8ºC
Pyruvate Control	600 µl	2-8ºC
Pyruvate Dilution Buffer	2 x 1.8 ml	2-8ºC

MATERIALS REQUIRED BUT NOT PROVIDED

Microtiter plate reader (340 nm).
Centrifuge to prepare serum samples.
Deionized or distilled water.
1.5 ml microfuge tubes.
Multichannel pipette or repeating pipettor (*recommended but not required*).

SENSITIVITY (Serum Detection Limit)

20 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.

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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 ALT 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.
6. Use 10 µl of serum in the assay.

ASSAY PROCEDURE

Set up

Allow all reagents and the microtitre plate to warm up to room temperature before use.

If a temperature controlled plate reader is available, adjust the plate reader temperature control to 37°C and equilibrate the Master Mix at 37°C to obtain even higher sensitivity measurements.

Preparation of Reagent Mix

Reconstitution: Add exactly 27 ml of deionized or distilled water to the Reagent Mix powder. Mix by swirling or inverting the bottle 10 times. Allow contents to dissolve for 10 minutes at room temperature. The reconstituted Reagent Mix can be left at room temperature for short periods (30-60 minutes) prior to use. Between uses the Reagent Mix should be stored at 4°C for up to 3 months. Allow the mix to warm up to room temperature for 30 – 60 minutes before use. Discard the mix 3 months after reconstitution. If a temperature controlled plate reader is available, adjust the plate reader temperature control to 37°C and equilibrate the Reagent Mix at 37°C to obtain even higher sensitivity measurements.

Preparation of Pyruvate Control Dilutions for Standard Curve – (optional)

Label six microfuge tubes: 1, 2, 3, 4, 5, Neg. Then make 6 serial dilutions of the Pyruvate Control (3 concentration increments per log) using the Pyruvate Dilution Buffer as described in the table below.

NOTE: There is enough material to construct 4 Standard Curves. Make the Pyruvate Control Dilutions for the Standard Curve fresh each time that the Standard Curve is performed. After each dilution, briefly mix the tube before performing the next dilution.

Standard Tube #	Preparation	Relative Dilution*
1	Add 150 µl of Pyruvate Control.	1
2	Add 100 µl from Standard Tube # 1 + 115 µl of Pyruvate Dilution Buffer. Mix thoroughly.	2.15
3	Add 100 µl from Standard Tube # 2 + 115 µl of Pyruvate Dilution Buffer. Mix thoroughly.	4.63

4	Add 100 µl from Standard Tube # 3 + 115 µl of Pyruvate Dilution Buffer. Mix thoroughly.	10
5	Add 100 µl from Standard Tube # 4 + 115 µl of Pyruvate Dilution Buffer. Mix thoroughly.	21.5
6 (Neg)	Add 100 µl of Pyruvate Dilution Buffer.	NA

*Only needed for the generation of the Standard Curve.

Sample Test Procedure

1. Add 10 µl of each sample or standard (in duplicate) to the microplate wells.
2. Add 240 µl of Master Mix to the wells.
3. Measure the absorbance of each sample at 340 nm. Exactly 5 minutes later, measure absorbance again.

CALCULATION OF RESULTS

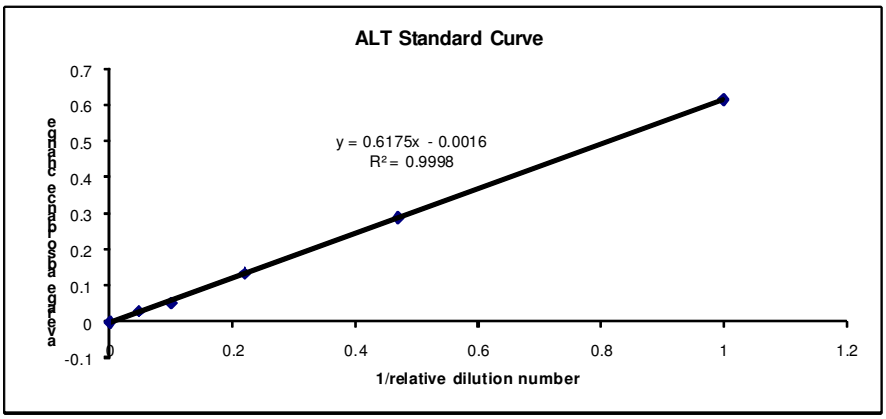
Standard Curve Construction

NOTE: This optional Standard Curve provides a reference for the linear range of the assay. It is simply used as a test to show that the experiment was carried out correctly; e.g. proper dilutions, temperatures, times, etc. The Standard Curve IS NOT USED to determine the concentration of ALT in the samples; see **Determination of Alanine Transaminase Activity in Serum Samples** section below.

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

1. For each calibration point, calculate the average absorbance change. To do this, subtract the average 5 minute absorbance value of each point from the average 5 minute absorbance value of the "Neg" (no pyruvate) point. This calculation should include subtracting the average 5 minute absorbance of the "Neg" value from itself, which is approximately zero.

2. For each standard, plot the average corrected absorbance along the y-axis (from lowest in value to highest in value) and the inverse value of the relative dilution number* (i.e. 0, 0.047, 0.1, 0.22, 0.47 and 1) on the x-axis.



*Relative dilution numbers can be found in the table above.

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Determination of Alanine Transaminase Activity in Serum Samples

Using the supplied materials and the procedure described above (for measurements performed at 37°C), the concentration of ALT (units per liter) can be determined by multiplying the decrease in absorbance in 5 minutes by 1072.

For example, if an absorbance decrease of 0.1 is observed over the 5 minute interval, the ALT enzyme concentration in the sample would be 1072 x 0.1 = 107.2 U/l.

References

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