IDTox™ Aspartate Transaminase (AST) Enzyme Assay Kit

SUP6002

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

PRODUCT DESCRIPTION

The ID Labs Aspartate Transaminase (AST) Enzymatic Assay Kit is a plate-based colorimetric enzymatic assay for the determination of the aspartate transaminase enzyme in serum samples. Aspartate transaminase (AST) also known as aspartate aminotransferase or (sGOT) is a metabolic enzyme expressed primarily in the liver. Elevation of AST levels is an indication of liver damage and has been associated with liver injury. AST levels are monitored routinely in patients with liver diseases. AST is also a very useful tool for preclinical investigation of experimental drug formulations and AST 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 aspartate 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
- Only Requires 10 µl of serum

Aspartate transaminase (AST) also known as aspartate aminotransferase or (sGOT) is a metabolic enzyme expressed primarily in the liver. Damage to the liver causes the release of the enzyme into the blood. AST is often determined routinely in patients with liver diseases. Elevation of AST is an indication of damage to liver and has been associated with liver injury. AST is also a very useful tool for preclinical investigation of experimental drug formulations since determination of AST levels into sera is a commonly used practice to monitor and attenuate the hepatotoxic effects of experimental drugs in rodents.

PROCEDURE OVERVIEW

The Aspartate Transaminase (AST) Enzymatic Assay Kit uses a coupled enzymatic reaction scheme: aspartate and α-ketoglutarate are first converted to glutamate and oxaloacetate which is converted by malate dehydrogenase to make malate and NAD^+. The conversion of the NADH chromophore to NAD^+ product, measured at 340 nm, is proportional to the level of AST enzyme in the sample. The absorbance of each well at 340 nm is measured using a plate reader. The concentration of AST in each sample is then directly determined from the change in absorbance at 340 nm within 5 minutes time. Dilutions of the AST 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 Aspartate Transaminase (AST) Enzymatic Assay Kit has the capacity for 96 determinations or testing of 42 samples in duplicate (using 12 wells for standards). Store the kit (except for the microplate) at 4°C. The kit also contains enough material to construct four standard curves. 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)	4ºC
Reagent Mix	bottle	4ºC
AST Enzyme Control	4 tubes	4ºC
AST Dilution Buffer	2 x 1.8 ml	4ºC

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

ASSAY PROCEDURE
Set up

IMPORTANT: Make sure you read "Warnings and Precautions" section above. All reagents and the microtiter plate should be brought up to room temperature before use (30 min - 1 hour at 20–25°C/68–77°F).

Preparation of Reagent Mix

To reconstitute the Reagent Mix, 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. IMPORTANT: The reconstituted Reagent Mix can be left at room temperature for short periods (30 – 60 min) prior to use. Between uses, the reconstituted Reagent Mix should be stored at 4 °C (for up to 4 months).

To obtain higher sensitivity measurements use a temperature controlled plate reader, if available. Adjust the plate reader temperature control to 37°C and equilibrate the Reagent Mix to 37°C for 10 minutes before use.

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

1. Add 190 µl AST Dilution Buffer to the AST Enzyme Control tube. Invert tube two times to dissolve any contents at the top of tube. Vortex or tap briefly to ensure mixing.

NOTE: There is enough material to construct 4 Standard Curves. Use a fresh tube of AST Enzyme Control for each Standard Curve. Discard any remaining diluted AST Enzyme Control after using it to make the dilutions, in Step 2, for the Standard Curve.

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

NOTE: Make the AST Enzyme Control Dilutions for the Standard Curve fresh each time that the Standard Curve is performed.

Std Tube #	Preparation	Relative Dilution*
1	Add 10 µl diluted AST Enzyme Control to 190 µl AST Dilution Buffer. Mix.	1
2	Add 100 µl of Standard Tube #1 +115 µl of AST Dilution Buffer. Mix.	2.15
3	Add 100 µl of Standard Tube #2 +115 µl of AST Dilution Buffer. Mix.	4.63
4	Add 100 µl of Standard Tube #3 +115 µl of AST Dilution Buffer. Mix.	10
5	Add 100 µl of Standard Tube #4 +115 µl of AST Dilution Buffer. Mix.	21.5
6 (Neg)	Add 150 µl of AST 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 Reagent Mix to the wells.
3. Measure the absorbance of each sample at 340 nm. Exactly 5 minutes later, measure absorbance again.

CALCULATION OF RESULTS

Determination of Aspartate Transaminase Activity in Serum Samples

Using the supplied materials and the procedure described above (for measurements performed at 37°C), the concentration of AST (units per liter) can be determined by multiplying the decrease in absorbance in 5 min by 1072.
For example, if an absorbance decrease of 0.1 is observed over the 5 min interval, the AST enzyme concentration in the sample would be 1072 x 0.1 = 107.2 U/L.

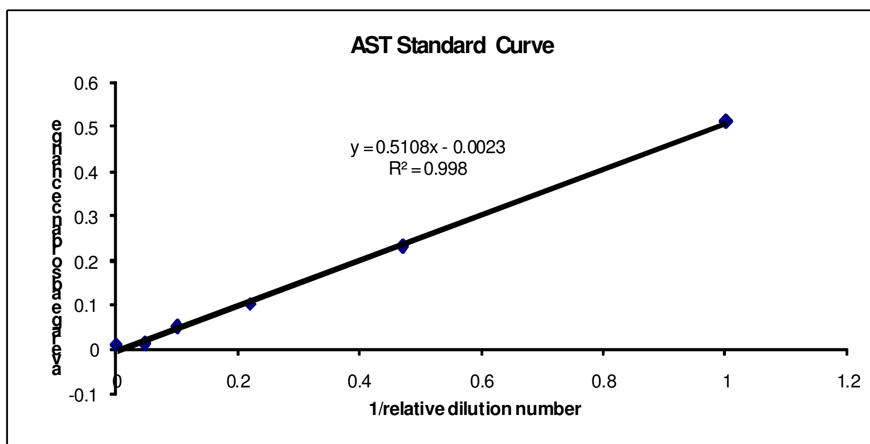
Standard Curve Construction

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 AST in the samples; see **Determination of Aspartate Transaminase Activity in Serum Samples** .

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 change in absorbance by subtracting the average 5 minute absorbance of each point from its corresponding average initial absorbance.

2. For each standard, plot the average change in absorbance along the y-axis (from lowest in value to highest in value) and the inverse value of the relative dilution number* (i.e. 0.047, 0.1, 0.22, 0.47 and 1) on the x-axis. For Tube # 6 (neg) use “0”.



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