PO Box 1145, Station CSC, London ON N6A 5K2 Canada
Tel: +1 519 434 5057 Fax: +1 519 434 2639
www.idlabs.com idinfo@idlabs.com

IDTox™ Aspartate Transaminase (AST) Color Endpoint Assay Kit

SUP6002-C

Color Endpoint Assay for the determination of the Aspartate Transaminase enzyme in serum samples.

PRODUCT DESCRIPTION

The IDTox™ Aspartate Transaminase (AST) Color Endpoint 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 colorimetric assay to detect changes in aspartate transaminase levels directly from serum samples. The features of the kit are:

- High sensitivity and low detection limit (10 U/L)
- Convenient, colorimetric, end-point assay
- Does not require expensive instrumentation
- High reproducibility
- Only 5 µl of serum sample is needed

PROCEDURE OVERVIEW

The IDTox™ Aspartate Transaminase (AST) Color Endpoint Assay Kit uses a colored reaction scheme to detect AST enzymatic activity. In this method aspartate and α-ketoglutarate are first converted to glutamate and oxaloacetate. The oxaloacetate then reacts with diazonium salt to form a colored product. The concentration of AST in each sample is then directly determined from the absorbance at 510 nm measured with a plate reader. Dilutions of the oxaloacetate 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 IDTox™ AST Color Endpoint 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. For more details, see "Preparation of Reagent Mix"

Kit Contents	Amount	Storage
Microtiter Plate	1 x 96-well Plate (8 wells x 12 strips)	RT
Oxaloacetate Control	4 tubes	-20ºC
Oxaloacetate Dilution Buffer	4 x 1.0 ml	4ºC
AST Reagent Solution	7 ml	4ºC
AST Color Reagent Mix	Bottle	4ºC

MATERIALS REQUIRED BUT NOT PROVIDED

Microtiter plate reader (510 nm).
Microfuge to prepare serum samples.
Deionized or distilled water.
0.5M NaOH
1.5 ml microfuge 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 pipette or repeating pipettor (*recommended but not required*).

SENSITIVITY (Serum Detection Limit)
10 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.

Do not use the kit past the expiration date.
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 by placing several layers of paper towel or some other insulation material under the assay plates during incubation.
Make sure to use 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.

ID Labs™ makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. There is no warranty of merchantability of this product, or of the fitness of the product for any purpose. ID Labs™ shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product. This product is For In Vitro Research Use Only.

ASSAY PROCEDURE

PREPARATION OF SAMPLE
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. Transfer serum layer to a clean 1.5ml microfuge 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 5 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 (for at least 30 minutes).
Turn on the plate reader, allow light source to warm up, and set the absorbance wavelength to 510 nm.

Reagent Preparation

1. Reconstitute AST Color Reagent Mix with 30 ml of ddH_2O. Gently swirl to mix. Reconstituted AST Color Reagent should be brought to room temperature before use. The AST Color Reagent should be stored at 4°C between uses.

2. Warm the AST Reagent Solution to 37°C for 10 minutes before use. The AST Reagent Solution can be left at room temperature for short periods (30 – 60 min) prior to use. Between uses, the AST Reagent Solution should be stored at 4°C (for up to 4 months).

PO Box 1145, Station CSC, London ON N6A 5K2 Canada
Tel: +1 519 434 5057 Fax: +1 519 434 2639
www.idlabs.com idinfo@idlabs.com

Preparation of Oxaloacetate Control Dilutions for Standard Curve

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

1. Prepare Oxaloacetate Standard by adding 250 µl of ddH_2O to the standard vial.
2. Label six microfuge tubes: 1, 2, 3, 4, 5, Neg.Then make 6 serial dilutions of the Oxaloacetate Control using the Oxaloacetate Dilution Buffer as described in the table below.
   NOTE: Make the Oxaloacetate Control Dilutions for the Standard Curve fresh each time that the Standard Curve is performed.

Standard Tube #	Preparation *	Equivalent AST conc. (U/L)
1	Add 200 µl diluted Oxaloacetate Control	800
2	Add 100 µl of Standard Tube #1 + 100 µl of Dilution Buffer. Mix.	400
3	Add 100 µl of Standard Tube #2 +100 µl of Dilution Buffer. Mix.	200
4	Add 100 µl of Standard Tube #3 +100 µl of Dilution Buffer. Mix.	100
5	Add 100 µl of Standard Tube #4 +100 µl of Dilution Buffer. Mix.	50
6 (Neg)	Add 150 µl of Oxaloacetate Dilution Buffer.	N/A

*Only needed for the generation of the Standard Curve.

Assay Procedure

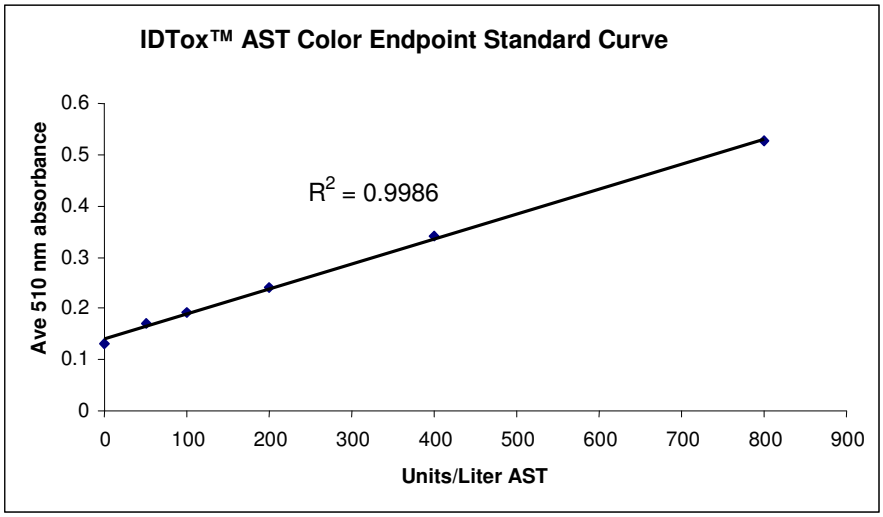
1. Add 5 µl of each sample or standard (in duplicate) to the microplate wells.
2. Add 50 µl of AST Reagent Solution to the wells. (Using a multichannel pipet or repeating pipettor is recommended). Cover wells with the adhesive film and incubate at 37°C for 10 min.
3. Carefully remove adhesive film and add 50 µl AST Color Reagent to the wells. Use second film to re-cover wells and incubate for 10 min at 37°C.
4. Remove adhesive and add 200 µl 0.1 M HCl to each well.
5. Read 510 nm absorbance in plate reader.

PO Box 1145, Station CSC, London ON N6A 5K2 Canada
Tel: +1 519 434 5057 Fax: +1 519 434 2639
www.idlabs.com idinfo@idlabs.com

CALCULATION OF RESULTS

Standard Curve Construction

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



Calculate the slope and the y-intercept for the line which best fits the standard curve data plot.

The AST concentration in each sample can be described by the equation:
AST concentration = (mean absorbance – y-intercept)/slope

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