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BIOO SCIENTIFIC
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MAX DISCOVERY™

Aspartate Transaminase (AST) Color Endpoint Assay Kit Manual

Catalog #: 5605-01

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GENERAL INFORMATION

Product Description

The *MaxDiscovery™ 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 spectrophotometric, colorimetric assay to detect changes in aspartate transaminase levels directly from serum samples. The unique features of the kit are:

- High sensitivity and low detection limit (10 U/L)
- A rapid (20 min), robust enzyme-based assay which does not require expensive instrumentation
- High reproducibility
- Only requires 5 μL of serum

Procedure Overview

The *MaxDiscovery™ Alanine 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 Contents, Storage and Shelf Life

The *MaxDiscovery™ Aspartate Transaminase (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. The shelf life of the kit is 6 months, after receipt, when the kit is properly stored.

Kit Contents	Amount	Storage
Microtiter Plate	1 x 96-well Plate (8 wells x 12 strips)	Room temp
Microplate cover sheets	2 adhesive films	Room temp
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

Required Materials Not Provided With the Kit

- Microtiter plate reader (*510 nm*)
- 37°C Incubator (*for heating microplates*)
- Microfuge (*to prepare serum samples*)
- Deionized or distilled water
- 0.1 M HCl
- 1.5 mL microfuge tubes
- Multichannel pipet or repeating pipettor (*Optional*)

Sensitivity (Detection Limit)

Sample Type	**Detection Limit** (U/L)
Serum	10

Warnings and Precautions

BIOO strongly recommends 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 included with the kit. If you need further assistance, you may contact your local distributor or BIOO at techsupport2@biooscientific.com.

- 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 be avoided by placing several layers of paper towel or some other insulation material under the assay plates during incubation.
- Make sure you are using only distilled 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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SAMPLE PREPARATION

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 5 µL of serum in the assay.

ASPARTATE TRANSAMINASE (AST) DETECTION PROTOCOL

Reagent Preparation

Preparation of Reagents

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).

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.*

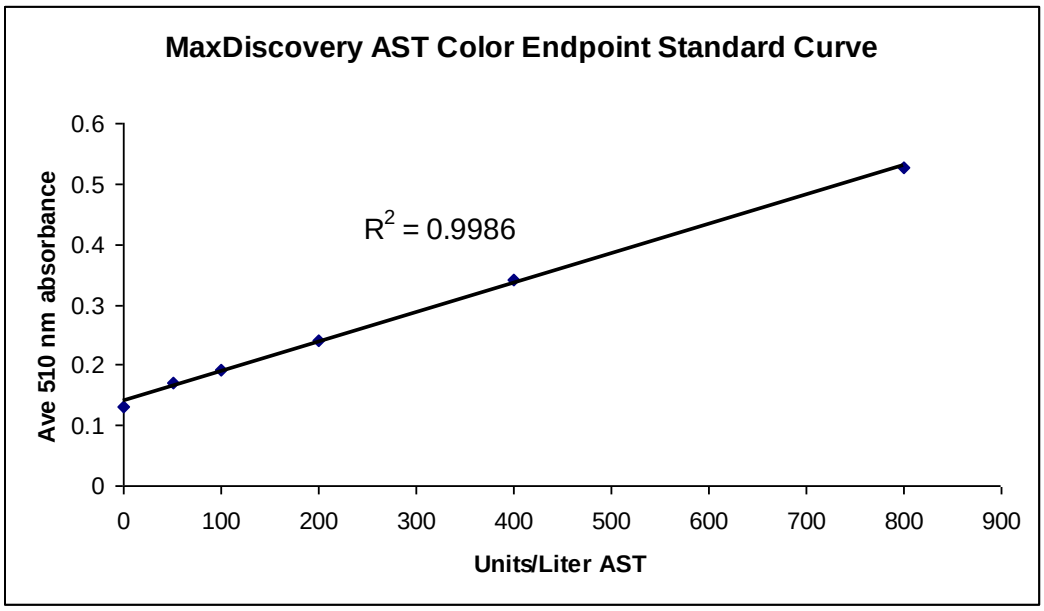
Assay Protocol

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.

DATA ANALYSIS

Standard Curve Construction

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



Determination of AST Concentration in Serum Samples

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.

RELATED PRODUCTS

Toxicity Assays:

Product	Catalog Number
MaxDiscovery™ Alanine Transaminase Color Endpoint Assay Kit	3460-08
MaxDiscovery™ Alanine Transaminase Enzymatic Assay Kit	3460-01
MaxDiscovery™ Alkaline Phosphatase Color Endpoint Assay Kit	3460-09
MaxDiscovery™ Alkaline Phosphatase Enzymatic Assay Kit	3460-03
MaxDiscovery™ Aspartate Transaminase Color Endpoint Assay Kit	5605-01
MaxDiscovery™ Aspartate Transaminase Enzymatic Assay Kit	3460-02
MaxDiscovery™ Creatine Kinase Enzymatic Assay Kit	3460-07
MaxDiscovery™ γ-Glutamyl Transferase Enzymatic Assay Kit	5601-01
MaxDiscovery™ Lactate Dehydrogenase Color Endpoint Assay Kit	5604-01
MaxDiscovery™ Lactate Dehydrogenase Enzymatic Assay Kit	3460-04
MaxDiscovery™ Total Bilirubin Assay Kit	3460-10

Blood Chemistry Assays:

Product	Catalog Number
MaxDiscovery™ Cholesterol Enzymatic Assay Kit	3460-06
MaxDiscovery™ Blood Urea Nitrogen (BUN) Enzymatic Assay Kit	5602-01
MaxDiscovery™ Triglycerides Enzymatic Assay Kit	5603-01

Cytotoxicity Assays

Product	Catalog Number
MaxDiscovery™ Aspartate Transaminase (AST) Cytotoxicity Kit	3460-11
MaxDiscovery™ Lactate Dehydrogenase Cytotoxicity Assay Kit	3460-12

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