# Aspartate Aminotransferase (AST/GOT) Assay Kit

**Catalog No.:** abx096003

**Size:** 96 tests

**Detection Range:** 1.1 IU/L – 72.3 IU/L

**Sensitivity:** 1.1 IU/L

**Storage:** Store all components at 4°C for up to 12 months. Store the Chromogenic Reagent in the dark.

**Application:** For detection and quantification of Aspartate Aminotransferase activity in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants, and other biological fluids.



## Introduction

Aspartate Aminotransferase (AST/GOT) is an enzyme involved in nitrogen metabolism, catalyzing the reaction between alpha-ketoglutaric acid and aspartic acid to form the reactive intermediate oxaloacetic acid. Oxaloacetic acid undergoes spontaneous decarboxylation to form pyruvic acid. Pyruvic acid is reactive with 2,4-DNPH, forming a red-brown compound.

Aspartate Aminotransferase (AST/GOT) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating AST activity. The absorbance should be measured at 510 nm. The intensity of the color is proportional to the activity of the AST enzymes, which can then be calculated.

## Kit components

1. 96-well microplate
2. Assay Buffer: 0.5 ml
3. Standard Solution (2 mmol/L): 0.5 ml
4. Substrate Solution: 5 ml
5. Chromogenic Reagent: 5 ml
6. Alkali Reagent: 5 ml
7. Plate sealer: 2

## Materials required but not provided

1. Microplate reader (510 nm)
2. Distilled water
3. Normal saline (0.9% NaCl) or 1X PBS (0.01 M, pH 7.4)
4. Multichannel pipette, pipette, and pipette tips
5. 1.5 ml microcentrifuge tubes
6. Centrifuge
7. Vortex mixer
8. Incubator

## Protocol

### A. Preparation of samples and reagents

#### 1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

Samples should be prepared according to conventional methods. The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or for 30 minutes at 25°C. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately. Serum can be stored for up to 7 days at 4°C, or up to 20 days at -20°C, but fresh samples are recommended.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge at approximately 1000 × g for 10 mins at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Weigh the tissue homogenate. For each 1 g of homogenate, add 9 ml ice-cold homogenization medium (for example, PBS (0.01 M pH 7.4) with 0.1 mM EDTA). Homogenize by hand, using a mechanical homogenizer, or by ultrasonication on ice . Centrifuge the homogenate at 1500 x g for 10 minutes at 4°C. Collect the supernatant and assay immediately.

  **For tissue homogenate analysis:** Determine the total protein concentration in the supernatant. The total protein concentration will be used in **C. Calculation of Results**.
- **Cell Lysates:** Detach adherent cells with trypsin, collect, and wash 2 times with homogenization medium (for example, PBS (0.01 M pH 7.4)). Centrifuge at 1000 × g for 10 minutes, and discard the supernatant. Add homogenization medium to the pellet in a ratio of 500 µl : 1 × $10^6$ cells. Homogenize by hand, using a mechanical homogenizer, or by ultrasonication, on ice. Centrifuge at 10,000 × g for 10 minutes, then carefully take the supernatant, keep on ice, and assay immediately.
- **Other biological fluids:** Collect fresh samples and centrifuge at 10,000 × g for 15 minutes at 4°C. Take the supernatant, keep on ice, and assay immediately.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

| Sample Type | Dilution Factor |
|---|---|
| Human serum | 1 |
| Human plasma | 1 |
| Porcine serum | 1 |
| Rat serum | 1 |
| 10% Rat liver tissue homogenate | 15 – 30 |
| 10% Rat lung tissue homogenate | 2 – 8 |



**Note:**

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

## 2. Reagents

- **Alkali Reagent Working Solution**: Dilute the Alkali Reagent with distilled water in a ratio of 1:9 (i.e. to 5 ml of Alkali Reagent, add 45 ml of distilled water). Mix fully. Prepare this solution just before use, and do not store after the assay is complete.
- **Activated Substrate Solution**: Incubate the Substrate Solution for 10 minutes at 37°C just before use.

**Note:**

- Allow all reagents to equilibrate to room temperature before use.
- The results of this assay are extremely sensitive to the volumes of Alkali Reagent Working Solution added during the assay procedure. It is recommended to use a multi-channel pipette when adding this reagent to minimize any variation in the volumes added. Use checked and calibrated pipettes, pipette carefully, and make every effort to reduce sources of error.

## B. Assay Procedure

1. Mark positions on the 96-well microplate for each sample and corresponding control, and mark out 5 standard wells. Each sample requires a corresponding control. *It is strongly recommended to prepare all wells in duplicate.*
2. Add 5 µl of Assay Buffer to the standard wells.
3. To the 5 marked standard wells, add 20 µl, 18 µl, 16 µl, 14 µl, and 12 µl of Activated Substrate Solution, followed by 0 µl, 2 µl, 4 µl, 6 µl, 8 µl of Standard Solution (2 mmol/L). All of these volumes are summarized in the table below:

| **Well No.** | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Volume of **Assay Buffer (µl)** | 5 | 5 | 5 | 5 | 5 |
| Volume of **Activated Substrate Solution (µl)** | 20 | 18 | 16 | 14 | 12 |
| Volume of **Standard Solution (2 mmol/L)** | 0 | 2 | 4 | 6 | 8 |

4. Add 20 µl of Activated Substrate Solution to the sample wells.
5. Add 20 µl of Activated Substrate Solution to the control wells.
6. Add 5 µl of sample to the corresponding sample wells.
7. Mix fully, then incubate at 37°C for 30 minutes. *It is essential that the liquid in the wells is mixed fully. If possible, use a shaking microplate reader or a dedicated microplate shaker to ensure thorough mixing. Mix for up to 10 seconds.*
8. Add 20 µl of Chromogenic Reagent to all wells.
9. Add 5 µl of sample to the corresponding control wells.
10. Mix fully for 10 seconds with a microplate shaker, then incubate at 37°C for 20 minutes.
11. Add 200 µl of Alkali Reagent Working Solution to all wells. *It is recommended that a multi-channel pipette is used for this step.*
12. Mix fully for 10 seconds with a microplate shaker, then stand at room temperature for 15 minutes.
13. Measure the OD of each well with a microplate reader at 510 nm.

### C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank, which is the standard point that contains 0 µl Standard Solution (2 mmol/L), in Well 1) on the x-axis, and their corresponding Carmen units on the y-axis. The Carmen values of each standard are: 0, 24, 61, 114, and 190 for Wells 1, 2, 3, 4, and 5 respectively (see the table in **Section B. 3 – Assay Procedure**). As the standard in Well 1 has been used as a blank, it is not plotted. The final plotted standard curve will contain 4 data points.

The curve should form a line described by the formula $y = ax^2 + bx + c$. It is recommended to fit this curve with a 2nd order polynomial model. Based on this curve, the concentration of Aspartate Aminotransferase in each sample well can be derived with the following formulae:

#### 1. Serum and Plasma samples:

1 International Unit (IU) is defined as the amount of Aspartate Aminotransferase required to oxidize 1 µmol of NADH in 1 ml of sample per minute at 25°C.

$$\mathrm{AST\ (IU/L)} = \mathrm{F} \times \mathbf{0.482} \times \left[\mathrm{a} \times \left(\mathrm{OD_{Sample}} - \mathrm{OD_{Control}}\right)^2 + \mathrm{b} \times \left(\mathrm{OD_{Sample}} - \mathrm{OD_{Control}}\right) + \mathrm{c}\right]$$

#### 2. Tissue Homogenate and Cell Lysate samples:

Aspartate Aminotransferase activity in tissue and cell samples is calculated according to total protein concentration (which must be assayed separately).

1 International Unit (IU) is defined as the amount of Aspartate Aminotransferase required to oxidize 1 µmol of NADH in 1 g of tissue protein per minute at 25°C.

$$\mathrm{AST\ (IU/L)} = \frac{\mathrm{F} \times 0.482}{\mathrm{C_{Protein}}} \times \left[\mathrm{a} \times \left(\mathrm{OD_{Sample}} - \mathrm{OD_{Control}}\right)^2 + \mathrm{b} \times \left(\mathrm{OD_{Sample}} - \mathrm{OD_{Control}}\right) + \mathrm{c}\right]$$

where:

| | |
|---|---|
| $OD_{Sample}$ | OD value of sample |
| $OD_{Control}$ | OD value of corresponding control to the sample |
| $C_{Protein}$ | Concentration of protein in sample (g/L) |
| a | Quadratic term of the standard curve ($y = \boldsymbol{a}x^2 + bx + c$) |
| b | Linear term of the standard curve ($y = ax^2 + \boldsymbol{b}x + c$) |
| c | Constant term of the standard curve ($y = ax^2 + bx + \boldsymbol{c}$) |
| F | The dilution factor of sample |

**Definition of a Carmen Unit:** A Carmen unit is defined as the amount of pyruvic acid required to change the absorbance of a sample by 0.001, in a 3 ml reaction volume with 1 ml of sample, optical path 1 cm, at wavelength 340 nm, reacting for 1 minute at 25°C. 1 Carmen Unit = 0.482 IU/L.