Aspartate Aminotransferase (AST/GOT) Assay Kit

Catalog No.: abx294136

Size: 96 tests

Detection Range: 0.38 IU/L – 72.3 IU/L

Sensitivity: 0.38 IU/L

Storage: Store all components at 4°C. Store the Chromogenic Reagent in the dark.

Application: For detection and quantification of Aspartate Aminotransferase activity in serum, plasma, tissue homogenates, cell lysates, 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 will then react with 2,4-DNPH, forming a red-brown compound with an absorbance maximum at 505 nm.

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 505 nm. The strength of the absorbance is proportional to the activity of the AST enzymes, which can then be calculated.

Kit components

1. Assay Buffer: 1.8 ml
2. Standard Solution (2 mmol/L): 1.8 ml
3. Substrate Solution: 2 × 30 ml
4. Chromogenic Reagent: 2 × 30 ml
5. Alkali Reagent: 2 × 30 ml

Materials required but not provided

1. Spectrophotometer (505 nm)
2. Double-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 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 1 month at -20°C, but fresh samples are recommended.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge at 1000 × g for 10 mins at 4°C. 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:** Carefully weigh up to 1 g of tissue. Add into ice-cold homogenization medium (for example, PBS (0.01 M pH 7.4) with 0.1 mM EDTA) in a ratio of 1:9 weight to volume (i.e. for each 1 g of homogenate, add 9 ml ice-cold homogenization medium). 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.

  For cell lysate analysis: Determine the total protein concentration in the supernatant. The total protein concentration will be used in **C. Calculation of Results**.
- **Other biological fluids (e.g. urine):** 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) 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
Mouse serum	1
Rat serum	1
10% Rat liver tissue homogenate	16 – 32
10% Rat heart tissue homogenate	1

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 double-distilled water in a ratio of 1:9 (i.e. to 1 ml of Alkali Reagent, add 9 ml of double-distilled water). Mix fully. Prepare this solution just before use, and do not store after the assay is complete. *It is recommended to aliquot and dilute only enough Alkali Reagent to meet the needs of the current experiment.*
- **Activated Substrate Solution:** Heat the Substrate Solution at 37°C for 10 minutes. Prepare this solution 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

Measurement of Standard Curve

1. Mark 5 microcentrifuge tubes for each standard point. Mark these tubes as A, B, C, D, and E. *It is strongly recommended to prepare all tubes in duplicate.*
2. Add 100 µl Assay Buffer to all standard tubes.
3. To the 5 standard tubes A, B, C, D, and E, add 0 µl, 50 µl, 100 µl, 150 µl, and 200 µl of Standard Solution (2 mmol/L) respectively.
4. To the 5 standard tubes A, B, C, D, and E, add 500 µl, 450 µl, 400 µl, 350 µl, and 300 µl of Activated Substrate Solution respectively.
5. Add 500 µl of Chromogenic Reagent to all standard tubes. The volumes thus far are summarized in the table below:

Tube	A	B	C	D	E
Volume of **Assay Buffer (µl)**	100	100	100	100	100
Volume of **Standard Solution (2 mmol/L)**	0	50	100	150	200
Volume of **Activated Substrate Solution (µl)**	500	450	400	350	300
Volume of **Chromogenic Reagent (µl)**	500	500	500	500	500

6. Mix fully, then incubate at 37°C for 20 minutes. *The timings of this incubation must be strictly controlled, and be the same for all tubes.*
7. Add 5 ml of Alkali Reagent Working Solution to all standard tubes.
8. Stand the tubes for 10 minutes at room temperature.
9. Transfer the contents of each standard tube to an optical cuvette. Measure the OD value of each standard cuvette with a spectrophotometer at 505 nm (set to zero using double-distilled water).

Measurement of Samples

1. Mark microcentrifuge tubes for each sample and control. *Each sample requires a corresponding control. It is recommended to prepare all tubes in duplicate.*
2. Add 100 µl of sample to the corresponding sample tubes.
3. Add 500 µl of Activated Substrate Solution to all the sample and control tubes.
4. Mix fully, then incubate all tubes at 37°C for 30 minutes.
5. Add 500 µl of Chromogenic Reagent to all tubes.
6. Add 100 µl of sample to the corresponding control tubes.
7. Mix fully and incubate all tubes at 37°C for 20 minutes.
8. Add 5 ml of Alkali Reagent Working Solution to all tubes.
9. Stand the tubes for 10 minutes at room temperature.

10. Transfer the contents of each sample tube to an optical cuvette. Measure the OD value of each sample cuvette with a spectrophotometer at 505 nm (set to zero using double-distilled water).

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 Tube A) on the x-axis, and their Karmen Units on the y-axis (see definitions at the end of this section). The Karmen units for the standard tubes A, B, C, D, and E are 0, 24, 61, 114, and 190 respectively. As the standard in Tube A 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 an International Unit: An International Unit is defined as the amount of enzyme required to consume 1 µmol NADH per minute at 25°C.

Definition of a Karmen Unit: A Karmen 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 Karmen Unit = 0.482 IU/L.

The Karmen units for standard tubes A, B, C, D, and E are 0, 24, 61, 114, and 190 respectively.