Product Manual

Aspartate Aminotransferase (AST) Activity Assay Kit (Colorimetric)

Catalog Number

MET-5127 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Aspartate Aminotransferase (AST), also known as serum glutamic-oxaloacetic transaminase (GOT, SGOT), is an enzyme that catalyzes the transfer of an alpha amino group from aspartate to α-ketoglutarate, thereby producing glutamate and oxaloacetic acid (Figure 1). The reaction is important in amino acid biosynthesis and degradation. This pyridoxal phosphate (PLP) cofactor dependent transaminase is found in various tissues such as liver, heart, kidneys, brain, muscles, and red blood cells. AST is usually measured as a clinical marker of liver function to ascertain liver health in blood panel testing. Hepatocellular injury often leads to an increase in AST levels, which are usually measured in units per liter (U/L). Acute renal disease, musculoskeletal afflictions, myocardial infarction, pancreatitis, anemia, burns, and trauma have all displayed high levels of AST.

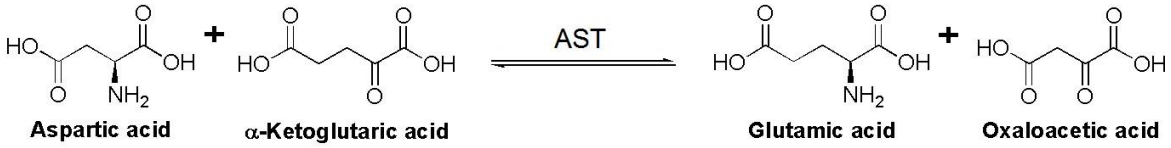

Figure 1: AST Reaction Principle.

Cell Biolabs' Aspartate Aminotransferase (AST) Assay Kit is a simple colorimetric assay that measures the activity of AST present within plasma, serum, tissue homogenates, or cell suspensions in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, standards and samples. An AST positive control is also provided.

Assay Principle

Cell Biolabs' Aspartate Aminotransferase (AST) Assay Kit measures AST activity through a series of enzyme driven reactions. AST within samples reacts with aspartate to transfer an amino group to α-ketoglutarate, producing glutamate and oxaloacetate. Glutamate is then detected with the colorimetric probe. Samples and standards are incubated for 60-120 minutes and then read with a standard 96-well spectrophotometric plate reader (450 nm). The AST activity is directly proportional to the amount of glutamate generated in the reaction. Sample AST levels are determined by comparison with the known glutamate standards.

Related Products

1. MET-5012: Lactate Assay (Colorimetric)
2. MET-5022: Glycogen Assay Kit (Colorimetric)
3. MET-5054: L-Amino Acid Assay Kit (Colorimetric)
4. MET-5093: Alanine Assay Kit (Colorimetric)
5. MET-5123: Alanine Aminotransferase (ALT) Activity assay Kit (Colorimetric)

Kit Components (shipped on blue ice)

1. AST Enzyme Mix (Part No. 51271B): One 150 µL amber tube
2. AST Substrate Mix (Part No. 51272C): One 1.5 mL tube
3. Glutamate Standard (Part No. 51273C): One 100 µL tube of a 100 mM solution
4. Colorimetric Probe (Part No. 50181C): Three 1.0 mL amber tubes
5. AST Positive Control (Part No. 51274B): One 50 µL tube of 100 U/mL enzyme

6. Assay Buffer (10X) (Part No. 51275A): One 25 mL bottle
7. Reaction Coenzyme (Part No. 51276D): One 300 µL amber tube

Materials Not Supplied

1. 1X PBS
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Standard 96-well microtiter plate
6. Spectrophotometric microplate reader capable of reading in the 450 nm absorbance range

Storage

Upon receipt, store the AST Enzyme Mix, AST Positive Control and 10X Assay Buffer at 4ºC and the Reaction Coenzyme at -80 ºC. Store the remaining components at -20ºC.

Preparation of Reagents

- 1X Assay Buffer: Warm the 10X Assay Buffer to room temperature prior to using. Dilute the Assay Buffer to 1X with deionized water by diluting the 25 mL Buffer with 225 mL deionized water for 250 mL total. Mix to homogeneity. Store the 1X Assay Buffer at 4ºC up to six months.
- AST Positive Control: Prior to use, dilute the positive control 1:100 in 1X Assay Buffer. Use only what is needed.
- Reaction Reagent: Prepare a Reaction Reagent by diluting the kit components accordingly. AST Enzyme Mix 1:100, AST Substrate Mix 1:10, and Colorimetric Probe 1:5 in 1X Assay Buffer. See Table 1 below for examples of Reaction Reagent preparation based on the number of assays employed. Mix thoroughly before use. For best results, use within 30 minutes of preparation. Do not store the Reaction Reagent solution.
- Reaction Coenzyme: Immediately prior to use, dilute the Reaction Coenzyme 1:10 in deionized water and mix. Use only what is needed. For best results, use within 30 minutes of preparation.

AST Enzyme Mix (mL)	AST Substrate Mix (mL)	Colorimetric Probe (mL)	1X Assay Buffer (mL)	**Number of Assays (0.150 mL/well)**
0.150	1.5	3	10.35	**100**
0.075	0.750	1.5	5.175	**50**
0.0375	0.375	0.750	2.588	**25**

Table 1. Preparation of Reaction Reagent

Preparation of Samples

Samples should be assayed immediately or stored at -80ºC prior to performing the assay. Optimal experimental conditions for samples must be determined by the investigator. The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design. A set of serial dilutions is recommended for samples to achieve optimal assay results and minimize possible interfering compounds. Run proper controls as necessary. Always run a standard curve with samples.

- Tissues: Weigh 500-1000 mg of sample and mince with scissors and a dounce until tissue is thoroughly liquified. Add 2 mL of 1X Assay Buffer or PBS and further sonicate the homogenate for several cycles on ice. Centrifuge 10 minutes at 12,000 x g to remove debris. Recover the supernatant and recentrifuge in a separate tube to clarify it further. Recover supernatant in a fresh microcentrifuge tube and incubate on ice. Prepare samples for testing and store the remaining supernatant at -80ºC. Prepare further dilutions in 1X Assay Buffer.
- Cell Suspensions: Prepare cells at 1 x 10^6 cells/mL and rapidly homogenize the cell pellet with 0.2 mL cold PBS or 1X Assay Buffer. Centrifuge 10 minutes at 12,000 x g to remove debris. Recover supernatant in a fresh microcentrifuge tube and incubate on ice. Prepare samples for testing and store the remaining supernatant at -80ºC. Prepare further dilutions in 1X Assay Buffer.
- Serum: Collect blood without using an anticoagulant. Allow blood to clot for 30 minutes at room temperature. Centrifuge at 2000 x g and 4ºC for 10 minutes. Remove the serum layer and store on ice. Take care to avoid disturbing the white buffy layer. Aliquot samples for testing and store remaining solution at -80ºC. Perform serum dilutions in 1X Assay Buffer or PBS. Perform several serial dilutions to ensure values are within the range of the standard curve.
- Plasma: Collect blood with heparin or citrate (EDTA could cause a quenching effect) and centrifuge at 1000 x g and 4ºC for 10 minutes. Remove the plasma layer and store on ice. Take care to avoid disturbing the white buffy layer. Aliquot samples for testing and store remaining solution at -80ºC. Perform plasma dilutions in 1X Assay Buffer or PBS. Perform several serial dilutions to ensure values are within the range of the standard curve.

Preparation of Glutamate Standard Curve

1. Prepare fresh glutamate standards by **diluting the Glutamate Standard stock tube from 100 mM to 1 mM (1:100) in 1X Assay Buffer**. (Example: Add 5 µL of Glutamate Standard stock tube to 0.495 mL of 1X Assay Buffer). **Vortex thoroughly.**
2. Prepare a series of the remaining glutamate standards in the concentration range of 0-250 µM by diluting the 1 mM (1000 µM) glutamate solution according to Table 2.

Tubes	1 mM Glutamate Solution (µL)	1X Assay Buffer (µL)	Final Glutamate Concentration (µM)	Glutamate Quantity (nmoles/well) *
1	125	375	250	12.5
2	250 of Tube #1	250	125	6.25
3	250 of Tube #2	250	62.5	3.13
4	250 of Tube #3	250	31.3	1.57
5	250 of Tube #4	250	15.6	0.78
6	250 of Tube #5	250	7.8	0.39
7	250 of Tube #6	250	3.9	0.20
8	0	250	0	0

Table 2. Preparation of Glutamate Standards.

*Note: *Based on 50 µL volume/well. Do not store diluted glutamate standard solutions.*

Assay Protocol

Each glutamate standard, AST positive control and sample should be assayed in duplicate. A freshly prepared standard curve and positive control should be used each time the assay is performed.

Note: The assay is continuous, thereby allowing for readings at multiple time points. This may be necessary in order to ensure the values of unknowns fall within the linear range of the standard curve.

1. Add 50 µL of the diluted glutamate standards, positive control or samples to each well of a 96-well microtiter plate.
2. Add 150 µL of the prepared Reaction Reagent to each well and mix the well contents thoroughly.
3. Add 25 µL of the prepared diluted Reaction Coenzyme solution to each well to be tested. Mix thoroughly.
4. Immediately read the absorbance of each microwell used on a spectrophotometric microplate reader using 450 nm absorbance. The initial time point is ($T_{Initial}$) and the initial plate absorbance reading is ($A_{Initial}$). Cover plate to protect from light and continue to incubate at 37ºC for up to 60-120 minutes.

Note: If measuring multiple time points, begin reading samples after adding the Reaction Reagent at every set time point (e.g., every 5 minutes). An initial lag phase (~2-5 minutes) may precede color development. After this lag phase and color begins to develop, take the initial measurement ($A_{Initial}$). Continue taking measurements until the reaction is complete, which is indicated by one of the following:

- *The absorbance (OD) of the most active sample exceeds the high end of the linear range of the standard curve. The penultimate (immediately prior) reading, where the absorbance value still falls within the linear range of the curve, is used to determine AST activity. This penultimate reading is (A_{Final}) at (T_{Final}) time point. The absorbance values for the initial and final measurements must fall within the linear range of the standard to be accurate.*
- *The absorbance (OD) of the most active sample does not significantly change from the prior reading. This indicates the reaction has reached a plateau and is not likely to continue.*

5. Once the assay is complete, read the absorbance of each microwell on a spectrophotometric microplate reader using 450 nm absorbance. This is the final time point (T_{Final}) plate reading (A_{Final}).

Example of Results

The following figures demonstrate typical AST Activity Assay results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

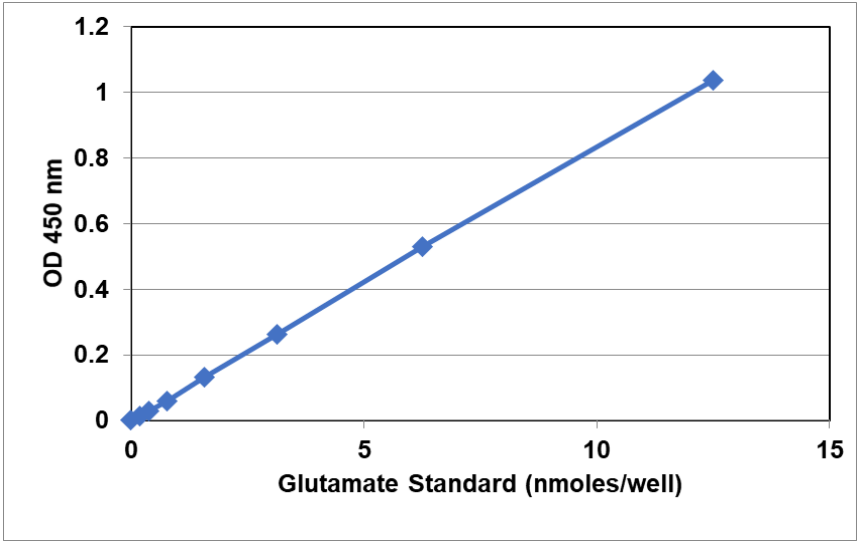

Figure 2: Example Glutamate Standard Curve.

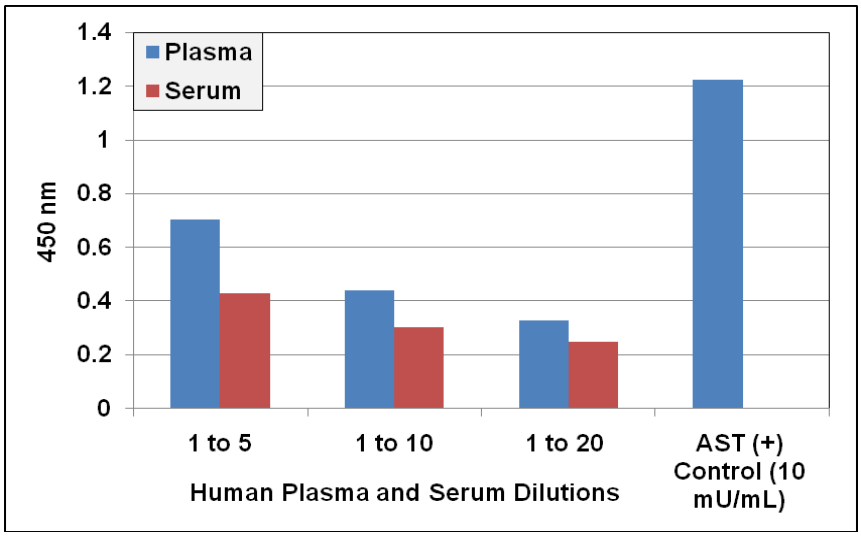

Figure 3: Plasma and Serum Samples. Human plasma and serum were diluted in 1X Assay Buffer and tested according to the assay instructions.

Calculation of Results

1. Determine the average absorbance values for every sample, control, and standard. Subtract the average zero standard value from itself and all standard and sample values. This is the background corrected absorbance. Use the T_{Final} readings to plot the glutamate standard curve graph.
2. Graph the standard curve with the corrected absorbance values (see Figure 2 for an example standard curve).
3. Calculate the change in sample absorbance values (ΔA) between the initial absorbance $(A_{Initial})$ and the final absorbance (A_{Final}):

$$\mathbf{(\Delta A) = (A_{Final}) - (A_{Initial})}$$

4. Compare the change in absorbance (ΔA) of each sample to the glutamate standard curve to determine the amount of glutamate produced within the assay. Only use values within the linear range of the standard curve.
5. Determine the AST activity in milliunits/mL (mU/mL) of a sample using the equation:

Q = Quantity (in nmoles/well) of glutamate produced as determined from standard graph
T = Reaction time (in minutes) determined by $T_{Final} - T_{Initial}$

$$AST\ Activity\ \left(\frac{mU}{mL}\right) = \left[\frac{Q}{(T_{Final} - T_{initial})\ x\ 0.05\ mL\ *}\right]$$

**Note: 50 µL sample volume. Be sure to account for any dilution factors made on unknown samples prior to the assay.*

AST activity is quantified as nmole/min/mL = milliunit/mL (mU/mL), where 1 milliunit of AST is the amount of enzyme that generates 1.0 nmole of glutamate per minute at 37ºC.

References

1. Dajnowicz, S., et al. (2017) *J. Biol. Chem.* **292**: 5970-5980.
2. Hirotsu, K., et al. (2005) *Chem. Rec.* **5(3)**: 160-172.
3. Karmen, A., et al. (1955) *J. Clin. Invest.* **34(1)**: 126-133.

Recent Product Citation

Elsyade, R. et al. (2021). Hazards of Chronic Exposure to Nonylphenol: Concomitant Effect on Non-alcoholic Fatty Liver Disease in Male Albino Rats. *Open Access Maced J Med Sci*. **9**(A):548-555 doi: 10.3889/oamjms.2021.6237.

Warranty

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