Amplite™ Colorimetric Aspartate Aminotransferase (AST) Assay Kit

Catalog number: 13801
Unit size: 200 Tests

Component	Storage	Amount
Component A: AST Enzyme Mixture	Freeze (<-15 °C), Minimize light exposure	1 bottle (lyophilized powder)
Component B: AST Assay Buffer	Freeze (<-15 °C), Minimize light exposure	1 bottle (10 mL)
Component C: NAD	Freeze (<-15 °C), Minimize light exposure	1 vial
Component D: AST Positive Control	Freeze (<-15 °C), Minimize light exposure	1 vial (10 U, lyophilized powder)

OVERVIEW

Aspartate aminotransferase (AST), also called serum glutamic oxaloacetic transaminase (GOT), is a member of transferase family. It catalyzes the reversible transfer of an alpha-amino group between aspartate and glutamate, and is an important enzyme in amino acid metabolism. AST is found in many body tissues such as liver, heart, muscle, kidneys, brain. In healthy subjects, serum AST levels are low. However, when cells are damaged, such as acute and chronic hepatitis, obstructive jaundice, carcinoma of liver, myocardial infarction, AST may leak into the blood stream and the AST levels are significantly elevated. Therefore, determination of serum AST level has great clinical and diagnostic significance. Amplite™ Colorimetric Aspartate Aminotransferase (AST) assay kit provides a quick and sensitive method for the measurement of AST in various biological samples. Aspartate transaminase catalyzes the reaction of aspartate and α-ketoglutarate to oxaloacetate and glutamate. The product L-glutamate is measured by the generation of a blue color product through an enzyme coupled reaction cycle. The signal can be read by an absorbance microplate reader at an absorbance ratio of A570 nm to A610 nm. With the Amplite™ Colorimetric Aspartate Aminotransferase Assay Kit, we have detected as little as 2 mU/mL AST in a 100 µL reaction volume. The assay is robust, and can be readily adapted for a wide variety of applications.

AT A GLANCE

Protocol summary

1. Prepare AST working solution (50 µL)
2. Add AST standards or test samples (50 µL)
3. Incubate at 37ºC for 30 - 120 minutes
4. Monitor absorbance increase at the absrobance ratio of A_{570nm} /A_{610nm}

Important Thaw the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Absorbance microplate reader
Absorbance:	570/610 nm
Recommended plate:	Clear bottom

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. AST standard solution (100 U/ml):
Add 100 µL DPBS buffer into the vial of AST Positive Control (Component D) to make 100 U/mL AST standard solution.

PREPARATION OF STANDARD SOLUTION

AST standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/13801

Add 5 µL of 100 U/mL AST standard solution into 997 µL DPBS buffer with 0.1% BSA to generate 500 mU/mL AST standard solution (AST7). Take 500 mU/mL AST standard solution (AST7) and perform 1:2 serial dilutions in DPBS buffer with 0.1% BSA to get serial diluted AST standards (AST6 - AST1).

PREPARATION OF WORKING SOLUTION

1. Add 100 µL of ddH2O into the vial of NAD (Component C) to have 100X NAD solution.

2. Add 10 mL of AST Assay Buffer (Component B) into the bottle of AST Enzyme Mixture (Component A), and mix well. Then, add the whole vial of 100X NAD solution into the AST Enzyme Mixture solution to make AST working solution.

Note This AST working solution is enough for two 96-well plates. It is unstable at room temperature, and should be used promptly within 2 hours. Avoid exposure to light.

Note Alternatively, one can make a 50X of AST Enzyme Mixture stock solution by adding 200 µL of H2O into the bottle of AST Enzyme Mixture (Component A), and then prepare the AST working solution by mix the stock solution with Assay Buffer (Component B) and 100X NAD solution proportionally.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of AST standards and test samples in a clear bottom 96-well microplate. AST= AST Standards (AST1 - AST7, 7.8 to 500 mU/mL), BL=Blank Control, TS=Test Samples.

BL	BL	TS	TS
AST1	AST1	...	...
AST2	AST2	...	...
AST3	AST3		
AST4	AST4		
AST5	AST5		
AST6	AST6		
AST7	AST7		

Table 2. Reagent composition for each well.

Note The AST standards are for positive control only, and should not be relied on as a quantitation standard for enzyme activity.

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Well	Volume	Reagent
AST1 - AST7	50 µL	Serial Dilutions (7.8 to 500 mU/mL)
BL	50 µL	DPBS with 0.1% BSA
TS	50 µL	test sample

1. Prepare AST standards (AST), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.
2. Add 50 µL of AST working solution to each well of AST standard, blank control, and test samples to make the total AST assay volume of 100 µL/well. For a 384-well plate, add 25 µL of AST working solution into each well instead, for a total volume of 50 µL/well.
3. Incubate the reaction at 37ºC for 30 - 120 minutes, protected from light.

   Note The background of Blank Control increases with time and temperature.

4. Monitor the absorbance increase with an absorbance plate reader at the absorbance ratio of A_{570nm} /A_{610nm}.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Abs 575/ Abs 610) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate AST samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

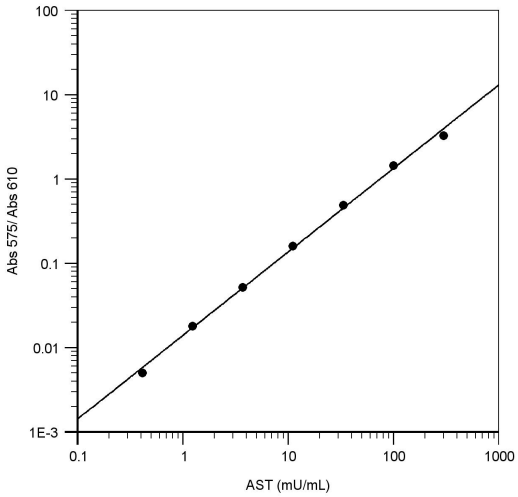

Figure 1. AST dose response was measured with Amplite™ Colorimetric Aspartate Aminotransferase (AST) Assay Kit in a 96-well clear bottom plate using a SpectraMax microplate reader (Molecular Devices).

DISCLAIMER