Amplite™ Fluorimetric Aspartate Aminotransferase (AST) Assay Kit

Catalog number: 13800
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 Enzyme 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™ Fluorimetric 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 red fluorescent product through an enzyme coupled reaction cycle. The signal can be read by a fluorescence microplate reader. With the Amplite™ Fluorimetric 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 37 °C for 20 - 30 min or RT for 60 min
4. Monitor fluorescence increase at Ex/Em = 540/590 nm

Important Thaw one bottle each of Component A and B at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 nm
Recommended plate:	Solid black

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 to 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/13800

Add 3 µL of 100 U/mL AST standard solution into 997 µL DPBS buffer with 0.1% BSA to generate 300 mU/mL AST standard solution (AST7). Take 300 µL of 300 mU/mL AST standard solution to perform 1:3 serial dilutions to get serial dilutions of AST standard (AST6 - AST1).

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

PREPARATION OF WORKING SOLUTION

1. NAD solution (100X):
Add 100 µL of ddH_2O into the vial of NAD (Component C).

2. AST Enzyme Mixture solution:
Add 10 mL of AST Assay Buffer (Component B) into the bottle of AST Enzyme Mixture (Component A), and mix well.

3. AST working solution:
Add the whole vial of 100X NAD solution (from 1) into the AST Enzyme Mixture solution (from 2) to have 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. Alternatively, one can make a 50X of AST Enzyme Mixture stock solution by adding 200 µL of H_2O into the bottle of Component A, and then prepare the AST working solution by mixing 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 solid black 96-well microplate. AST= AST Standards (AST1 - AST7, 0.3 to 300 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.

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Well	Volume	Reagent
AST1-AST7	50 µL	Serial Dilution (0.3 to 300 mU/mL)
BL	50 µL	DPBS with 0.1% BSA
TS	50 µL	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 37°C for 20 - 30 minutes or room temperature for 60 minutes, protected from light.

   Note The background of Blank Control increases with time and temperature.
4. Monitor the fluorescence increase with a fluorescence plate reader at Excitation = 530 - 570 nm, Emission = 590 - 600 nm (optimal Ex/Em = 540/590 nm, cut off at 570 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) 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

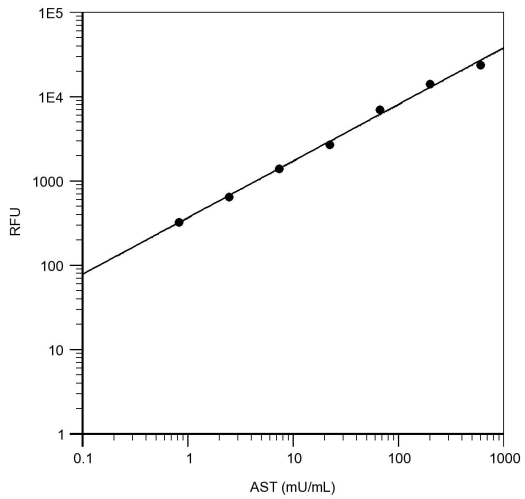

Figure 1. AST dose response was measured with Amplite™ Fluorimetric Aspartate Aminotransferase Assay Kit in a 96-well black plate using a Gemini fluorescence microplate reader (Molecular Devices).

DISCLAIMER