Amplite™ Fluorimetric Aspartate Aminotransferase Assay Kit
Red Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 13800 (200 assays)	Keep in freezer and avoid exposure to light	Fluorescence microplate readers

Introduction

Aspartate aminotransferase (AST), also called serum glutamic oxaloacetic transaminase (GOT), is a member of transferase family. It catalyzes the reversible transfer of an α-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:

Aspartate (Asp) + α-ketoglutarate ⇌ oxaloacetate + glutamate (Glu)

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 at Ex/Em = 530-570 nm/590-600 nm (optimal Ex/Em = 540 nm/590 nm). 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.

Kit Components

Components	Amount	Storage Conditions
Component A: AST Enzyme Mixture	1 bottle (lyophilized powder)	-20 °C
Component B: AST Assay Buffer	1 bottle (10 mL)	-20 °C
Component C: NAD	1 vial	-20 °C
Component D: AST Positive Control	1 vial (10 U, lyophilized powder)	-20 °C

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare AST reaction mixture (50 µL) → Add AST standards or test samples (50 µL) → Incubate at 37 °C for 20-30 min or RT for 60 min → Monitor fluorescence increase at Ex/Em = 540/590 nm

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

1. Prepare serial dilutions of AST standard (1 to 300 mU/mL):

1.1 Add 100 µL DPBS Buffer to AST Positive Control (Component D) to make 100 U/mL AST standard solution.
Note: The unused AST Positive Control (Component D) divided into single use aliquots and stored at -20°C.

1.2 Add 3 µL of 100 U/mL AST standard solution (from Step 1.1) into 997 µL DPBS buffer with 0.1% BSA to generate 300 mU/mL AST standard solution.

1.3 Take 300 µL of 300 mU/mL AST standard solution to perform 1:3 serial dilutions to get 100, 30, 10, 3, 1, 0.3 and 0 mU/mL serial dilutions of AST standard.

1.4 Add serial dilutions of AST standard and AST containing test samples into a solid black 96-well microplate as described in Tables 1 and 2.
Note: Dilute the test samples to the concentration range in DPBS buffer with 0.1% BSA if needed.

Table 1 Layout of AST standards and test samples in a solid black 96-well microplate

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

Note: AST= AST Standards, BL=Blank Control, TS=Test Samples.

Table 2 Reagent composition for each well

AST Standard	**Blank Control**	**Test Sample**
Serial Dilutions*: 50 µL	DPBS with 0.1% BSA: 50 µL	50 µL

Note1: Add the serially diluted AST standards from 0.3 mU/mL to 300 mU/mL into wells from AST1 to AST 7 in duplicate.
Note 2: The AST standards are for positive control only, and should not be relied on as a quantitation standard for enzyme activity.

2. Prepare AST assay mixture:

2.1 Add 100 µL of ddH_2O into the vial of NAD (Component C) to have 100X NAD solution.

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

2.3 Add whole vial of 100X NAD solution (from Step 2.1) into the AST Enzyme Mixture solution (from Step 2.2) to have AST assay mixture.
Note1: This AST assay mixture is enough for two 96-well plates. It is unstable at room temperature, and should be used promptly within 2 hours and avoid exposure to light.

Note2: 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 assay mixture by mix the stock solution with assay buffer (Component B) and 100 X NAD solution proportionally. Aliquot and store the unused 50X AST Enzyme Mixture stock solution and 100X NAD solution at -20°C, and avoid freeze-thaw cycles.

3. Run AST assay:

3.1 Add 50 µL of AST assay mixture (from Step 2.3) to each well of AST standard, blank control, and test samples (see Step 1.4) to make the total AST assay volume of 100 µL/well.
Note: For a 384-well plate, add 25 µL of sample and 25 µL of AST assay mixture into each well.

3.2 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.

3.3 Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 530 - 570/590 - 600 nm (optimal Ex/Em = 540/590 nm, cut off at 570 nm).

Data Analysis

The fluorescence in blank wells (with the DPBS buffer with 0.1% BSA only) is used as a control, and is subtracted from the values for those wells with the AST reactions. An AST standard curve is shown in Figure 1. *Note: The fluorescence background increases with time, thus it is important to subtract the intensity value of the blank wells from that of each data point.*

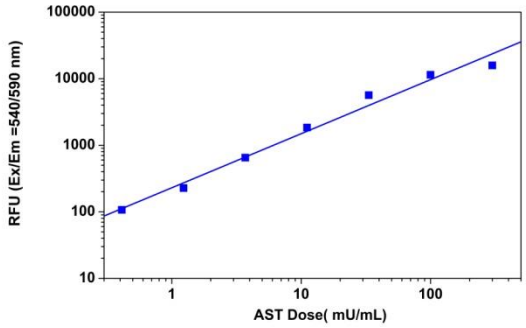

Figure 1. AST dose response was measured with Amplite™ Fluorimetric Aspartate Aminotransferase Assay Kit in a 96-well black plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 2 mU/mL AST can be detected with 60 min incubation (n=3) at room temperature.

References

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