Amplite™ Fluorimetric Alanine Aminotransferase Assay Kit

Catalog number: 13802
Unit size: 200 Tests

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

OVERVIEW

Alanine aminotransferase (ALT), also called serum glutamate pyruvic transaminase (GPT), is a member of transferase family. It catalyzes the reversible transfer of an alpha-amino group between alanine and glutamate, and is an important enzyme in amino acid metabolism. ALT is found mainly in liver and small amount in heart, muscle, and kidneys. In healthy subjects, serum ALT levels are low. However, when cells are damaged, such as acute and chronic hepatitis, obstructive jaundice, carcinoma of liver, myocardial infarction, ALT may leak into the blood stream and the ALT levels are significantly elevated. Therefore, determination of serum ALT level has great clinical and diagnostic significance. Amplite™ Fluorimetric Alanine Aminotransferase Assay Kit provides a quick and sensitive method for the measurement of ALT in various biological samples. ALT catalyzes the reaction of alanine and α-ketoglutarate to pyruvate and glutamate. The product 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 Alanine Aminotransferase Assay Kit as little as 4 mU/mL ALT was detected 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 ALT working solution (50 µL)
2. Add ALT standards or test samples (50 µL)
3. Incubate at 37°C for 30 min to 60 min
4. Monitor fluorescence increase at Ex/Em = 540/590 nm

Important Thaw one bottle 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. ALT standard solution:
Add 100 µL DPBS into the vial of ALT Positive Control (Component D) to make 100 U/mL ALT stock solution.

PREPARATION OF STANDARD SOLUTION

ALT standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/13802

Add 10 µL of 100 U/mL ALT standard solution into 990 µL DPBS buffer with 0.1% BSA to generate 1000 mU/mL ALT standard solution (ALT7). Take the 1000 mU/mL ALT standard solution and perform 1:3 serial dilutions to get serial dilutions of ALT standard (ALT6 - ALT1).

PREPARATION OF WORKING SOLUTION

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

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

3. ALT working solution:
Add the whole vial of 100X NAD solution (from 1) into the ALT Enzyme Mixture solution (from 2) to have ALT working solution.

Note This ALT working solution 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. Alternatively, one can make a 50X of ALT Enzyme Mixture stock solution by adding 200 µL of H_2O into the bottle of Component A, and then prepare the ALT working solution by mixing the stock solution with assay buffer (Component B) and 100x NAD solution proportionally.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of ALT standards and test samples in a solid black 96-well microplate. ALT = ALT standard (ALT1 - ALT7, 1 to 1000 mU/mL); BL = blank control; TS = test sample.

BL	BL	TS	TS
ALT1	ALT1	...	...
ALT2	ALT2	...	...
ALT3	ALT3		
ALT4	ALT4		
ALT5	ALT5		
ALT6	ALT6		
ALT7	ALT7		

Table 2. Layout of ALT standards and test samples in a solid black 96-well microplate.

Well	Volume	Reagent
ALT1 - ALT7	50 µL	Serial Dilution (1 to 1000 mU/mL)
BL	50 µL	DPBS with 0.1% BSA

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TS	50 µL	Test Sample

1. Prepare ALT standards (ALT), blank controls (BL), and test samples (TS) into a solid black 96-well microplate according the the layout provided in Table 1 and Table 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

   Note Dilute the test samples to the appropriate concentration range (indicated by the ALT standards) in DPBS buffer with 0.1% BSA if needed.

2. Add 50 µL of ALT working solution into each well of ALT standard, blank control, and test samples to make the total ALT assay volume of 100 µL/well. For a 384-well microplate, add 25 µL of ALT working solution into each well instead, for a total volume of 50 µL/well.

3. Incubate the reaction at 37°C for 30 min to 60 minutes, protected from light.

   Note The background of Blank Control will increase with time.

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 ALT 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

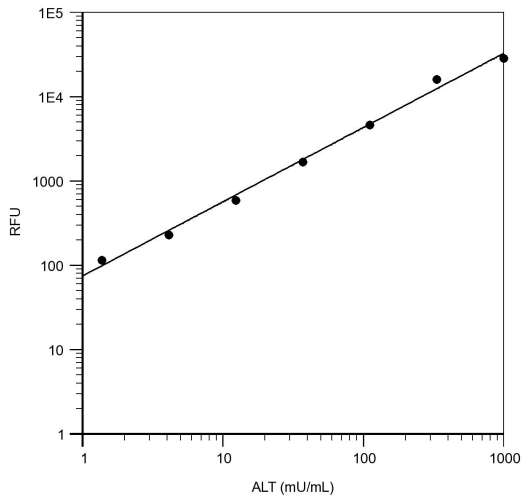

Figure 1. ALT dose response was measured with Amplite™ Fluorimetric Alanine Aminotransferase Assay Kit in a 96-well black plate using a Gemini fluorescence microplate reader (Molecular Devices).

DISCLAIMER