

## Amplite™ Fluorimetric Alanine Aminotransferase Assay Kit

### *\*Red Fluorescence\**

Ordering Information	Storage Conditions	Instrument Platform
Product Number: 13802 (200 assays)	Keep frozen and avoid light	Fluorescence microplate readers

### Introduction

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  $\alpha$ -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 at Ex/Em = 530-570 nm/590-600 nm (optimal Ex/Em = 540 nm/590 nm). With the Amplite™ Fluorimetric Alanine Aminotransferase Assay Kit, as little as 4 mU/mL ALT was detected in a 100  $\mu$ L reaction volume. The assay is robust, and can be readily adapted for a wide variety of applications.

### Kit Components

Components	Amount
Component A: ALT Enzyme Mixture	1 bottle (lyophilized powder)
Component B: ALT Assay Buffer	bottle (10 mL)
Component C: NAD	1 vial
Component D: ALT Positive Control	1 vial (10 U)

### Assay Protocol for One 96-Well Plate

#### Brief Summary

**Prepare ALT reaction mixture (50  $\mu$ L) → Add ALT standards or test samples (50  $\mu$ L) → Incubate at 37 °C for 30 min to 60 min → Monitor fluorescence increase at Ex/Em = 540/590 nm**

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

#### 1. Prepare serial dilutions of ALT standard (1 to 1000 mU/mL):

- 1.1 Add 100  $\mu$ L DPBS into the vial of ALT Positive Control (Component D) to make 100U/mL ALT stock solution.

*Note: The unused ALT stock solution should be divided into single use aliquots and stored at -20 °C.*

- 1.2 Add 10  $\mu$ L of 100 U/mL ALT standard solution (from Step 1.1) into 990  $\mu$ L DPBS buffer with 0.1% BSA to generate 1 U/mL ALT standard solution.

- 1.3 Take 300  $\mu$ L of 1 U/mL ALT standard solution to perform 1:3 serial dilutions to get 300, 100, 30, 10, 3, 1 and 0 mU/mL serial dilutions of ALT standard.
- 1.4 Add serial dilutions of ALT standard and ALT 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 ALT standards and test samples in a solid black 96-well microplate

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

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

**Table 2** Reagent composition for each well

ALT Standard	Blank Control	Test Sample
Serial Dilutions*: 50 $\mu$ L	DPBS with 0.1% BSA: 50 $\mu$ L	50 $\mu$ L

*Note: Add the serially diluted ALT standards from 1 mU/mL to 1U/mL into wells from ALT1 to ALT 7 in duplicate.*

## 2. Prepare ALT assay mixture:

- 2.1 Add 100  $\mu$ L of ddH<sub>2</sub>O into the vial of NAD (Component C) to have 100X NAD solution.
- 2.2 Add 10 mL of ALT Assay Buffer (Component B) into the bottle of ALT Enzyme Mixture (Component A), and mix well.
- 2.3 Add whole vial of 100X NAD solution (from Step 2.1) into the ALT Enzyme Mixture solution (from Step 2.2) to have ALT assay mixture.

*Note1: This ALT 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 ALT Enzyme Mixture stock solution by adding 200  $\mu$ L of H<sub>2</sub>O into the bottle of Component A, and then prepare the ALT assay mixture by mix the stock solution with assay buffer (Component B) and 100x NAD solution proportionally. Aliquot and store the unused 50X ALT Enzyme Mixture stock solution and 100X NAD solution at -20°C, and avoid freeze-thaw cycles.*

## 3. Run ALT assay:

- 3.1 Add 50  $\mu$ L of ALT assay mixture (from Step 2.3) to each well of ALT standard, blank control, and test samples (see Step 1.4) to make the total ALT assay volume of 100  $\mu$ L/well.

*Note: For a 384-well plate, add 25  $\mu$ L of sample and 25  $\mu$ L of ALT assay mixture into each well.*

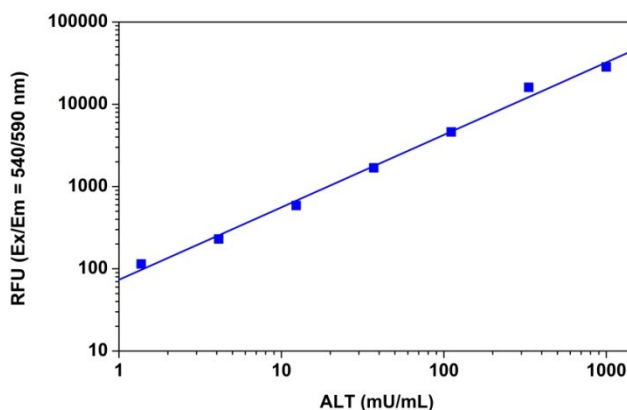
- 3.2 Incubate the reaction at 37 °C for 30 min to 60 minutes, protected from light.

*Note: The background of Blank Control increases with time.*

- 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 ALT reactions. An ALT 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.** 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). As low as 4 mU/mL ALT can be detected with 60 min incubation (n=3) at 37°C.

## References

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2. Gaze DC (2007). "The role of existing and novel cardiac biomarkers for cardioprotection". *Curr. Opin. Invest. Drugs* 8 (9): 711–7.
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4. Nalpas B, Vassault A, Charpin S, Lacour B, Berthelot P (1986). "Serum mitochondrial aspartate aminotransferase as a marker of chronic alcoholism: diagnostic value and interpretation in a liver unit". *Hepatology* 6 (4): 608–614.
5. Almo SC, Smith DL, Danishefsky AT, Ringe D (March 1994). "The structural basis for the altered substrate specificity of the R292D active site mutant of aspartate aminotransferase from *E. coli*". *Protein Eng.* 7 (3): 405–412.

**Warning:** This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at [info@aatbio.com](mailto:info@aatbio.com) if you have any questions.