# Amplite™ Colorimetric Alanine Aminotransferase Assay Kit

**Blue Color**

| *Ordering Information* | *Storage Conditions* | *Instrument Platform* |
|---|---|---|
| Product Number: 13803 (200 assays) | Keep in frozen and avoid light | Absorbance 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 α-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™ Colorimetric 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:

**Alanine (Ala) + α-ketoglutarate ⇌ pyruvate + glutamate (Glu)**

The product 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 the absorbance ratio of $A_{570nm}$ to $A_{610nm}$. With the Amplite™ Colorimetric Alanine Aminotransferase Assay Kit, as little as 10 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.

## Kit Components

| Components | Amount |
|---|---|
| Component A: ALT Enzyme Mixture | 1 bottle (lyophilized powder) |
| Component B: ALT Assay Buffer | 1 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 µL) → Add ALT standards or test samples (50 µL) → Incubate at 37ºC for 60 min to 120 min → Monitor absorbance increase at the absorbance ratio of $A_{570nm}/A_{610nm}$**

*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 µL 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 -20ºC.*

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


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1.3 Take 400 µL of 1 U/mL ALT standard solution to perform 1:2 serial dilutions to get 500, 250, 125, 62.5, 31.25, 15.6, and 0 mU/mL serial dilutions of ALT standard.

1.4 Add serial dilutions of ALT standard and ALT containing test samples into a white/clear bottom 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 clear, white, or black with clear bottom 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 µL | DPBS with 0.1% BSA: 50 µL | 50 µL |

*Note: Add the serially diluted ALT standards from 15.6 mU/mL to 1U/mL into wells from ALT1 to ALT 7 in duplicate.*

**2. Prepare ALT assay mixture:**

2.1 Add 100 µL of dd$H_2O$ 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 µL of $H_2O$ 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 µL 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 µL/well.
*Note: For a 384-well plate, add 25 µL of sample and 25 µL of ALT assay mixture into each well.*

3.2 Incubate the reaction at 37°C for 60 min to 120 minutes, protected from light.
*Note: The background of Blank Control increases with time.*

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

## Data Analysis

The absorbance 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.*

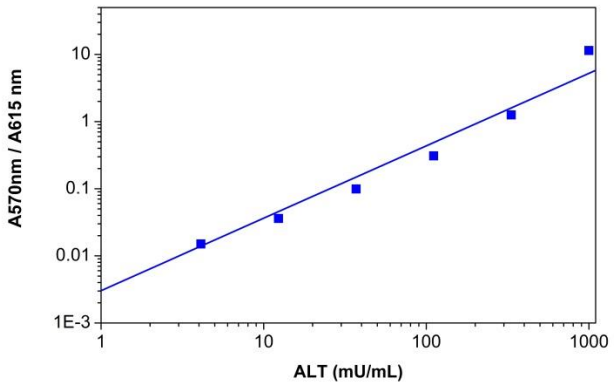


**Figure 1.** ALT dose response was measured with Amplite™ Colorimetric Alanine Aminotransferase Assay Kit in a 96-well black/clear bottom plate using a SpectraMax microplate reader (Molecular Devices). As low as 15.6 mU/mL ALT can be detected with 90 min incubation (n=3) at 37°C.

## References

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