Amplite™ Colorimetric L-Alanine Assay Kit

Catalog number: 13826
Unit size: 200 Tests

Component	Storage	Amount
Component A: Quest Fluor™ L-Alanine Sensor	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B1: Enzyme Mix 1	Freeze (<-15 °C), Minimize light exposure	2 bottles (lyophilized powder)
Component B2: Enzyme Mix 2	Freeze (<-15 °C), Minimize light exposure	2 vials (lyophilized powder)
Component C: Assay Buffer	Freeze (<-15 °C)	1 bottle (10 mL)
Component D: L-Alanine Standard	Freeze (<-15 °C), Minimize light exposure	100 mM (100 µL)
Component E: DMSO	Freeze (<-15 °C)	1 vial (100 µL)

OVERVIEW

L-alanine (L-Ala) plays a crucial role as a building block of important proteins. L-alanine is mostly synthesized by the muscle cells from lactic acid and absorbed into blood via the liver. It is converted into pyruvate by glutamic-pyruvic transaminase to enter the metabolic mainstream. L-Ala is critical for the production of glucose and hence blood sugar management, and plays an important role on the immune system and prevention of kidney stones. Insufficiency of L-alanine is usually a sign of poor nutrition, low protein diet, as well as stress. AAT Bioquest's Amplite™ Colorimetric L-Alanine Assay Kit offers a sensitive colorimetric assay for quantifying L-alanine in biological samples. It utilizes an enzyme coupled reaction that releases hydrogen peroxide, which can be detected by Quest Fluor™ L-Alanine Sensor in an absorbance microplate reader at 575 nm.

AT A GLANCE

Protocol summary

1. Prepare L-Alanine working solution (50 µL)
2. Add L-Alanine standards or test samples (50 µL)
3. Incubate at 37°C for 30 minutes to 1 hour
4. Monitor absorbance intensity at 575 nm

Important To achieve the best result, it is strongly recommended to use the white clear plates. Thaw kit components at room temperature before use.

KEY PARAMETERS

Instrument:	Absorbance microplate reader
Absorbance:	575 nm
Recommended plate:	Clear bottom

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. Quest Fluor™ L-Alanine Sensor stock solution (200X):
Add 55 µL of DMSO (Component E) into Quest Fluor™ L-Alanine Sensor (Component A) to make 200X Quest Fluor™ L-Alanine Sensor stock solution.

2. L-Alanine standard solution (1 mM):
Add 10 µL of 100 mM L-Alanine Standard (Component D) into 990 µL of PBS (pH 7.0) to get 1 mM L-Alanine standard solution.

PREPARATION OF STANDARD SOLUTION

L-Alanine standard

For convenience, use the Serial Dilution Planner:

https://www.aatbio.com/tools/serial-dilution/13826

Add 100 µL of 1 mM L-Alanine standard solution into 900 µL PBS to make 100 µM L-Alanine standard solution (AS7). Take 100 µM L-Alanine standard solution (AS7) and perform 1:2 serial dilutions in PBS to get serially diluted L-Alanine standards (AS6 - AS1).

PREPARATION OF WORKING SOLUTION

1. Add 5 mL Assay Buffer (Component C) into one Enzyme Mix1 bottle (Component B1) and mix well.

2. Add 100 µL of ddH_2O into one Enzyme Mix2 vial (Component B2) and mix well.

3. Transfer entire vial (100 µL) of Enzyme Mix2 and 25 uL of 200X L-Alanine Sensor stock solution into the Enzyme Mix1 bottle and mix well to make L-Alanine working solution.

Note L-Alanine working solution is not stable - use it promptly and avoid direct exposure to light.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of L-Alanine standards and test samples in a white clear 96-well microplate. AS= L-Alanine Standard (AS1 - AS7, 1.563 to 100 µM), BL=Blank Control, TS=Test Sample.

BL	BL	TS	TS
AS1	AS1	...	...
AS2	AS2	...	...
AS3	AS3		
AS4	AS4		
AS5	AS5		
AS6	AS6		
AS7	AS7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
AS1 - AS7	50 µL	Serial Dilutions (1.563 to 100 µM)
BL	50 µL	PBS
TS	50 µL	test sample

1. Prepare L-Alanine standards (AS), 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.

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

   Note Run the L-Alanine assay at pH 6.5 to 7.0.

3. Incubate the reaction at 37°C for 30 minutes to 1 hour.

4. Monitor the absorbance increase with an absorbance plate reader at 575 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Absorbance) 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 L-Alanine 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

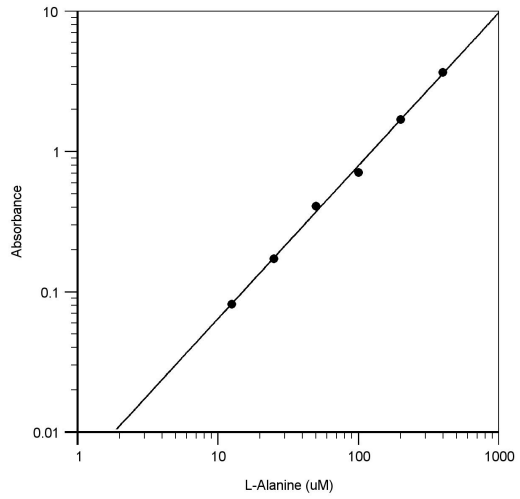

Figure 1. L-alanine dose response was measured with Amplite™ Colorimetric L-Alanine Assay Kit on a white clear 96-well plate using a SpectraMax microplate reader (Molecular Devices).

DISCLAIMER