Amplite® Colorimetric L-Alanine Assay Kit

Catalog number: 13826
Unit size: 200 Tests

Component	Storage	Amount (Cat No. 13826)
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	1 vial (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 standards or test samples (50 µL)
2. Add L-Alanine working solution (50 µL)
3. Incubate at 37°C for 1 hour to 2 hours
4. Monitor absorbance intensity at 575 nm

Important Note

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

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

Quest Fluor™ L-Alanine Sensor stock solution (200X)

Add 50 µL of DMSO (Component E) into Quest Fluor™ L-Alanine Sensor (Component A) to make 200X Quest Fluor™ L-Alanine Sensor stock solution.

L-Alanine standard solution

Add 10 µL of 100 mM L-Alanine Standard (Component D) into 490 µL of PBS (pH 7.0) to get 2 mM L-Alanine standard solution. Add 7.5 µL of 100 mM L-Alanine Standard (Component D) into 492.5 µL of PBS (pH 7.0) to get 1.5 mM L-Alanine standard solution.

PREPARATION OF WORKING SOLUTION

L- Alanine standard serial dilutions

Use 2 mM L-Alanine standard stock solution and perform 1:2 serial dilutions in PBS to get serially diluted L-Alanine standards (2000, 1000, 500, 250, 125 uM). Use 1.5 mM L-Alanine standard stock solution and perform 1:2 serial dilutions in PBS to get serially diluted L-Alanine standards (1500, 750 uM).

L-Alanine 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, 125 to 2000 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 (125 to 2000 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.
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 1 hour to 2 hours.
4. Monitor the absorbance increase with an absorbance plate reader at 575 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

Placeholder for image details

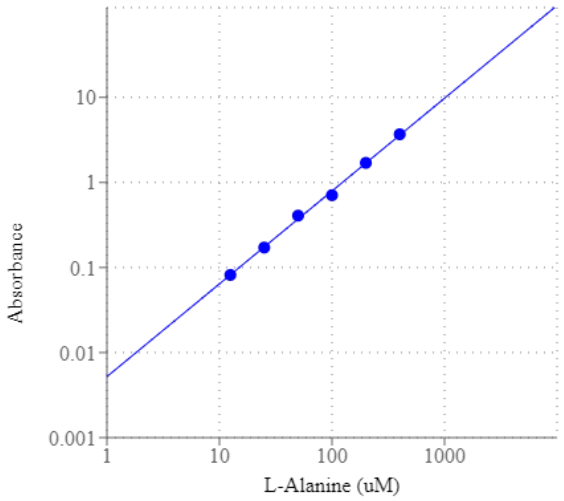

Figure 1. L-alanine dose response was measured with Amplite® Colorimetric L-Alanine Assay Kit on a white clear 96-well plate using a SpectraMax microplate reader (Molecular Devices).

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.