Amplite™ Fluorimetric L-Alanine Assay Kit

Catalog number: 13825
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 in 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™ Fluorimetric L-Alanine Assay Kit offers a sensitive fluorescent assay for quantifying L-alanine in biological samples. It utilizes an enzyme coupled reaction that releases hydrogen peroxide, which is detected by Quest Fluor™ L-Alanine Sensor with a fluorescence microplate reader.

AT A GLANCE

Protocol summary

1. Prepare test samples along with serially diluted L-Alanine standards (50 µL)
2. Add equal volume of L-Alanine working solution (50 µL)
3. Incubate at 37°C for 30 minutes to 1 hour
4. Monitor fluorescence intensity at Ex/Em = 540/590 nm

Important To achieve the best result, it's strongly recommended to use the black plates. Thaw kit components at room temperature before use.

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. 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 (Component D) into 990 µL PBS (pH 7.0) to get 1 mM L-alanine solution.

PREPARATION OF STANDARD SOLUTION

L-Alanine standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/13825

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

PREPARATION OF WORKING SOLUTION

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

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

3. Transfer entire vial (100 µL) of Enzyme Mix 2 and 25 uL of 200X L-alanine sensor stock solution into the Enzyme Mix 1 bottle; mix well.

Note The working solution is not stable. Use promptly and avoid direct exposure to light.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of L-alanine standards and test samples in a solid black 96-well microplate. AS = L-Alanine standard (AS1 - AS7, 1.5 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 Dilution (1.5 to 100 µM)
BL	50 µL	1X PBS Buffer
TS	50	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 mixture at 37°C for 30 minutes to 1 hour.

4. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 540/590 nm (cut off: 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 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

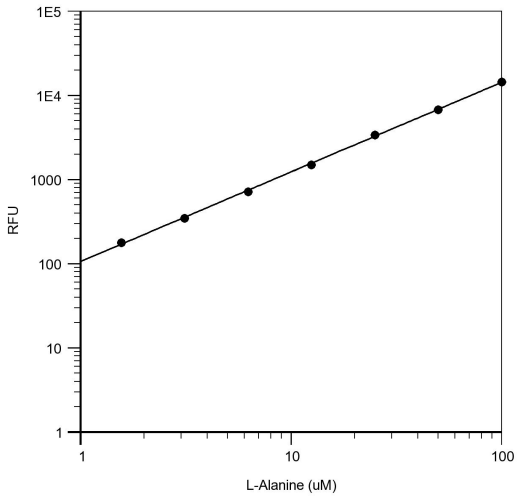

Figure 1. L-alanine dose response was measured with Amplite™ Fluorimetric L-Alanine Assay Kit on a solid black 96-well plate using a Gemini microplate reader (Molecular Devices).

DISCLAIMER