Amplite™ Fluorimetric L-Alanine Assay Kit

Red Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Cat#: 13825 (200 Assays)	Keep in freezer and protect from light	Fluorescence microplate readers

Introduction

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 Ex/Em = 540/590 nm.

Kit Components

Components	Amount
Component A: Quest Fluor™ L-Alanine Sensor	1 vial
Component B1: Enzyme Mix1	2 bottles (lyophilized powder)
Component B2: Enzyme Mix2	2 vials (lyophilized powder)
Component C: Assay Buffer	1 bottle (10 mL)
Component D: L-Alanine Standard	100 mM (100 µL)
Component E: DMSO	1 vial (100 µL)

Assay Protocol for One 96-Well Plate

Brief Summary

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

Note: To achieve the best result, it's strongly recommended to use the black plates.

1. Prepare L-Alanine Assay Mixture:

1.1 Thaw kit components at room temperature before use.

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

1.3 Make Assay Mixture:

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

1.3.2 Add100 µL of ddH_2O into one Enzyme Mix2 vial (Component B2) mix well.

1.3.3 Transfer entire vial (100 µL) of Enzyme Mix2 (from Step 1.3.2), and 25 uL of 200X L-alanine sensor stock solution (from Step 1.2) into the Enzyme Mix1 bottle (from Step 1.3.1) and mix well.

Note1: The assay mixture is not stable, use it promptly, and avoid direct exposure to light.

Note2: Store unused 200 X Quest Fluor™ L-alanine sensor stock solution (from Step 1.2) at -20° C, avoid light and repeated freeze-thaw cycles.

2. Prepare Serially Diuted L-Alanine Standards and Test Samples:

2.1 Prepare L-Alanine Standard: Add 10 µL of 100 mM L-alanine (Component D) into 990 µL PBS (pH 7.0) to get 1mM L-alanine solution. Add 100 µL of 1mM L-alanine standard solution into 900 µL PBS to make 100 µM L-alanine solution. Perform 1:2 serial dilutions to get 50, 25, 12.5, 6.25, 3.125 and 1.563 µM serially diluted L-alanine standards.

2.2 Add L-alanine containing samples and serially diluted L-alanine standards into a solid black 96-well microplate according to Tables 1.

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Table 1 Layout of L-alanine standards and test samples in a solid black 96-well microplate

BL	BL	TS	TS	….	….						
AS 1	AS 1	….	….	….	….						
AS 2	AS 2										
AS 3	AS 3										
AS 4	AS 4										
AS 5	AS 5										
AS 6	AS 6										
AS 7	AS 7										

*Note 1: AS= L-Alanine Standard, BL=Blank Control (*PBS*), TS=Test Sample.*
Note 2: Add the serial dilutions of L-Alanine Standard from 1.5 µM to 100 µM into wells from AS1 to AS7.

3. Run L-Alanine Assay:

3.1 Add 50 µL of Assay Mixture (from Step 1.3.3) into each well of L-alanine standard, blank control and test samples (see Step 2.2) to make the total L-alanine assay volume of 100 µL/well.
Note 1: For a 384-well plate, add 25 µL of sample, 25 µL of Assay mixture (from Step 1.3) into each well.
Note 2: Run the L-alanine assay at pH 6.5 to 7.0.
3.2 Incubate the reaction mixture at 37°C for 30 minutes to 1 hour.
3.3 Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 540/590 nm (cut off: 570 nm).

Data Analysis

The fluorescence reading in blank wells (with assay buffer only) is used as a control, and is subtracted from the values of the wells with the L-alanine standards and test samples. L-alanine standard curve is shown in Figure 1. Calculate the L-alanine concentrations of the samples according to the L-alanine standard curve.

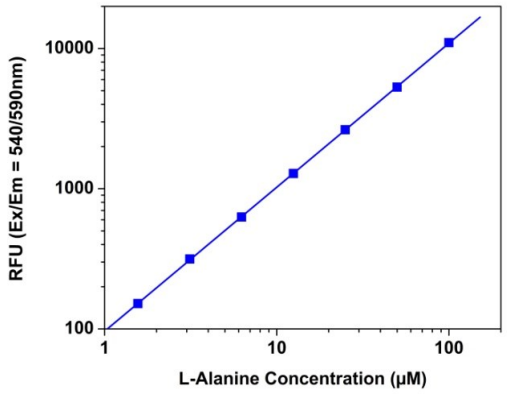

Figure1. L-alanine dose response was measured with the Amplite™ Fluorimetric L-Alanine Assay Kit in a solid black 96-well plate using a Gemini microplate reader (Molecular Devices). As low as 1.5 µM L-Alanine can be detected with 30 min incubation at 37°C. (*Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point).*

References

1. De Sousa, C. A. F., and L. Sodek. "Alanine metabolism and alanine aminotransferase activity in during hypoxia of the root system and subsequent return to normoxia." *Environmental and Experimental Botany* 50.1 (2003): 1-8.
2. Cunningham GA, McClenaghan NH, Flatt PR, Newsholme P. "L-alanine induces changes in metabolic and signal transduction gene expression in a clonal rat pancreatic beta-cell line and protects from pro-inflammatory cytokine-induced apoptosis." *Clin Sci (Lond)*. 2005 Nov;109 (5):447-55.
3. Sann L, Ruitton A, Mathieu M, Bourgeois J, Genoud J. "Effect of intravenous L-alanine administration on plasma glucose, insulin and glucagon, blood pyruvate, lactate and beta-hydroxybutyrate concentrations in newborn infants. Study in term and preterm newborn infants." Acta Paediatr Scand. 1978 May;67(3):297-302.