Amplite™ Fluorimetric L-Aspartate (Aspartic Acid) Assay Kit

Catalog number: 13827
Unit size: 200 Tests

Component	Storage	Amount
Component A: Amplite™ Red Substrate (light sensitive)	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: Conversion Mix	Freeze (<-15 °C), Minimize light exposure	2 vials (lyophilized powder)
Component E: Aspartate Standard	Freeze (<-15 °C), Minimize light exposure	1 vial (lyophilized powder)
Component F: DMSO	Freeze (<-15 °C)	1 vial (100 µL)

OVERVIEW

Aspartate (or Aspartic acid) is a negatively charged, polar amino acid. Aspartate is involved in the control point of pyrimidine biosynthesis, in transamination reactions, interconversions with asparagine, in the metabolic pathway leading to AMP, in the urea cycle, and is a precursor to homoserine, threonine, isoleucine, and methionine. It is also involved in the malate aspartate shuttle. Amplite™ Fluorimetric Aspartate Assay Kit offers a sensitive fluorescent assay for quantifying aspartate in biological samples. Aspartate is converted to pyruvate that generates hydrogen peroxide through an enzyme coupled reaction. The amount of hydrogen peroxide generated by aspartate is monitored with Amplite™ Red substrate for quantifying aspartate by a fluorescence microplate reader.

AT A GLANCE

Protocol summary

1. Prepare test samples along with diluted aspartate standards (50 µL)
2. Add equal volume of working solution (50 µL)
3. Incubate at 37°C for 20 - 30 minutes
4. Monitor fluorescence intensity at Ex/Em = 540/590 nm

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

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. Aspartate standard solution (10 mM):
Add 100 uL of ddH_2O into Aspartate Standard vial (Component E) to make 10 mM aspartate standard solution.

2. Amplite™ Red substrate stock solution (200X):
Add 50 µL of DMSO (Component F) into Amplite™ Red substrate (Component A) to make 200X Amplite™ Red substrate stock solution.

PREPARATION OF STANDARD SOLUTION

Aspartate standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/13827

Add 10 µL of 10 mM aspartate standard into 990 µL of 1X PBS buffer to get 100 µM aspartate solution (ASP7). Then perform 1:3 serial dilutions in 1X PBS buffer to get serially diluted aspartate standards (ASP6 - ASP1).

PREPARATION OF WORKING SOLUTION

1. Conversion Mix solution (100X):
Add 50 µL of ddH_2O into Conversion Mix (Component D) to make 100X Conversion Mix stock solution.

2. Amplite™ Red working solution:
Add 5 mL Assay Buffer (Component C) into one Enzyme Mix 1 bottle (Component B1); mix well. Add 100 µL of ddH_2O into one Enzyme Mix 2 vial (Component B2); mix well. Transfer the entire vial (100 µL) of Enzyme Mix 2, 25 uL of 200X Amplite™ Red substrate stock solution, and 50 µL of 100X Conversion Mix solution into the Enzyme Mix 1 bottle and mix well.

Note The working solution is not stable, use it promptly, and avoid direct exposure to light.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of aspartate standards and test samples in a solid black 96-well microplate. ASP = aspartate standard (ASP1 - ASP7, 0.1 to 100 µM); BL = blank control; TS = test sample.

BL	BL	TS	TS
ASP1	ASP1	...	...
ASP2	ASP2	...	...
ASP3	ASP3		
ASP4	ASP4		
ASP5	ASP5		
ASP6	ASP6		
ASP7	ASP7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent

Tel: 408-733-1055 | Fax: 408-733-1304 | Email: support@aatbio.com

ASP1 - ASP7	50 µL	Serial Dilution (0.1 to 100 µM)
BL	50 µL	1X PBS Buffer
TS	50	Test Sample

1. Prepare aspartate standards (ASP), blank controls (BL), and test samples (TS) according to the layout provided in Table 1 and Table 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

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

   Note Run the aspartate assay at pH 6.5 to 7.0.

3. Incubate the reaction mixture at 37°C for 20 - 30 minutes.

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 Aspartate 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

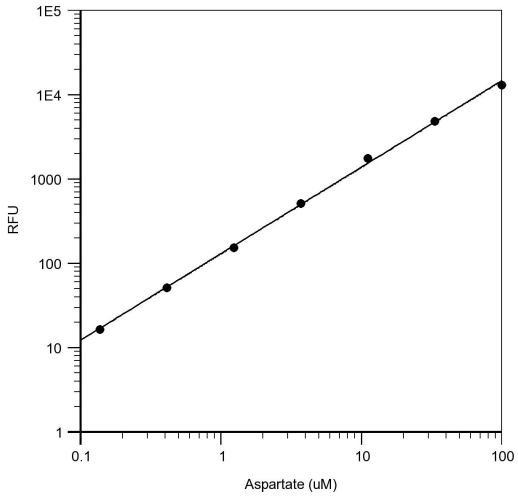

Figure 1. Aspartate dose response was measured with Amplite™ Fluorimetric Aspartate Assay Kit on a solid black 96-well plate using a Gemini microplate reader (Molecular Devices).

DISCLAIMER