Amplite® Colorimetric L-Aspartate (Aspartic Acid) Assay Kit

Catalog number: 13828
Unit size: 200 Tests

Component	Storage	Amount (Cat No. 13828)
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® Colorimetric Aspartate Assay Kit offers a sensitive colorimetric 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 in an absorbance microplate reader at 575 nm.

AT A GLANCE

Protocol Summary

1. Prepare test samples (50 µL) along with serially diluted aspartate standards (50 µL)
2. Add equal volume of working solution (50 µL)
3. Incubate at 37 °C for 30 - 60 minutes
4. Monitor absorbance increase at OD of 575±5 nm

Important Note

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

KEY PARAMETERS

Absorbance microplate reader

Absorbance	575 ± 5 nm
Recommended plate	Clear bottom
Instrument specification(s)	Path check

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

Aspartate standard solution (10 mM)

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

Amplite™ Red substrate stock solution

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

Note Store unused 200X Amplite™ Red stock solution at -20 °C, avoid light and repeated freeze-thaw cycles.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/13828

Aspartate standard

Add 32 µL of 10 mM aspartate standard into 368 µL of 1X PBS buffer to get 800 µM aspartate solution (ASP7). Then perform 1:2 serial dilutions in 1X PBS buffer to get serially diluted aspartate standards (ASP6 - ASP1).

PREPARATION OF WORKING SOLUTION

Conversion Mix solution (100X)

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

Amplite Red™ working solution

Add 5 mL Assay Buffer (Component C) into one Enzyme Mix 1 bottle (Component B1) and mix well. Add 100 µL of ddH_2O into one Enzyme Mix 2 vial (Component B2) and mix well. Transfer entire vial (100 µL) of Enzyme Mix 2, 25 µL 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 clear bottom 96-well microplate. ASP= Aspartate Standard (ASP1 - ASP7, 12.5 to 100 µM), BL=Blank Control (1×PBS buffer), 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
ASP1-ASP7	50 µL	Serial Dilution (12.5 to 100 µM)
BL	50 µL	1X PBS Buffer
TS	50 µL	Test Sample

1. Prepare aspartate standards (ASP), blank control (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 Amplite™ Red working solution to 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 Amplite™ Red 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 30 - 60 minutes.
4. Monitor the absorbance increase with an absorbance plate reader with path check at OD of 575 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

Placeholder for image details

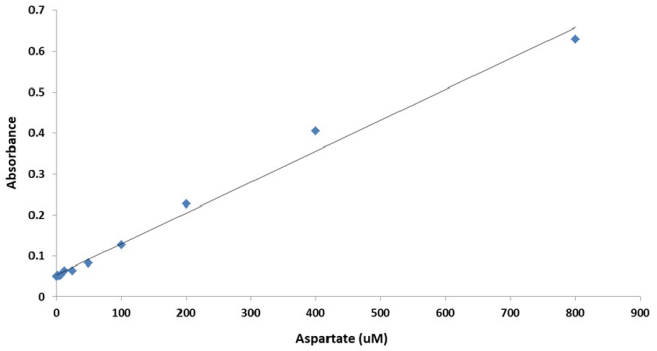

Figure 1. Aspartate dose response was measured with Amplite® Colorimetric Aspartate Assay Kit on a white clear bottom 96-well plate using a SpectraMax microplate reader with Path check ON (Molecular Devices).

DISCLAIMER

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