Amplite™ Fluorimetric Ascorbic Acid Assay Kit

Catalog number: 13835
Unit size: 200 Tests

Component	Storage	Amount
Component A: Ascorbrite™ Blue	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Enzyme Mix	Freeze (<-15 °C), Minimize light exposure	2 bottles (lyophilized powder)
Component C: Assay Buffer	Freeze (<-15 °C)	1 bottle (20 mL)
Component D: Ascorbic Acid Standard (MW=176.1)	Freeze (<-15 °C), Minimize light exposure	3.52 mg
Component E: DMSO	Freeze (<-15 °C)	1 vial (100 µL)

OVERVIEW

L-Ascorbic Acid (also called Vitamin C) is a critical metabolite for both plant and animals in cell division, growth and defense. Ascorbate is produced from glucose in the liver of most mammalian species. For humans ascorbate has to be obtained from food to survive, and a lack of sufficient Vitamin C can result in scurvy, and may eventually lead to death. As an antioxidant ascorbate can reduce the risk of developing chronic disease such as cancer and cardiovascular disease. In food industry, ascorbic acid and its sodium, potassium, and calcium salts are commonly used as antioxidant food additives to prevent undesired color and taste. AAT Bioquest's Amplite™ Fluorimetric Ascorbic Acid Assay Kit offers a sensitive fluorescent assay for quantifying total ascorbic acid and the ratio of dehydroascorbic acid (DHA) to ascorbic acid in biological samples. It utilizes an enzyme reaction that oxidize ascorbic acid to DHA, which can be detected by Ascorbrite™ Blue with a fluorescence microplate reader. The assay can detect 1uM of total ascorbate in a sample.

AT A GLANCE

Protocol summary

1. Prepare test samples with diluted ascorbic acid standards (50 µL)
2. Add equal volume of working solution (50 µL)
3. Incubate at room temperature for 30 minutes to 1 hour
4. Monitor fluorescence intensity at Ex/Em = 340/430 nm

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	340 nm
Emission:	430 nm
Cutoff:	420 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. Ascorbrite™ Blue stock solution (200X):
Add 50 µL of DMSO (Component E) into Ascorbrite™ Blue (Component A) to make 200X Ascorbrite™ Blue stock solution.

2. Ascorbic Acid standard solution (100 mM):
Add 200 µL of ddH_2O into ascorbic acid standard vial (Component D) to make 100 mM ascorbic acid standard solution.

PREPARATION OF STANDARD SOLUTION

Ascorbic Acid standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/13835

Add 10 µL of 100 mM ascorbic acid into 990 µL of assay buffer to get 1000 µM ascorbic acid solution (AA7). Then take the 1000 µM ascorbic acid standard solution and perform 1:3 serial dilutions to get serially diluted ascorbic acid standards (AA1 - AA6).

Note Ascorbic acid aqueous solution is not stable and will oxidize into DHA.

PREPARATION OF WORKING SOLUTION

Total AA Assay
Add 5 mL of Assay Buffer (Component C) into one bottle of Enzyme Mix (Component B). Then add 25 uL of Ascorbrite™ Blue stock Solution (200X) into the same bottle. Mix well.

DHA Assay
In an appropriate container, add 5 mL of Assay Buffer (Component C) with 25 uL of Ascorbrite™ Blue stock Solution (200X). Mix well.

Note Alternatively, one can make enzyme stock solution by adding 100 µL ddH_2O into one Enzyme Mix bottle (Component B) to make 50X enzyme stock solution, and use it proportionally for total AA assay (for example, for 1mL total AA assay working solution, add 20 µL 50X enzyme stock solution and 5 µL 200X Ascorbrite™ Blue stock Solution into 1 mL Assay Buffer).

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

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of ascorbic acid standards and test samples in a solid black 96-well microplate. AA = Ascorbic Acid Standard (AA1-AA7, 1 to 1000 µM); BL = blank control; TS = test sample.

BL	BL	TS	TS
AA1	AA1	...	...
AA2	AA2	...	...
AA3	AA3		
AA4	AA4		
AA5	AA5		
AA6	AA6		
AA7	AA7		

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Table 2. Reagent composition for each well.

Well	Volume	Reagent
AA1-AA7	50 µL	Serial Dilution (1 to 1000 µM)
BL	50 µL	Assay Buffer (Component C)
TS	50 µL	Test Sample

1. Prepare ascorbic acid standards (AA), blank control (BL), and test samples (TS) according to the layout of 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 working solution into each well of ascorbic acid standard, blank control, and test samples to make the total ascorbic acid 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.
3. Incubate the reaction at room temperature for 30 minutes to 1 hour.
4. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 340/430 nm (cut off: 420 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 Ascorbic Acid 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

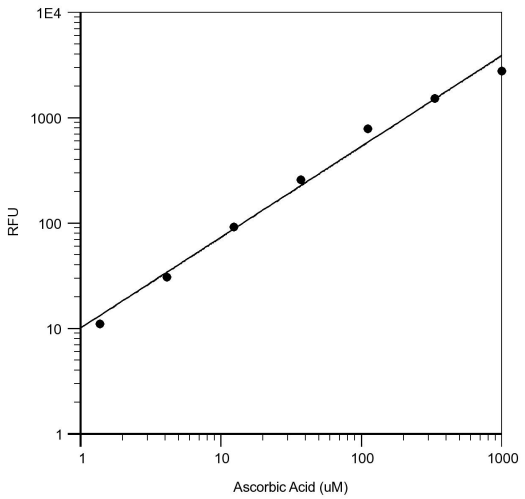

Figure 1. Ascorbic acid dose response was measured with Amplite™ Fluorimetric Ascorbic Acid Assay Kit on a solid black 96-well plate using a Gemini microplate reader (Molecular Devices).

DISCLAIMER