ab65346

Ascorbic Acid Assay Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Ascorbic Acid in various samples

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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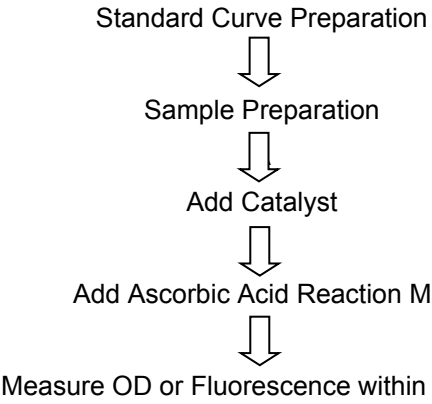
1. Overview

Ascorbic Acid (Vitamin C) plays an important role in many biological processes. It is a potent anti-oxidant, anti-inflammatory, anti-viral agent, and an immune stimulant and is present is a wide variety of foods and biological specimens. It is important to be able to monitor ascorbic acid content in these different samples

Abcam's Ascorbic Acid Assay Kit provides a rapid, simple, and sensitive means of detecting ascorbic acid in various biological samples. In this assay, our proprietary catalyst oxidizes ascorbic acid to produce a product that interacts with the OxiRed Probe/ascorbic acid probe, generating color and fluorescence. Ascorbic acid can be easily determined by either colorimetric (spectrophotometry at λ = 570 nm) or fluorometric (Ex/Em = 535/587 nm) methods. The assay can detect 0.01-10 nmol of ascorbic acid per assay in various samples.

2. Protocol Summary



Components and Storage

A. Kit Components

Item	Quantity
Assay Buffer II/Ascorbate Acid Buffer	25 mL
OxiRed Probe/Ascorbic Acid Probe (DMSO)	0.2 mL
Catalyst Reagent/Catalyst	0.5 mL
Developer Solution V/Ascorbic Acid Enzyme Mix (Lyophilized)	1 vial
Ascorbic Acid Standard/Ascorbic Acid Standard (20 µmol)	1 vial

* Store kit at -20°C, protect from light. Warm Assay Buffer II/Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening.

OxiRed Probe/ASCORBIC PROBE: Ready to use as supplied. Warm to room temperature prior to use to completely melt frozen DMSO, then vortex to ensure uniformity. Store at -20°C, protect from light and moisture. Use within two months.

Developer Solution V/ASCORBIC ACID ENZYME MIX: Dissolve in 220 µl Assay Buffer II/Ascorbic Acid Assay Buffer. Aliquot and store at -20°C. Use within two months.

ASCORBIC STANDARD: Dissolve in 200 µl of distilled water to generate 100 mM Ascorbic Standard stock solution. Store at -20°C. Use within two months.

Catalyst Reagent/CATALYST: Ready to use as supplied

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent or colorimetric microplate reader
- 96 well plate
- Orbital shaker

3. Assay Protocol

1. Standard Curve Preparation:

a. For the colorimetric assay:

Dilute the standard to 1 mM by adding 10 µl of the 100 mM Ascorbic Acid Standard to 990 µl of distilled water, mix well. Add 0, 2, 4, 6, 8, 10 µl into each well individually. Adjust volume to 120 µl/well with Assay Buffer II/Ascorbic Acid Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of Ascorbic Acid Standard.

b. For the fluorometric assay:

Dilute the Ascorbic Acid Standard to 0.01- 0.1 mM with the Assay Buffer II/Ascorbic Acid Assay Buffer (Detection sensitivity is 10 to 100 fold higher for a fluorometric than a colorimetric assay). Follow the procedure for the colorimetric assay.

Note:

Diluted ascorbic acid standard is unstable, use fresh dilution each time.

2. Sample Preparation:

Prepare test samples to a final volume of 120 µl/well with Assay Buffer II/Ascorbic Acid Assay Buffer in a 96-well plate.

We suggest testing several doses of your sample to make sure the readings are within the standard curve range.

Notes:

a) Due to high protein content and other compounds present in plasma and serum we recommend using ab65656 (Ascorbic Acid Assay Kit (Biological Samples)) for plasma and serum samples.

b) Ascorbate is easily oxidized during sample preparation and great care must be exercised to achieve quantitative recovery.

3. Add 100 µl of Catalyst Reagent/catalyst to 900 µl of distilled water and vortex well. Add 30 µl of Catalyst Reagent/catalyst to each standard and sample well.

4. Ascorbic Acid Reaction Mix: Mix enough reagent for the number of samples and standards to be performed: For each well, prepare a total 50 µl Reaction Mix containing:

Assay Buffer II/Ascorbic Acid Assay Buffer	46 µl
OxiRed Probe/Ascorbic Acid Probe	2 µl
Developer Solution V/Ascorbic Acid Enzyme Mix	2 µl

The fluorometric assay is ~10 times more sensitive than the colorimetric assay. Use 0.4 µl of the OxiRed Probe/probe per reaction to decrease the background reading/increase detection sensitivity significantly.

Mix well. Add 50 µl of the Reaction Mix to each well containing the Ascorbic Acid Standard and test samples. Mix well.

Note: Protect from light, Color is developed within 3 min and stable for an hour.

5. Measure OD_{570nm} for colorimetric assay or Ex/Em = 535/590 nm for fluorometric assay in a micro-plate reader.

Data Analysis

Correct background by subtracting the value derived from the zero ascorbic acid standard from all sample readings. The background reading can be significant and must be subtracted from sample readings.

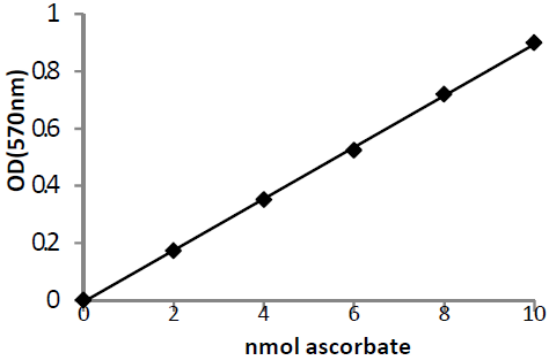
Apply sample readings to the generated standard curve.

Ascorbic Acid concentration can then be calculated:

Concentration = As / Sv (nmol/µl or µmol/ml or mM)

Where:
As is ascorbic acid amount from standard curve (nmol).
Sv is the sample volume added in sample wells (µl).

Ascorbic Acid molecular weight: 176.12.



Standard Curve performed according to assay protocol.

4. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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