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Product Information

Ascorbic Acid Assay Kit

Catalog Number **MAK074**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Ascorbic Acid, also known as Vitamin C, is a six-carbon lactone produced by plants and some animal species but not by humans and other primates. Ascorbic acid functions as an enzymatic cofactor for multiple enzymes, serving as an electron donor for monooxygenases and dioxygenases. Ascorbic acid also functions as a powerful antioxidant, particularly in regards to reactive oxygen species.

In this assay, Ascorbic Acid concentration is determined by a coupled enzyme reaction, which results in a colorimetric (570 nm)/fluorometric (λ_{ex} = 535/ λ_{em} = 587 nm) product, proportional to the ascorbic acid present.

This kit is suitable for use with biological fluids, food samples, and growth media. Proteins present in the sample can interfere with this assay. Protein containing samples are best analyzed using the Ascorbic Acid Assay II (MAK075). Alternatively, samples can be deproteinized using a 10 kDa Molecular Weight Cut-Off Spin Filter.

Components

The kit is sufficient for 100 assays in 96 well plates.

Ascorbic Acid Assay Buffer Catalog Number MAK074A	25 mL
Ascorbic Acid Probe, in DMSO Catalog Number MAK074B	0.2 mL
Catalyst Catalog Number MAK074C	0.5 mL
Ascorbic Acid Enzyme Mix Catalog Number MAK074D	1 vl
Ascorbic Acid Standard, 20 µmole Catalog Number MAK074E	1 vl

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Ascorbic Acid Assay Buffer – Allow buffer to come to room temperature before use.

Ascorbic Acid Probe – Thaw at room temperature to melt the solution prior to use and mix well by pipetting. Aliquot and store protected from light and moisture at –20 °C. Upon thawing, the Ascorbic Acid Probe is ready-to-use in the colorimetric assay.

For the fluorescence assay, dilute an aliquot of the Ascorbic Acid Probe Solution 5 to 10-fold with Ascorbic Acid Assay Buffer, just prior to use. This will reduce the background of the fluorescence assay.

Ascorbic Acid Enzyme Mix – Reconstitute with 220 µL of Ascorbic Acid Assay Buffer. Mix well by pipetting, then aliquot and store, protected from light, at –20 °C. Use within 2 months of reconstitution and keep cold while in use.

Ascorbic Acid Standard – Reconstitute in 200 µL of water to generate a 100 mM (100 nmole/µL) standard solution. Mix well by pipetting, then aliquot and store at -20 °C. Use within 2 months of reconstitution and keep cold while in use.

Storage/Stability
The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Ascorbic Acid Standards for Colorimetric Detection
Dilute 10 µL of the 100 mM (100 nmole/µL) Ascorbic Acid Standard Solution with 990 µL of Ascorbic Acid Assay Buffer to prepare a 1 mM (1 nmole/µL) standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM Ascorbic Acid standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Ascorbic Acid Assay Buffer to each well to bring the volume to 120 µL.
Note: The diluted ascorbic acid standard is not stable and should be prepared fresh for each time.

Ascorbic Acid Standards for Fluorometric Detection
Dilute 10 µL of the 100 mM (100 nmole/µL) Ascorbic Acid Standard Solution with 990 µL of Ascorbic Acid Assay Buffer to prepare a 1 mM (1 nmole/µL) standard solution. Dilute 10 µL of the 1 mM standard solution with 90 µL of water to generate a 0.1 mM (0.1 nmole/µL). Add 0, 2, 4, 6, 8, and 10 µL of the 0.1 mM Ascorbic Acid standard solution into a 96 well plate, generating 0 (blank), 0.2, 0.4, 0.6, 0.8, and 1.0 nmole/well standards. Add Ascorbic Acid Assay Buffer to each well to bring the volume to 120 µL.
Note: The diluted ascorbic acid standard is not stable and should be prepared fresh for each time.

Sample Preparation
Tissue (10 mg) or cells (2×10^6) should be rapidly homogenized in 4 volumes of cold Ascorbic Acid Assay buffer. Centrifuge at 13,000 × *g* for 10 minutes at 4 °C to remove insoluble material. High concentrations of proteins may interfere with the assay and should be removed with a 10 kDa MWCO spin filter. The soluble fraction may be assayed directly.

Serum samples should be deproteinized with a 10 kDa MWCO spin filter. The soluble fraction may be assayed directly.

Liquid samples can be added to the wells directly.

Bring samples to a final volume of 120 µL with Ascorbic Acid Assay Buffer.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.
Note: Ascorbate is easily oxidized during sample preparation and great care must be taken during sample preparation to minimize sample oxidation.

Assay Reaction
1. Dilute Catalyst 10-fold with water and mix well. Add 30 µL of diluted catalyst to each standard and sample well.

2. Set up the Master Reaction Mix according to the scheme in Table 1. 50 µL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Volume
Ascorbic Acid Buffer	46 µL
Ascorbic Acid Probe	2 µL
Ascorbic Acid Enzyme Mix	2 µL

3. Add 50 µL of the Master Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 3-5 minutes at room temperature. Protect the plate from light during the incubation. Developed color is stable for an hour.

4. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity (λ_{ex} = 535/λ_{em} = 587 nm).

Results

Calculations

The background for the assays is the value obtained for the 0 (blank) Ascorbic Acid Standard. Correct for the background by subtracting the 0 (blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Ascorbic Acid standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Concentration of Ascorbic Acid

$$S_a/S_v = C$$

S_a = Amount of Ascorbic Acid in unknown sample (nmole) from standard curve

S_v = Sample volume (μL) added into the wells

C = Concentration of Ascorbic Acid in sample

Ascorbic Acid molecular weight: 176.12 g/mole

Sample Calculation

Amount of Ascorbic Acid (S_a) = 5.84 nmole (from standard curve)

Sample volume (S_v) = 120 μL

Concentration of Ascorbic Acid in sample

$$5.84 \text{ nmole}/120 \text{ μL} = 0.0487 \text{ nmole/μL}$$

$$0.0487 \text{ nmole/μL} \times 176.12 \text{ ng/nmole} = 8.57 \text{ ng/μL}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Ice Cold Assay Buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples were not deproteinized	Use a 10 kDa MWCO spin filter to deproteinize samples
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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