Product Information

Ascorbic Acid Colorimetric/Fluorometric Assay Kit

I. Kit Contents:

Components	K2211-100 100 assays	Cap Color	Part Number
Ascorbic Acid Assay Buffer	25 ml	WM	K2211-C-1
Ascorbic Acid Probe (in DMSO)	0.2 ml	Red	K2211-C-2
Catalyst	0.5 ml	Blue	K2211-C-3
Ascorbic Acid Enzyme Mix (lyophilized)	1 vial	Green	K2211-C-4
Ascorbic Acid Standard (20 µmole)	1vial	Yellow	A8158

II. Introduction:

Ascorbic Acid is a reductone and plays important roles in many biological processes. Ascorbic Acid is present in a wide variety of biological specimens and foods. Ascorbic Acid is a potent anti-oxidant, anti-viral and anti-inflammatory agent, and acts as an immune stimulant. Measurement of ascorbic acid content in different samples is important.

The Ascorbic Acid Colorimetric/Fluorometric Assay Kit provides a sensitive, fast and convenient way for detection of ascorbic acid levels in various samples based on colorimetric and fluorometric method. In the assay, the Catalyst oxidizes ascorbic acid to generate a product that interacts with the ascorbic acid probe, generating fluorescence (Ex/Em = 535/587 nm) and color (spectrophotometry at λ = 570 nm). The kit can detect 0.01-10 nmol ascorbic acid in various samples.

III. Storage and Handling:

Store kit at –20°C, protect from light. Warm Ascorbic Acid Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening.

IV. Reagent Preparation:

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 , protect from light and moisture. Use within two months.

Ascorbic Acid Enzyme Mix: Dissolve in 220 µl 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: Ready to use as supplied

V. Ascorbic Acid Assay Protocol:

1. Standard Curve Preparations:

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 Ascorbic Acid Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of Ascorbic Acid Standard.

For the fluorometric assay, dilute the Ascorbic Acid Standard to 0.01 - 0.1 mM with the Ascorbic Acid Assay Buffer (Note: 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 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:

1) Due to high protein content and other compounds present in serum we recommend using FRASC Ascorbic Acid Kit for serum samples.

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

3. Catalyst: Add 100 µl of catalyst to 900 µl of distilled water and vortex well.

4. Add 30 µl of catalyst to each standard and sample well.

5. 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:

Ascorbic Acid Assay Buffer	46 µl
Ascorbic Acid Probe	2 µl
Ascorbic Acid Enzyme Mix	2 µl

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

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

8. Measure OD 570nm for colorimetric assay or Ex/Em = 535/590 nm for fluorometric assay in a micro-plate reader.

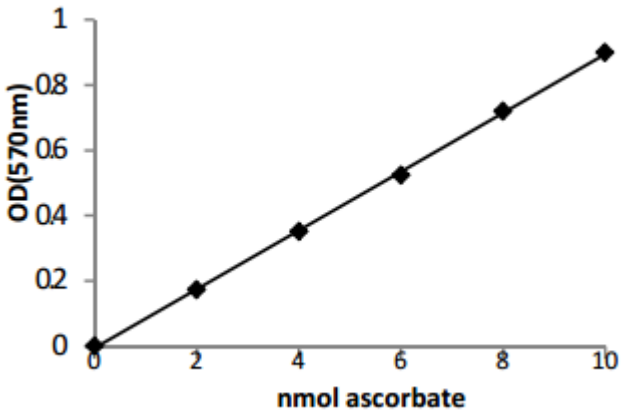
9. Correct background by subtracting the value derived from the 0 ascorbic acid standard from all sample readings (Note: The background reading can be significant and must be subtracted from sample readings). Apply sample readings to the generated standard curve. Ascorbic Acid concentration can then be calculated:

C = 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.



General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument

		• Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in d atasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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