Ascorbic Acid Colorimetric/Fluorometric Assay Kit

(Catalog #K661-100; 100 assays; Store at -20°C)

I. Introduction:

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. BioVision'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 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.

II. Kit Contents:

Components	K661-100	Cap Code	Part Number
Ascorbic Acid Assay Buffer	25 ml	WM	K661-100-1
Ascorbic Acid Probe (in DMSO)	0.2 ml	Red	K661-100-2A
Catalyst	0.5 ml	Blue	K661-100-4
Ascorbic Acid Enzyme Mix (lyophilized)	1 vial	Green	K661-100-5
Ascorbic Acid Standard (20 µmole)	1vial	Yellow	K661-100-6

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°C, 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 (K671-100) for serum samples.
2) Ascorbate is easily oxidized during sample preparation and great care must be exercised to achieve quantitative recovery.

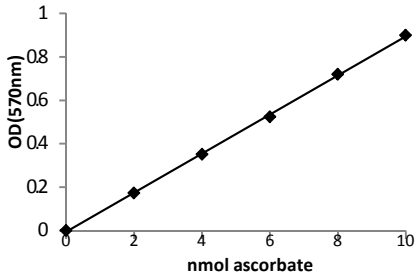
3. **Catalyst:** Add 100 µl of catalyst to 900 µl of distilled water and vortex well.
4. Add 30 µl of catalyst to each standard and sample well.
5. **Ascorbic Acid Reaction Mix:** Mix enough reagent for the number of samples and standards to be performed: For each well, prepare a total 50 µl Reaction Mix containing:

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

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.



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GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till 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	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• 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 an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• 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.		