

# Ascorbic Acid Assay Kit

For colorimetric & fluorometric detection of ascorbic acid in various biological samples

## Instruction Manual

Catalog Number	PK-CA577-K661																		
Description	<p>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 in a wide variety of foods and biological specimens. It is important to be able to monitor ascorbic acid content in these different samples.</p> <p>PromoKine's Ascorbic Acid Assay Kit provides a rapid, simple, and sensitive means of detecting ascorbic acid in various biological samples such as tissue and cell extracts, growth media, serum and other body fluids, and food products. 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 <math>\lambda = 570</math> 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.</p> <p>We also provide L-Ascorbic Acid separately (Cat.Nos. PK-CA577-2269-100 &amp; -1000).</p>																		
Quantity	100 assays																		
Kit Components	<table border="1"><thead><tr><th>Components</th><th>Quantity</th><th>Color Code</th></tr></thead><tbody><tr><td>Ascorbic Acid Assay Buffer</td><td>25 ml</td><td>WM</td></tr><tr><td>Ascorbic Acid Probe (in DMSO)</td><td>0.2 ml</td><td>Red</td></tr><tr><td>Catalyst</td><td>0.5 ml</td><td>Blue</td></tr><tr><td>Ascorbic Acid Enzyme Mix (lyophilized)</td><td>1 vial</td><td>Green</td></tr><tr><td>Ascorbic Acid Standard (20 <math>\mu</math>mole)</td><td>1 vial</td><td>Yellow</td></tr></tbody></table>	Components	Quantity	Color Code	Ascorbic Acid Assay Buffer	25 ml	WM	Ascorbic Acid Probe (in DMSO)	0.2 ml	Red	Catalyst	0.5 ml	Blue	Ascorbic Acid Enzyme Mix (lyophilized)	1 vial	Green	Ascorbic Acid Standard (20 $\mu$ mole)	1 vial	Yellow
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Storage and Reagents Preparation	<p>Store kit at <math>-20^{\circ}\text{C}</math>, protect from light. Warm Ascorbic Acid Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay.</p> <ul style="list-style-type: none"><li>• <b>Ascorbic Probe:</b> Ready to use as supplied. Warm to room temperature prior to use to completely melt frozen DMSO, then vortex to ensure uniformity. Store at <math>-20^{\circ}\text{C}</math>, protect from light and moisture. Use within two months.</li><li>• <b>Ascorbic Acid Enzyme Mix:</b> Dissolve in 220 <math>\mu</math>l Ascorbic Acid Assay Buffer. Aliquot and store at <math>-20^{\circ}\text{C}</math>. Use within two months.</li><li>• <b>Ascorbic Standard:</b> Dissolve in 200 <math>\mu</math>l of distilled water to generate 100 mM Ascorbic Standard stock solution. Store at <math>-20^{\circ}\text{C}</math>. Use within two months.</li><li>• <b>Catalyst:</b> Ready to use as supplied</li></ul>																		
Assay Protocol	<p><b>1. Standard Curve Preparations:</b></p> <p><u>For the colorimetric assay</u>, dilute the standard to 1 mM by adding 10 <math>\mu</math>l of the 100 mM Ascorbic Acid Standard to 990 <math>\mu</math>l of distilled water, mix well. Add 0, 2, 4, 6, 8, 10 <math>\mu</math>l into each well individually. Adjust volume to 120 <math>\mu</math>l/well with Ascorbic Acid Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of Ascorbic Acid Standard.</p> <p><u>For the fluorometric assay</u>, 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.</p> <p>Note: Diluted ascorbic acid standard is unstable, use fresh dilution each time.</p> <p><b>2. Sample Preparation:</b> Prepare test samples to a final volume of 120 <math>\mu</math>l/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.</p> <p>NOTES:</p> <ol style="list-style-type: none"><li>1) Due to high protein content and other compounds present in serum the assay is not optimally suited for serum samples.</li><li>2) Ascorbate is easily oxidized during sample preparation and great care must be exercised to achieve quantitative recovery.</li></ol> <p><b>3. Catalyst:</b> Add 100 <math>\mu</math>l of catalyst to 900 <math>\mu</math>l of distilled water and vortex well. Add 30 <math>\mu</math>l of catalyst to each standard and sample well.</p>																		

**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:

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

Mix well. Add 50 µl of the Reaction Mix to each well containing the Ascorbic Acid Standard and test samples. Mix well. Protect from light. Color is developed within 3 minutes and stable for an hour.

**5. Measurement:** Measure OD 570 nm for colorimetric assay or Ex/Em = 535/590 nm for fluorometric assay in a microwell plate reader.

**6. Calculation:** 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:

$$C = A_s / S_v \text{ nmol}/\mu\text{l or } \mu\text{mol}/\text{ml or mM}$$

Where: **A<sub>s</sub>** is ascorbic acid amount from standard curve (nmol).

**S<sub>v</sub>** is the sample volume added in sample wells (µl).

Ascorbic Acid molecular weight: 176.12.

Intended Use

For in vitro research use only. Not for diagnostic or therapeutic procedures.

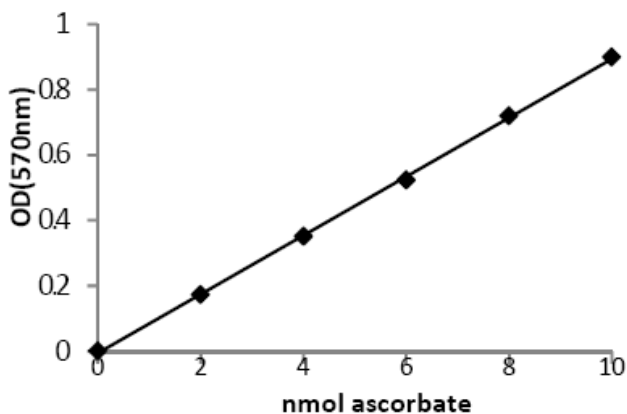


Figure: Ascorbate Standard Curve.

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