

## Ascorbic Acid 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 in 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  $\lambda = 570$  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 (lyophilized)	1 vial	Red	K661-100-2
Dimethylsulfoxide (DMSO, Anhydrous)	0.4 ml	Brown	K661-100-3
Catalyst	0.5 ml	Blue	K661-100-4
Ascorbic Acid Enzyme Mix (lyophilized)	1 vial	Green	K661-100-5
Ascorbic Acid Standard (20 $\mu$ mole)	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:** Dissolve in 220  $\mu$ l anhydrous DMSO (provided) before use. Store at -20°C, protect from light and moisture. Use within two months.

**Ascorbic Enzyme Mix:** Dissolve in 220  $\mu$ l Ascorbic Acid Assay Buffer. Aliquot and store at -20°C. Use within two months.

**Ascorbic Standard:** Dissolve in 200  $\mu$ l 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  $\mu$ l of the 100 mM Ascorbic Acid Standard to 990  $\mu$ l of distilled water, mix well. Add 0, 2, 4, 6, 8, 10  $\mu$ l into each well individually. Adjust volume to 120  $\mu$ l/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.**

- Sample Preparation:** Prepare test samples to a final volume of 120  $\mu$ 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.

#### NOTE:

- Due to high protein contents and other compounds present in serum we recommend using FRASC Ascorbic Acid Kit (K671-100) for serum samples.
- Ascorbate is easily oxidized during sample preparation and great care must be exercised to achieve quantitative recovery.

- Catalyst:** Add 100  $\mu$ l of catalyst to 900  $\mu$ l of distilled water and vortex well.

- Add 30  $\mu$ l of catalyst to each standard and sample well.

- Ascorbic Acid Reaction Mix:** Mix enough reagents for the number of samples and standards to be performed: For each well, prepare a total 50  $\mu$ l Reaction Mix containing:

46  $\mu$ l Ascorbic Acid Assay Buffer  
2  $\mu$ l Ascorbic Acid Probe  
2  $\mu$ l Ascorbic Acid Enzyme Mix

- Mix well. Add 50  $\mu$ l 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.

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

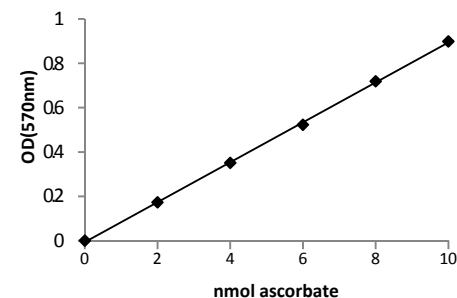
- Correct background by subtracting the value derived from the 0 ascorbic acid standard from all sample readings (Note: The background reading can be significant and must be subtracted from sample readings). Apply sample readings to the generated standard curve. Ascorbic Acid concentration can then be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

$$C = \text{As} / \text{Sv} \text{ nmol}/\mu\text{l or } \mu\text{mol}/\text{ml or mM}$$

Where: **As** is ascorbic acid amount from standard curve (nmol).

**Sv** is the sample volume added in sample wells ( $\mu$ l).

Ascorbic Acid molecular weight: 176.12.



### VI. RELATED PRODUCTS:

Apoptosis Detection Kits & Reagents

Cholesterol, LDL/HDL Assay Kits

Ethanol and Uric Acid Assay Kit

Pyruvate and Lactate Assay Kits

Glucose and Sucrose Assay Kit

Glutathione Assay Kit

NAD/NADH and NADP/NADPH Assay Kit

cAMP/cGMP Kits