

## **Technical Manual**

## **ColorFluor Ascorbic Acid Assay Kit**

**Catalogue Code: BA0077**

**Pack Size: 100 assays**

**Research Use Only**

## DESCRIPTION

Ascorbic acid (the *L*-enantiomer commonly known as vitamin C) is an important antioxidant found in living organisms and applied as additives in food and other industrial processes. By reacting with reactive oxygen species, it protects the cell from oxidative damages. The Assay Genie ColorFluor Ascorbic Acid Assay Kit provides a simple, direct and high-throughput assay for measuring ascorbic acid. In this assay, ascorbic acid is oxidized by ascorbate oxidase resulting in the production of H<sub>2</sub>O<sub>2</sub> which reacts with a specific dye to form a pink colored product. The color intensity at 570nm or fluorescence intensity (530/585 nm) is directly proportional to the ascorbic acid concentration in the sample.

## KEY FEATURES

Use 20  $\mu$ L samples. Linear detection range: colorimetric assay 6 to 1000  $\mu$ M, fluorimetric assay 1 to 100  $\mu$ M ascorbic acid.

## APPLICATIONS

**Assays:** ascorbic acid in biological samples such as serum, plasma, urine, saliva, milk, tissue, and cell culture.

**Drug Discovery/Pharmacology:** effects of drugs on ascorbic acid metabolism.

## KIT CONTENTS

**Assay Buffer:** 10 mL                      **Enzyme Mix:** 120  $\mu$ L  
**Dye Reagent:** 120  $\mu$ L                      **Standard:** 400  $\mu$ L 10 mM ascorbic acid

**Storage conditions.** The kit is shipped on ice. Store all components at -20°C. Shelf life of 12 months after receipt.

**Precautions:** reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

## COLORIMETRIC ASSAY

*Note: SH-containing reagents (e.g.  $\beta$ -mercaptoethanol, dithiothreitol, > 5  $\mu$ M) are known to interfere in this assay and should be avoided in sample preparation.*

**Sample treatment:** liquid samples such as serum and plasma can be assayed directly. Tissue and cell ( $10^6$ - $10^7$ ) lysates can be prepared by homogenization in cold 1 x PBS and centrifugation (5 min at 14,000 rpm). Use clear supernatants for assay. Milk samples should be cleared by mixing 600  $\mu$ L milk with 100  $\mu$ L 6 N HCl. Centrifuge 5 min at 14,000 rpm. Transfer 300  $\mu$ L supernatant into a clean tube and neutralize with 50  $\mu$ L 6 N NaOH. The neutralized supernatant is ready for assay (dilution factor  $n = 1.36$ ).

1. Equilibrate all components to room temperature. Briefly centrifuge the tubes before opening. Keep thawed tubes on ice during assay.
2. **Standards:** mix 22  $\mu$ L 10 mM Standard with 198  $\mu$ L dH<sub>2</sub>O (final 1000  $\mu$ M). Dilute standard in dH<sub>2</sub>O as follows.

No	1000 $\mu$ M STD + H <sub>2</sub> O	Vol ( $\mu$ L)	Ascorbic acid ( $\mu$ M)
1	100 $\mu$ L + 0 $\mu$ L	100	1000
2	60 $\mu$ L + 40 $\mu$ L	100	600
3	30 $\mu$ L + 70 $\mu$ L	100	300
4	0 $\mu$ L + 100 $\mu$ L	100	0

Transfer 20  $\mu$ L diluted standards into separate wells of a clear flat-bottom 96-well plate.

**Samples:** transfer 20  $\mu$ L of each sample into separate wells of the plate.

3. **Color reaction.** Prepare enough Working Reagent by mixing, for each reaction well, 85  $\mu\text{L}$  Assay Buffer, 1  $\mu\text{L}$  Enzyme Mix and 1  $\mu\text{L}$  Dye Reagent. Add 80  $\mu\text{L}$  Working Reagent to each well. Tap plate to mix. Incubate 10 min at room temperature.
4. Read optical density at 570nm (550-585nm).

### FLUORIMETRIC ASSAY

The fluorimetric assay procedure is similar to the Colorimetric Assay except that (1) 0, 30, 60 and 100  $\mu\text{M}$  ascorbic acid standards and (2) a black 96-well plate are used. Read fluorescence intensity at  $\lambda_{\text{ex}} = 530 \text{ nm}$  and  $\lambda_{\text{em}} = 585 \text{ nm}$ .

*Note:* if the calculated Ascorbic acid concentration of a sample is higher than 1000  $\mu\text{M}$  in the Colorimetric Assay or 100  $\mu\text{M}$  in the Fluorimetric Assay, dilute sample in water and repeat the assay. Multiply result by the dilution factor  $n$ .

### CALCULATION

Subtract blank value (#4) from the standard values and plot the  $\Delta\text{OD}$  or  $\Delta\text{F}$  against standard concentrations. Determine the slope and calculate the ascorbic acid concentration of Sample,

$$[\text{Ascorbic Acid}] = \frac{R_{\text{SAMPLE}} - R_{\text{BLANK}}}{\text{Slope } (\mu\text{M}^{-1})} \times n \quad (\mu\text{M})$$

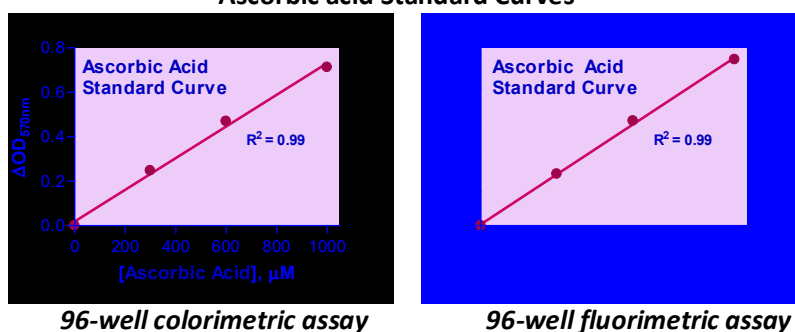
$R_{\text{SAMPLE}}$  and  $R_{\text{BLANK}}$  are optical density or fluorescence intensity readings of the Sample and  $\text{H}_2\text{O}$  Blank, respectively.  $n$  is the sample dilution factor.

**Conversions:** 1 mM ascorbic acid equals 17.6 mg/dL, 0.0176% or 176 ppm.

### MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom uncoated 96-well plates, optical density plate reader; black flat-bottom uncoated 96-well plates, fluorescence plate reader.

**Ascorbic acid Standard Curves**



### LITERATURE

1. Baker, W.L. and Lowe, T. (1985). Sensitive ascorbic acid assay for the analysis of pharmaceutical products and fruit juices. *Analyst* 110:1189-1191.
2. Chung, W.Y. et al (2001). Plasma ascorbic acid: measurement, stability and clinical utility revisited. *Clin Biochem.* 34:623-627.
3. Arya, S.P. et al (2002). A new method for the ascorbic acid assay using iron(II)-pyridine-2,6-dicarboxylic acid complex. *Ann Chim.* 92: 1159-1164.

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