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Technical Bulletin

Ascorbic Acid Assay Kit

Catalogue number MAK505

Product 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. This method 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_2O_2 which reacts with a specific dye to form a pink colored product. The color intensity at 570 nm or fluorescence intensity at λ_{Ex} =530 nm/λ_{Em} =585 nm is directly proportional to the ascorbic acid concentration in the sample.

The linear detection range of the kit is 6 to 1000 µM ascorbic acid for colorimetric assays and 1 to 100 µM for fluorometric assays. The kit is suitable for ascorbic acid activity determination in biological samples such as plasma, serum, urine, saliva, milk, tissue and culture media, as well as for studying the effects of drugs on ascorbic acid metabolism.

Components

The kit is sufficient for 100 colorimetric/Fluorometric assays in 96-well plates.

- Assay Buffer — 10 mL
  Catalogue Number MAK505A
- Enzyme Mix — 120 µL
  Catalogue Number MAK505B
- Dye reagent — 120 µL
  Catalogue Number MAK505C
- Standard (10 mM Ascorbic Acid) — 400 µL
  Catalogue Number MAK505D

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (for example multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates for colorimetric assay or black flat-bottom 96-well plates for fluorometric assay. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL centrifuge tubes
- PBS (Catalogue Number PPB006 or equivalent)
- Dounce tissue grinder set.
  (Catalogue Number D9063 or equivalent)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use.

Enzyme mix: Keep thawed tubes on ice during assay.

Procedure

Sample Preparation

Note: SH-containing reagents (for example β-mercaptoethanol, dithiothreitol, >5 µM) are known to interfere in this assay and should be avoided in Sample preparation.

Liquid Samples such as serum and plasma can be assayed directly.

Tissue and Cell Samples:

1. Homogenization tissue or cells (10^6-10^7) in cold 1× PBS.
2. Centrifuge at 14,000 rpm for 5 minutes.
3. Use clear supernatant for assay.

Milk Samples:

1. Clarify milk by mixing 600 µL milk with 100 µL of 6 N HCl.
2. Centrifuge mixture for 5 minutes at 14,000 rpm.
3. Transfer 300 µL supernatant into a clean tube and neutralize with 50 µL of 6 N NaOH. The neutralized supernatant is ready for assay (dilution factor n = 1.36).

Transfer 20 µL of each Sample into separate wells of the plate.

Colorimetric Standard Curve Preparation

1. Prepare a 1000 µM Standard of Ascorbic Acid by diluting 22 µL 10 mM Standard with 198 µL purified water.
2. Dilute Standards in purified water according to Table1.

Table 1.
Preparation of Colorimetric Standards

Well No.	1000 µM Standard	Purified Water	Ascorbic acid (µM)
1	100 µL	0 µL	1000
2	60 µL	40 µL	600
3	30 µL	70 µL	300
4	0 µL	100 µL	0

3. Transfer 20 µL of each Standard into separate wells of a clear flat-bottom 96-well plate.

Fluorometric Standard Curve Preparation

1. Prepare Standards according to Colorimetric Standard Curve Preparation section.
2. Mix 10 µL of the Standards from Colorimetric Procedure with 90 µL of purified water according to Table 2.

Table 2.
Preparation of Fluorometric Standards

Well No.	Colorimetric Standard	Assay Buffer	Ascorbic Acid (µM)
1	10 µL of 1000 µM Std	90 µL	100
2	10 µL of 600 µM Std	90 µL	60
3	10 µL of 300 µM Std	90 µL	30
4	-	100µL	0

3. Transfer 20 µL Standards separate wells of a black 96-well plate.

Working Reagent Preparation

1. Mix enough reagents for the number of assays to be performed. For each Standard and Sample well, prepare 87 µL of Working Reagent according to Table 3.

Table 3.
Preparation of Working Reagent

Reagent	Volume
Assay Buffer	85 µL
Enzyme mix	1 µL
Dye reagent	1 µL

2. Transfer 80 µL of appropriate Working Reagent into each Standard and Sample well. Tap plate to mix.
3. Incubate the plate for 10 minutes at room temperature.

Measurement

Measure the optical density (OD) at 570 nm or fluorescence intensity (F) at
λ_{Ex} = 530 nm/λ_{Em} = 585 nm.

Results

1. Calculate ΔOD or ΔF by subtracting the reading (OD or fluorescence intensity F) of Standard #4 (Blank) from the remaining Standard reading values.
2. Plot the ΔF or ΔOD against the standard concentrations and determine the slope of the Standard curve.
3. Calculate the Ascorbic acid concentration of Samples using the below equation:

$$\text{Ascorbic acid } (\mu M) = \frac{R_{Sample} - R_{Blank}}{\text{Slope}} \times n$$

Where:

R_{SAMPLE} = OD or fluorescence intensity (F) reading of Sample

R_{BLANK} = OD or fluorescence intensity (F) reading of Blank

n = Sample dilution factor (DF = 1 for undiluted Samples)

Note: If the calculated Ascorbic acid concentration of a Sample is higher than 1000 µM for the colorimetric assay or 100 µM in the fluorometric assay, dilute Sample in purified water and repeat the assay. Multiply result by the dilution factor (DF).

Figure 1.
Typical Colorimetric Ascorbic Acid Standard Curve

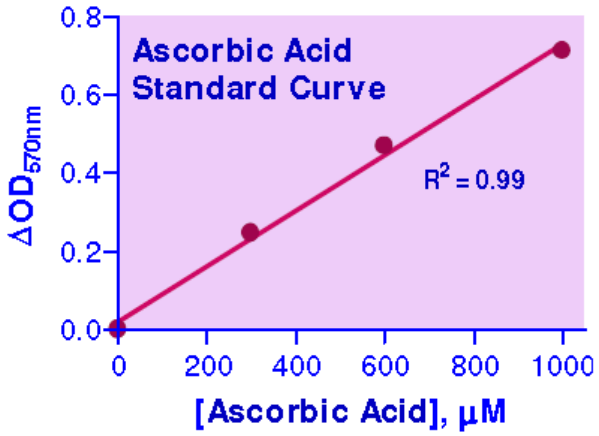
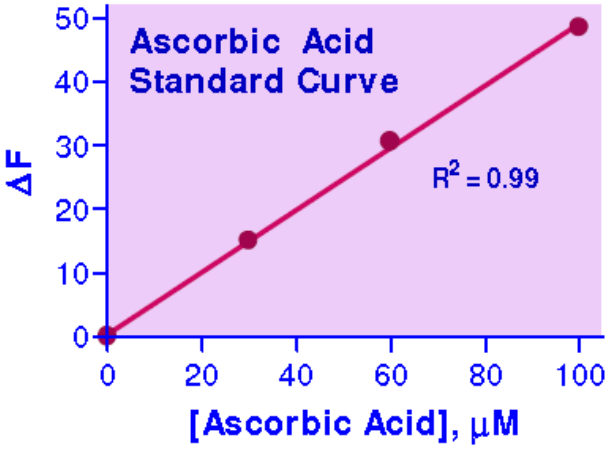


Figure 2.
Typical Fluorometric Ascorbic Acid Standard Curve



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