Ascorbic Acid Assay Kit

Catalog No.: abx298814

Size: 96 tests (80 samples)

Detection Range: 0.31 µg/ml - 20 µg/ml

Sensitivity: 0.31 µg/ml

Storage: Store all components at 2 – 8 °C for up to 6 months.

Application: For detection and quantification of Ascorbic Acid activity in serum, plasma, animal/plant tissue, and other biological fluids.

Introduction

Ascorbic Acid, also known as Vitamin C, is a six-carbon lactone produced by plants and some animal species but not by humans and other primates. Ascorbic acid functions as an enzymatic cofactor for multiple enzymes, serving as an electron donor for monooxygenases and dioxygenases. Ascorbic acid also functions as a powerful antioxidant, particularly in regard to reactive oxygen species.

Abbexa's Ascorbic Acid Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Ascorbic Acid content. Ascorbic Acid reacts with the reagents in this kit to produce an absorbance at between 536 nm. The intensity of the color is proportional to the concentration of Ascorbic Acid, which can then be calculated.

Kit components

1. 96-well microplate
2. Extracting solution: 5 ml
3. Buffer solution: 15 ml
4. Chromogenic agent: 6 ml
5. Ferrum reagent: 0.5 ml
6. Stop solution: 12 ml
7. Vitamin C standard: 2 vials
8. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (536 nm)
2. Double distilled water
3. Homogenization medium (Normal saline (0.9% NaCl) or PBS (0.01 M), pH 7.4)
4. Absolute ethanol
5. Pipette and pipette tips
6. Vials/tubes
7. Incubator
8. Centrifuge
9. Vortex mixer

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge for 10 mins at 1000-2000 x g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Weigh the tissue homogenate. For each 1 g of homogenate, add 9 ml homogenization medium. Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 10,000 x g at 4°C for 10 min. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately.

Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4), then carry out the assay procedure.

The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human Serum	1
Mouse Serum	1
Chicken Serum	1
Horse Serum	1
10% Mouse Liver Tissue Homogenate	1
10% Mouse Kidney Tissue Homogenate	1
10% Mouse Lung Tissue Homogenate	1
10% Mouse Spleen Tissue Homogenate	1
10% Rat Heart Tissue Homogenate	1
10% Rat Liver Tissue Homogenate	1
10% Plant Tissue Homogenate	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Extracting working solution:** Dilute the Stock solution at a ratio of 1:14 with double distilled water to prepare Extracting working solution. Prepare immediately before carrying out the assay. Unused solution can be stored at 2-8°C for up to 7 days.
- **Chromogenic working agent:** Dilute the chromogenic agent at a ratio of 1:9 with absolute ethanol to prepare Chromogenic working agent. Prepare immediately before carrying out the assay. Unused agent can be stored at 2-8°C for up to 7 days.
- **Ferrum working reagent:** Dilute the 0.15 ml of Ferrum reagent to a final volume of 25 ml with double distilled water to prepare Ferrum working reagent. Prepare immediately before carrying out the assay. Unused reagent can be stored at 2-8°C for up to 7 days.
- **6 mg/ml standard solution:** Dissolve a vial of Vitamin C Standard with 1 ml of Extracting working solution. Prepare immediately before carrying out the assay and mix fully.
- **0.06 mg/ml standard solution:** Dilute the 6 mg/ml standard solution 100-fold with Extracting working solution. Prepare immediately before carrying out the assay and mix fully. This solution is readily oxidized, it is therefore recommended to use within 30 minutes of preparation.

B. Assay Procedure

1. **Standard curve preparation:** Label 8 tubes with 0, 0.5, 7.5, 10, 12.5, 15, 17.5 and 20 µg/ml. Dilute the 0.06 mg/ml standard solution with Extracting working solution to concentrations of 0.5, 7.5, 10, 12.5, 15, 17.5 and 20 µg/ml. The Extracting working solution itself serves as the 0 µg/ml (blank) standard.

2. **Sample preparation:**

2.1. To 0.10 ml of sample, add 0.30 ml Extracting working solution.
2.2. For each tube, mix fully and allow to stand at room temperature for 15 minutes.
2.3. Centrifuge at 2000 × g for 10 minutes. Then take 100 µl of supernatant for the chromogenic reaction.

3. **Chromogenic Reaction:**

3.1. Add 100 µl of prepared standards to the Standard tubes.
3.2. Add 100 µl of sample supernatant to the Sample tubes.
3.3. Add 125 µl of Buffer solution to each tube.
3.4. Add 250 µl of Chromogenic working agent to each tube.

3.5. Add 65 µl of Ferrum working reagent to each tube.
3.6. Mix fully using a vortex mixer. Incubate at 37°C for 30 minutes.
3.7. Add 25 µl of Stop solution to each tube.
3.8. Mix fully using a vortex mixer and allow to stand at room temperature for 10 minutes.
3.9. Set the Blank, Standard and Sample wells on the well-plate.
3.10. Add 250 µl of standard reaction solution to standard wells.
3.11. Add 250 µl of sample reaction solution to sample wells.
3.12. Measure the OD of each well with a microplate reader at 536 nm.

4. Calculation of Results

The standard curve can be plotted as the absolute OD_{536} of each standard solution (*y*) vs. the respective concentration of the standard solution (*x*). A linear fit is recommended for the standard curve ($y = ax + b$). The Ascorbic acid concentration of the samples can be interpolated from the standard curve.

1. Ascorbic acid concentration in serum and plasma samples:

$$\text{Ascorbic Acid } (\mu g/ml) = \frac{\Delta A_{536} - b}{a} \times f \times 4^*$$

2. Ascorbic acid concentration in tissues samples:

$$\text{Ascorbic acid } (\mu g/mg \text{ protein}) = \frac{\Delta A_{536} - b}{a} \times f \times \frac{4^*}{C_P}$$

where:

ΔA_{536}	OD value of the sample ($OD_{Sample} - OD_{Blank}$)
a	gradient of the standard curve (linear fit)
b	y-intercept of the standard curve (linear fit)
f	dilution factor of the sample before carrying out the assay
4^*	dilution factor of the pretreatment of sample supernatant
C_P	concentration of protein in sample (mg protein/ml)