# Vitamin C Assay Kit

**(Cat/No.:BC106 Size 50T/48S)**

## 1. Composition and preparation (Kit Shelf life: 6 months)

**Reagent 1: Stock solution**, 5 mL × 1 bottle, store at 4 °C. **Preparation of Reagent 1 working solution:** dilute the **Reagent 1 stock solution with distilled water** at a ratio of 1:14 before use and mix well.

**Reagent 2:** Powder × 1 vial, store at 4 °C. **Preparation of Reagent 2 working solution:** dissolve 1 vial of powder in 0.36 mL glacial acetic acid, then add water to a final volume of 40 mL, dissolve completely, store at 4 °C.

**Reagent 3:** White crystalline powder × 1 vial, store at 4 °C. **Preparation of Reagent 3 working solution:** due to poor solubility, add 60 mL of 95% or absolute ethanol 2–4 hours before use to dissolve thoroughly, store at 4 °C.

**Reagent 4:** Yellow stock solution, 0.2 mL × 1 bottle, store at 4–8 °C protected from light. Before use, take 0.15 mL of **Reagent 4 stock solution** and add distilled water to 25 mL to prepare the **Reagent 4 working solution** (or prepare proportionally as needed).

**Reagent 5:** Liquid, 6 mL × 1 bottle, store at 4 °C.

**Reagent 6: Vc standard:** powder, 6 mg × 3 vials, each vial contains vitamin C, store at 4 °C protected from light. Before use, dissolve 1 vial of standard powder in 10 mL of **Reagent 1 working solution** to prepare a **600 μg/mL Vc standard working solution**, which can be stored at 4 °C for 1–2 weeks; then dilute the 600 μg/mL Vc standard working solution 100-fold with Reagent 1 working solution to prepare a **6μg/mL Vc standard working solution** for use. Note: Vitamin C standards are easily oxidized; therefore, it is best to prepare them after the sample supernatant has been prepared and allow them to react with reagents within 10 minutes after preparation.

## 2. Required instruments and reagents

Visible spectrophotometer and cuvettes (or microplate reader (536 ± 10 nm) and 96-well plate), centrifuge, vortex mixer, 37 °C water bath or air incubator, distilled water, absolute ethanol (analytical grade), a small amount of glacial acetic acid (0.5 mL is sufficient, analytical grade), protein assay reagents (for animal tissue samples).

## 3. Operation procedure

**1. Sample pretreatment:**

①. **Animal and plant tissue samples:** Accurately weigh the tissue sample, add 9 volumes of homogenization medium according to the ratio of weight (g): volume (mL) = 1:9 (recommended 0.1 mol/L phosphate buffer with pH 7.0–7.4 or 0.9% physiological

saline), mechanically homogenize in an ice-water bath, centrifuge at 2500 rpm for 10 minutes, and collect the supernatant (10% homogenate supernatant) for testing. **(Note: For animal tissue homogenate supernatant, the protein concentration must be determined (not required for plant tissues; use the second tissue calculation formula during calculation).)**

**②. Serum (plasma) and other liquid samples:** Use directly.

**2. Preparation of supernatant:** Take 0.15 mL of the above samples (or tissue homogenate supernatant) and add 0.45 mL of **Reagent 1 working solution**, vortex mix, incubate at room temperature for 15 minutes, centrifuge at 3500–4000 rpm for 10 minutes, and collect the clear upper liquid as the supernatant. **(Note: Tissue samples may also be directly homogenized using Reagent 1 working solution as the homogenization medium to prepare supernatant for direct use; refer to the second tissue calculation formula during calculation.)**

**3. Determination of Vc in the supernatant:**

| | Blank tube | Standard tube | Test tube |
|---|---|---|---|
| Reagent 1 working solution (mL) | 0.4 | | |
| 6 μg/mL Vc standard working solution (mL) | | 0.4 | |
| Supernatant (mL) | | | 0.4 |
| Reagent 2 working solution (mL) | 0.5 | 0.5 | 0.5 |
| Reagent 3 working solution (mL) | 1 | 1 | 1 |
| Reagent 4 working solution (mL) | 0.25 | 0.25 | 0.25 |
| Mix thoroughly, incubate in a 37 °C water bath for 30 minutes | | | |
| Reagent 5 (mL) | 0.1 | 0.1 | 0.1 |
| Mix thoroughly, incubate for 10 minutes, measure absorbance at a wavelength of 536 nm with an optical path length of 1 cm (or 0.5 cm), zero with distilled water, and measure the absorbance of each tube (or take 200 μL of reaction solution from each tube, add to a 96-well plate, and read at 536 nm using a microplate reader). | | | |

## 4. Calculation formula

**1. Calculation and examples for liquid samples:**

**①. Calculation formula:**

$$\text{VC content (μg/mL)} = \frac{A_{test} - A_{blank}}{A_{standard} - A_{blank}} \times C_{standard} \times N$$

**$C_{sample}$:** standard concentration, 6 g/mL;

**N:** dilution factor before sample measurement (including the pretreatment dilution factor of 4).

**②. Calculation example:**

**Example 1:** Take 0.15 mL serum and operate according to the operation table. The measured OD values were: blank tube OD = 0.031, standard tube OD = 0.184,

test tube OD = 0.144. The calculation result is:

$$\text{VC ontent}\ (\mu g/mL) = \frac{0.144 - 0.031}{0.184 - 0.031} \times 6 \times 4 = 17.73 \mu g/mL$$

**2. Calculation and examples for tissues:**

**①. Calculation formula:**

$$\text{Tissue VC content}\ (\mu g/mgprot) = \frac{A_{test} - A_{blank}}{A_{standard} - A_{blank}} \times C_{standard} \times N \div Cpr$$

$$\text{Tissue VC ontent}\ (\mu g/g\ \text{Tissue}) = \frac{A_{test} - A_{blank}}{A_{standard} - A_{blank}} \times C_{standard} \times N \div \frac{W}{V_{sample\ total}}$$

**$C_{standard}$:** standard concentration, 6 g/mL;

**N:** dilution factor before sample measurement (the first formula also includes the pretreatment dilution factor of 4 when Reagent 1 working solution is added);

**Cpr:** protein concentration of tissue homogenate, mgprot/mL (prot refers to protein);

**W:** sample weight, g;

**$V_{sample\ total}$:** total volume of homogenization medium added during sample homogenization, mL.

**②. Calculation examples:**

**Example 1:** Take 0.15 mL of 10% rat liver homogenate and operate according to the operation table. The measured OD values were: blank tube OD = 0.032, standard tube OD = 0.184, test tube OD = 0.146. The protein concentration of the 10% rat liver homogenate was measured as 8.5 mgprot/mL. The calculation result is:

$$\text{VC content in Rat liver}\ (\mu g/mgprot) = \frac{0.146 - 0.032}{0.184 - 0.032} \times 6 \times 4 \div 8.5 = 2.12 \mu g/mgprot$$

**Example 2:** Take 0.15 mL of 20% rotifer homogenate and operate according to the operation table. The measured OD values were: blank tube OD = 0.031, standard tube OD = 0.186, test tube OD = 0.177. The protein concentration of the 20% rotifer homogenate was measured as 0.854 mgprot/mL. The calculation result is:

$$\text{Rotifer VC content}\ (\mu g/mgprot) = \frac{0.177 - 0.031}{0.186 - 0.031} \times 6 \times 4 \div 0.854 = 26.48 \mu g/mgprot$$

## 5. Assay principle

In this method, $Fe^{3+}$ rapidly reacts with reduced ascorbic acid to generate $Fe^{2+}$ , which subsequently reacts with o-phenanthroline to produce a colored complex, allowing the determination of vitamin C content in plasma.

## 6. Assay significance

Vitamin C participates in collagen synthesis in organisms and is a cofactor for proline hydroxylase and lysine hydroxylase. It is also required for the copper-containing enzyme dopamine β-hydroxylase, which catalyzes the conversion of dopamine to norepinephrine. When vitamin C is deficient, synthesized collagen cannot be sufficiently hydroxylated and cannot

properly form fibers. The most prominent chemical activity of vitamin C is its role as a reducing agent, whereby it reduces $Fe^{3+}$ to $Fe^{2+}$, promoting intestinal iron absorption and enhancing iron storage and utilization. As a free radical scavenger, ascorbic acid can rapidly react with $O_2^-$ and $H_2O_2$, and even more rapidly with $OH^-$ to form ascorbyl radicals. It can also scavenge $O_2^-$, thereby protecting the organism from damage caused by endogenous oxygen free radicals. Vitamin C can reduce α-tocopheroxyl radicals back to vitamin E, indirectly acting as a chain-breaking antioxidant. Vitamin C is the most effective antioxidant in plasma and constitutes the first line of defense of the extracellular antioxidant defense system. It also inhibits ongoing lipid peroxidation in plasma.