Cysteine (Cys) Content Assay Kit

Cat. No. KITG-145 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	This kit quantitatively detects cysteine (Cys) content by measuring the absorbance change at 600 nm. Cysteine can reduce phosphotungstic acid to tungsten blue, and the product has a characteristic absorption peak at 600 nm.
Description	Cysteine (Cys) is a thiol-containing amino acid converted from methionine. It can be mutually converted with cystine, participates in the formation of disulfide bonds in protein active centers, and provides sulfhydryl groups for other physiological and biochemical reactions. Cysteine accumulates on the surfaces of the skin and mucosa, maintains the activity of sulfhydryl enzymes during keratin production, and regulates basal melanin production by epidermal pigment cells. It has whitening, detoxification, and inflammation- and allergic skin-improving effects.
Form	Size 100T/96S Kit Components Extraction Solution: liquid, 50 mL × 1 bottle; store at 4°C. Reagent I: liquid, 15 mL × 1 bottle; store at 4°C. Reagent II: powder, 2 bottles; store at 4°C. One day before use, add 2 mL distilled water and 0.5 mL phosphoric acid to each bottle, heat in a boiling water bath for 2 h, cool, and then add 8 mL distilled water. Seal the bottle to prevent moisture loss. After preparation, the solution can be stored at 4°C for 2 weeks. Standard: powder, 1 vial containing 10 mg cysteine standard; store at 4°C. Before use, add 4.13 mL distilled water and dissolve fully to obtain a 20 μmol/mL cysteine standard solution.
Applications	Detection Method Measure the absorbance change at 600 nm. Cysteine reduces phosphotungstic acid to tungsten blue, and the product has a characteristic absorption peak at 600 nm. Compatible Sample Types Tissue homogenates and

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	liquid samples.
Notes	Notes 1. If the measured absorbance is outside the linear range of the standard absorbance: if it is higher than the highest value, appropriately dilute the sample and measure again; if it is lower than the lowest value, appropriately increase the sample amount and measure again. Make the corresponding modifications in the calculation. 2. To ensure accurate results and avoid reagent loss, carefully read the instructions before measurement, confirm that reagent storage and preparation are sufficient, and make sure the operating steps are clear. Before the formal assay, select 2–3 samples expected to show large differences for a preliminary assay. If any problem occurs during the process, contact the relevant staff in time. Scientific Background Cysteine (Cys) is converted from methionine and is a thiol-containing amino acid. It can be converted to and from cystine, participates in the formation of disulfide bonds in protein active centers, and provides sulfhydryl groups for other physiological and biochemical reactions. Cysteine accumulates on the surfaces of the skin and mucosa, maintains sulfhydryl enzyme activity during keratin production, and regulates basal melanin production by epidermal pigment cells. It has whitening, detoxification, and inflammation- and allergic skin-improving effects. Cysteine reduces phosphotungstic acid to generate tungsten blue, which has a characteristic absorption peak at 600 nm; therefore, cysteine content can be quantitatively determined from the absorbance change. Technical Notes If the measured absorbance is outside the linear range of the standard absorbance: if it is higher than the highest value, appropriately dilute the sample and measure again; if it is lower than the lowest value, appropriately increase the sample amount and measure again. Make the corresponding modifications in the calculation. Introduction The Cysteine (Cys) Content Assay Kit provides the reagents required for quantitative detection of cysteine by a phosphotungstic acid tungsten blue colorimetric method. Cysteine reduces phosphotungstic acid to generate tungsten blue, which has a characteristic absorption peak at 600 nm. Cysteine content is calculated from the absorbance change using a standard curve. Handling Before measurement, preheat the visible spectrophotometer or microplate reader for

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	more than 30 min, set the wavelength to 600 nm, and zero with distilled water. Equipment required for the assay: visible spectrophotometer or microplate reader; micro glass cuvette with a 10 mm optical path or 96-well plate; mortar/homogenizer; adjustable pipette; benchtop centrifuge. Reagents required for the assay: phosphoric acid and distilled water. Warning For Research Use Only. Not for Use in Diagnostic Procedures.
Storage	Storage Store the kit at 2–8°C. Store Extraction Solution, Reagent I, Reagent II, and the Standard at 4°C. After Reagent II is prepared, seal it to prevent moisture loss; the prepared solution can be stored at 4°C for 2 weeks.
Reaction conditions	Preparation Sample preparation (adjust the sample amount and ratio appropriately according to preliminary experiment results): • Tissue: Weigh 0.2 g tissue, add 0.5 mL Extraction Solution, homogenize on ice, centrifuge at 12,000 × g and 4°C for 10 min, and collect the supernatant as the sample to be measured. • Liquid sample: Mix 200 µL liquid sample with 300 µL Extraction Solution thoroughly, centrifuge at 12,000 × g and 4°C for 10 min, and collect the supernatant as the sample to be measured. Standard preparation: Before use, add 4.13 mL distilled water to the 10 mg cysteine standard and dissolve fully to obtain a 20 µmol/mL cysteine standard solution. Prepare 2.0, 1.0, 0.5, 0.25, 0.125, and 0.0625 µmol/mL standard dilutions with distilled water as follows: mix 100 µL of the 20 µmol/mL standard solution with 900 µL distilled water to obtain the 2.0 µmol/mL dilution; mix 200 µL of the 2.0 µmol/mL standard solution with 200 µL distilled water to obtain the 1.0 µmol/mL dilution; mix 200 µL of the 1.0 µmol/mL standard solution with 200 µL distilled water to obtain the 0.5 µmol/mL dilution; mix 200 µL of the 0.5 µmol/mL standard solution with 200 µL distilled water to obtain the 0.25 µmol/mL dilution; mix 200 µL of the 0.25 µmol/mL standard solution with 200 µL distilled water to obtain the 0.125 µmol/mL dilution; mix 200 µL of the 0.125 µmol/mL standard solution with 200 µL distilled water to obtain the 0.0625 µmol/mL dilution. Assay Protocol 1. Preheat the visible spectrophotometer

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	or microplate reader for more than 30 min, set the wavelength to 600 nm, and zero with distilled water. 2. Add the following reagents to a 96-well plate or centrifuge tube: • Measurement group: 40 μL sample, 100 μL Reagent I, and 60 μL Reagent II. • Standard group: 40 μL standard dilution, 100 μL Reagent I, and 60 μL Reagent II. • Blank group: 40 μL distilled water, 100 μL Reagent I, and 60 μL Reagent II. 3. Mix thoroughly and let stand at room temperature for 15 min. 4. Measure the absorbance at 600 nm and record the values as A(measurement), A(standard), and A(blank). Calculate ΔA(measurement) = A(measurement) − A(blank) and ΔA(standard) = A(standard) − A(blank). The blank group needs to be measured only 1–2 times. 5. Prepare the standard curve using 2.0, 1.0, 0.5, 0.25, 0.125, and 0.0625 μmol/mL standard dilution concentrations as the x-axis and the corresponding ΔA(standard) values as the y-axis. Obtain the linear regression equation y = kx + b, substitute ΔA(measurement) into the equation, and calculate x (μmol/mL). Analysis 1. Calculation based on tissue sample mass: Cysteine content (μmol/g) = x × V(extraction) ÷ W. Here, x is the concentration calculated from the standard curve (μmol/mL); V(extraction) is the total volume of the sample solution, 0.5 mL; and W is the sample mass in g. 2. Calculation based on liquid sample volume: Cysteine content (μmol/mL) = x × V(extraction) ÷ V(sample) = 2.5 × x. Here, V(extraction) is the total volume of the sample solution, 0.5 mL; V(sample) is the original liquid sample volume, 0.2 mL; and x is the concentration calculated from the standard curve (μmol/mL).
Usage	Materials Required but Not Supplied Phosphoric acid (H_3PO_4; molecular weight = 98; CAS No. 7664-38-2) and distilled water.

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