CheKine™ Micro Cysteine (Cys) Assay Kit

Cat #: KTB1450 Size: 48 T/48 S 96 T/96 S

	Micro Cysteine (Cys) Assay Kit		
REF	**Cat #:** KTB1450	LOT	**Lot #:** Refer to product label
	Detection range: 0.156-10 μmol/mL		**Sensitivity:** 0.156 μmol/mL
	Applicable samples: Animal and Plant Tissues, Cells, Bacteria, Plasma, Serum or other Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Protein contains three sulfur-containing amino acids: Methionine, Cystine and Cysteine (Cys). Among them, Cys is the only sulfur-containing amino acid containing a sulfhydryl group, which is converted from methionine and can be mutually converted with cystine. Cys participates in the formation of protein disulfide bonds and is often a component of the active center of proteins. It can also provide sulfhydryl groups for other physiological and biochemical reactions. Cys accumulates in large amounts on the surface of the skin and mucous membranes, maintains the activity of important sulfhydryl enzymes during keratin production, and supplements sulfhydryl groups to maintain the normal metabolism of the skin and regulate the underlying melanin produced by the pigment cells in the lowermost layer of the epidermis. It has the functions of whitening, detoxification, improving inflammation and allergic skin. CheKine™ Micro Cysteine (Cys) Assay Kit provides a simple method for detecting Cys concentration in a variety of biological samples such as animal and plant tissues, cells, bacteria, liquid sample such as plasma and serum. Cys reduces phosphotungstic acid to produce tungsten blue, which has an absorption peak at 600 nm; the Cys content is calculated by the absorbance at 600 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	120 mL	4℃
Assay Buffer	7 mL	14 mL	4℃
Chromogen	Powder×1 vial	Powder×1 vial	4℃, protected from light
Cys Standard	Powder×1 vial (10 mg)	Powder×1 vial (10 mg)	4℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 600 nm

- 96-well plate or microglass cuvette
- Freezing centrifuge, ice maker, incubator
- Precision pipettes, disposable pipette tips
- Deionized water, phosphoric acid
- Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Working Chromogen Reagent: Prepare the day before use, add 3 mL deionized water to Chromogen for full dissolution, then add 0.75 mL phosphoric acid, mix well, take boiling water bath for 2 h (cover tightly to prevent water loss). After cooling, add 12 mL deionized water. Equilibrate to room temperature before use. Store at 4℃, protected from light. The remaining working solution can be stored at 4℃, protected from light for 2 weeks.

Working Cys Standard: Before use, add 8.264 mL deionized water to prepare 10 μmol/mL Cys standard. Store at 4℃, protected from light. The remaining standard solution can be stored at 4℃, protected from light for 3 days.

Setting of Standard Curves: Further dilute the 10 μmol/mL standard to 5, 2.5, 1.25, 0.625, 0.3125, 0.156 μmol/mL Standard with deionized water, as shown in the following table.

Num.	Volume of Working Cys Standard	Volume of Deionized Water (μL)	Standard Concentration (μmol/mL)
Std.1	200 μL 10 μmol/mL	0	10
Std.2	100 μL of Std.1 (10 μmol/mL)	100	5
Std.3	100 μL of Std.2 (5 μmol/mL)	100	2.5
Std.4	100 μL of Std.3 (2.5 μmol/mL)	100	1.25
Std.5	100 μL of Std.4 (1.25 μmol/mL)	100	0.625
Std.6	100 μL of Std.5 (0.625 μmol/mL)	100	0.3125
Std.7	100 μL of Std.6 (0.3125 μmol/mL)	100	0.156

Notes: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for one month.

1. Animal Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 11,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Plant Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and mash. Ultrasonic break in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 11,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Cells or Bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 11,000 g for 10 minutes at 4℃. Use supernatant for assay, and place it on ice to be tested.
4. Liquid samples: Take 0.1 mL liquid sample, add 0.3 mL Extraction Buffer, then mix well. Centrifuge at 11,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

Note: It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 600 nm, visible spectrophotometer was returned to zero with deionized water.

2. Sample measurement (the following operations are operated in the 96-well plate or microglass cuvette).

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Sample	0	0	20
Standard	0	20	0
Deionized Water	20	0	0
Assay Buffer	100	100	100
Working Chromogen Solution	100	100	100

3. Mix well and kept at room temperature for 15 min. The absorbance value (OD value) is measured at 600 nm. The blank well is recorded as A_{Blank}, the standard well is recorded as $A_{Standard}$, and the test well is recorded as A_{Test}. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Blank well only needs to measure 1times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.001, increase the sample quantity appropriately. If ΔA_{Test} is greater than 1.5, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1.Drawing of standard curve

With the concentration of the standard solution as the y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve.

2.Calculation of Cys content

Bring the ΔA_{Test} of the sample into the equation to get the y value (μmol/mL).

(1) Calculated by volumet of liquid samples

Cys (μmol/mL)**=4×y**

(2) Calculated by fresh weight of samples

Cys (μmol/g fresh weight)=$y\times V_{Sample\ Total}\div W$**=y÷W**

(3) Calculated by cells or bacteria numbers

Cys (μmol/10^4) =$y\times V_{Sample\ Total}\div 500$**=y÷500**

(4) Calculated by protein concentration

Cys (μmol/mg prot) **=y÷Cpr**

Where:4: Dilution ratio of liquid samples during extraction; $V_{Sample\ Total}$:Volume of Extraction Buffer added to samples, 1 mL; Cpr: Sample protein concentration, mg/mL; W: Sample weight, g; 500: Total number of cells or bacteria, 500×10^4.

Typical Data

Typical standard curve

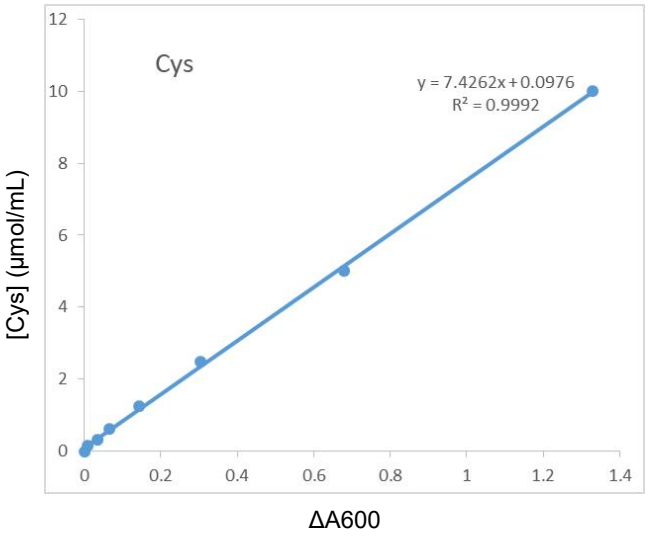

Figure 1. Standard curve of Cys, data provided for demonstration purposes only. A new standard curve must be generated for each assay.

Recommended Products

Catalog No.	Product Name
KTB1410	CheKine™ Micro Alanine Aminotransferase (ALT/GPT) Activity Assay Kit
KTB1420	CheKine™ Micro Aspartate Aminotransferase (AST/GOT) Activity Assay Kit
KTB1430	CheKine™ Micro Proline (PRO) Assay Kit
KTB1440	CheKine™ Micro Glutamate (Glu) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.