Elabscience®

8th Edition, revised in February, 2018

（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Cysteine (Cys) Assay Kit

Catalog No: E-BC-K352
Method: Colorimetric method
Specification: 50 Assays

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) Fax: 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

It is recommended to take 2~3 samples which expected large difference to do pre-experiment before formal experiment.

Application

This kit can be used to measure the cysteine (cys) content in animal serum, plasma, tissue, cells and other samples. This kit (50 Assays) can detect 48 samples.

Detection significance

There are three kinds of sulfur-containing amino acids in protein: Methionine, Cystine and Cys. Cys is the only one sulfur amino acid which contains thiol. Cys is converted from methionine and can mutual transform with Cystine. Cys is involved in the formation of protein disulfide bonds, which usually acts as the component of protein activity center and can provide thiol for other physiological and biochemical reactions. In addition, Cys accumulated on the surface of skin and mucosal, and maintains the activity of important thiol enzyme in the formation of keratin. Cys also supplies the thiol to keep the normal metabolism of skin, and regulates the bottom melanin produced by the pigment cells in the lower epidermis, which has the function of whitening, detoxification, improve inflammation and allergies skin etc.

Detection principle

Phosphotungstic acid can be reduced by Cys and form tungsten blue, which has an absorption peak at 600 nm. Cys content can be calculated with the absorbance at 600 nm.

Reagent composition

Reagent 1: liquid, 55 mL ×1 vial. Store at 4℃.

Reagent 2: liquid, 25 mL ×1 vial. Store at 4℃.

Reagent 3: powder, 1 vial. Store at 4℃.

Preparation of Reagent 3 working solution: prepare fresh solution 1 day before experiment. Add 5 mL of distilled water to dissolve the powder fully, then add 1.25 mL of 85% phosphoric acid (self-prepared). Mix fully and cover the lid tightly. Incubate in boiling water for 2 hours. Add 20 mL of distilled water after cooling. It can be store at 4℃ for 2 weeks.

Standard: powder, 1 vial, Store at 4℃.

Preparation of 1 μmol/mL Standard solution: dissolve a vial of standard powder with 10 mL distilled water fully. The prepared Standard solution can be stored at 4℃ with shading light for 3 days.

Experimental instruments

Spectrophotometer (600 nm), Refrigerated centrifuge, Micropipettor, 1 mL cuvette

Operation steps

1. Extraction of Cys:

(1) Extraction of Cys in liquid sample: take 0.1 mL of liquid sample, add 0.9 mL of Reagent 1 and mix fully. Centrifuge at 8000g for 10 min at 4℃, then take the supernatant for measurement.

(2) Extraction of Cys in tissue sample: add the appropriate volume of **Reagent 1** according to the ratio of Weight (g): Volume (mL) =1: 5~10 (It is recommended to weigh 0.1 g of tissue, and add 1 mL of Reagent 1). Mechanical homogenate the sample in ice water bath. Centrifuge at 8000 g for 10 min at 4℃, then take the supernatant for measurement.

(3) Extraction of Cys in bacteria or culture cells: collect the bacteria or culture cells into the centrifuge tube, centrifuge and discard the supernatant. Add Reagent 1 into the sediment according to the ratio of **Bacteria or cells number: Reagent 1 (mL) =500~1000: 1**(it is recommended to add 1 mL of Reagent 1 into 5×10^6 cells), then treat the sample with sonication on ice (power: 20% or 200W, 3 seconds/time, interval for 10 seconds, repeat 30 times). Centrifuge at 8000 g for 10 min at 4℃. Take the supernatant and preserve it on ice for detection.

2. Operation table:

(1) Preheat the spectrophotometer for more than 30 min, set the wavelength at 600 nm and set to zero with distilled water.

(2) Operation table

	Blank tube	Standard tube	Sample tube
Double-distilled water (mL)	0.1		
Standard solution (mL)		0.1	
Sample(mL)			0.1
Reagent 2 (mL)	0.5	0.5	0.5
Reagent 3 working solution (mL)	0.5	0.5	0.5
Mix fully, stand for 15 min at room temperature. Measure OD value at 600 nm.			

Note: Blank and standard tubes need to be done only once.

Calculation of results

1. According to the volume of liquid sample:

Cys content (*μmol/mL*)

$$=\frac{OD_{Sample}-OD_{Blank}}{OD_{Standard}-OD_{Blank}}\times C_{Standard}\times V_{Standard}\times (V_{Sample\ total}\div V_{Sample})$$

$$=\frac{OD_{Sample}-OD_{Blank}}{OD_{Standard}-OD_{Blank}}\times 1$$

2. According to the weight of sample:

Cys content (*μmol/g*)

$$= \frac{OD_{Sample}-OD_{Blank}}{OD_{Standard}-OD_{Blank}} \times C_{Standard} \times V_{Standard} \times (V_{Sample\ total} \div V_{Sample}) \div W$$

$$= \frac{OD_{Sample}-OD_{Blank}}{OD_{Standard}-OD_{Blank}} \times 1 \div W$$

3. According to the protein concentration:

Cys content (*μmol/mg* prot)

$$= \frac{OD_{Sample}-OD_{Blank}}{OD_{Standard}-OD_{Blank}} \times C_{Standard} \times V_{Standard} \div (V_{Sample\ total} \times Cpr)$$

$$= \frac{OD_{Sample}-OD_{Blank}}{OD_{Standard}-OD_{Blank}} \times 1 \div Cpr$$

4. According to the amount of cell:

Cys content (*μmol*/10^4 cell)

$$= \frac{OD_{Sample}-OD_{Blank}}{OD_{Standard}-OD_{Blank}} \times C_{Standard} \times V_{Standard} \times (V_{Sample\ total} \div V_{Sample}) \div Amount\ of\ cells$$

$$= \frac{OD_{Sample}-OD_{Blank}}{OD_{Standard}-OD_{Blank}} \times 1 \div Amount\ of\ cells$$

$C_{Standard}$: concentration of standard, 1 μmol/mL.

$V_{Standard}$: the volume of standard in the reaction system, 0.1 mL.

V_{Sample}: volume of sample extraction in the reaction system, 0.1 mL.

$V_{Sample\ total}$: total volume of extraction solution, 1 mL.

W: weight of sample, g.

Cpr: protein concentration of sample, mg/mL.

Note:

1. The kit is for scientific research only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 3 months.
4. Do not use components from different batches of kit.
5. The minimum detection limit is 100 μmol/L.
6. If the Cys content is calculated by protein concentration, the protein concentration of the sample needs to be determined separately (E-BC-K318, E-BC-K168, E-BC-K165).