ELK Biotechnology

Cysteine (Cys) content test kit

(Cat/No.:BC146   Size:50T/48S)

1. Assay Significance

Proteins contain 3 types of sulfur-containing amino acids: Methionine, Cystine, and Cys. Cys is the only sulfur-containing amino acid that contains sulfhydryl groups, is derived from Methionine, and can be interconverted with Cystine. Cys is involved in the formation of disulfide bonds in proteins, is often a component of the active center of proteins, and can provide sulfhydryl groups for other physiological and biochemical reactions. In addition, Cys massively accumulates on the surface of the skin and mucous membranes, maintains the activity of important mercapto enzymes in keratinogenesis, and replenishes sulfhydryl groups to maintain normal metabolism of the skin and regulate the production of underlying melanin by the pigment cells in the lowermost layer of the epidermis. It has whitening, detoxifying, improving inflammatory and allergic skin effects.

2. Assay Principle (Colorimetric Method)

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.

3. Instrumentation (self-provided)

Visible spectrophotometer, cryogenic centrifuge, micropipette gun, phosphoric acid and distilled water.

4. Reagents composition

Reagent 1: liquid ×1 bottle, stored at 4°C.
Reagent 2: liquid ×1 bottle, stored at 4°C.
Reagent 3: powder ×1 bottle, stored at 4°C..Add 5mL of distilled water to Reagent 3 one day in advance to fully dissolve, then add 1.25mL of phosphoric acid, mix well, and cover tightly (to prevent water dissipation). Boiling water bath for 2h; after cooling, add 20mL of distilled water and store at 4°C. for 2 weeks.
Standard Buffer： 1μmol/mL Standard solution × 1 bottle, stored at 4°C.

5. Operation Procedure

1,Cysteine extraction

(1) Cysteine extraction from liquid samples: Take 0.1mL of liquid sample, add 0.9mL of Reagent 1, mix well, 8000g, centrifuge at 4°C for 10min, and take the supernatant to be measured.

2,Cysteine extraction in tissues: According to the ratio of tissue mass (g): volume of extraction solution (mL) = 1:5-10 (i.e., take about 0.1g of tissue and add 1 mL of Reagent 1),

homogenize in an ice bath, 8000 g, centrifuge at 4°C for 10 min, and take the supernatant to be measured.

3,Bacteria or cultured cells: Collect the bacteria or cells into the centrifuge tube first, and discard the supernatant after centrifugation. According to the ratio of the number of bacteria or cells (10^4): the volume of the extraction solution (mL) = 500~1000:1 (it is recommended to add 5 million bacteria or cells to 1mL of Reagent 1), ultrasonic breakage of bacteria or cells (ice bath, power 20% or 200W, ultrasonic 3s, 10s interval, repeat 30 times); 8000g, centrifuge at 4℃ for 10min, take the supernatant and put it on ice to be measured.

2,Operational Table

Reagent	Blank	Standard	Sample
Double-distilled water（μL）	100		
Standard Solution（μL）		100	
Sample（μL）			100
Reagent 2（μL）	800	800	800
Reagent 3（μL）	100	100	100
Well mixed, room temperature static 15min, wavelength 600nm, double-distilled water zero, measurement of absorbance values of each tube.			

Note: Blanks and Standards should only be made in 1-2 tubes.

3, Unit Definitions and Formulas

1) By volume of liquid sample:

$$\text{Cys} - \text{concentration} \ (\mu mol/mL) = \frac{A_{sample} - A_{blank}}{A_{standard} - A_{blank}} \times C_{standard}$$

2) By mass of sample:

$$\text{Cys} - \text{concentration} \ (\mu mol/gtissue) = \frac{A_{sample} - A_{blank}}{A_{standard} - A_{blank}} \times C_{standard} \div \frac{W}{V_{Total\ Sample\ Volume}}$$

3) by protein concentration:

$$\text{Cys} - \text{concentration} \ (\mu mol/mg\,prot) = \frac{A_{sample} - A_{blank}}{A_{standard} - A_{blank}} \times C_{standard} \div Cpr$$

4) by number of cells or bacteria:

$$\text{Cys} - \text{concentration} \ (\mu mol/10^4 cells) = \frac{A_{sample} - A_{blank}}{A_{standard} - A_{blank}} \times C_{standard} \div C_{cell}$$

$C_{standard}$: Concentration of standard solution, 1μmol/mL

W: Mass of sample, g

$V_{Total\ Sample\ Volume}$: Total volume of extraction solution, mL

Cpr: Sample Protein Concentration, mgprot/mL（prot is Protein）

C_{cell}: Cell concentration, 104 /mL.

4, detection limit

Minimum detectable limit: 0.05 μmol/mL.