

Cysteine (Cys) Content Assay Kit

Note: Take two or three different samples for prediction before test.

Detection equipment: Spectrophotometer/Microplate reader

Cat No: NA0614

Size: 100T/96S

Components:

Extract solution: Liquid 50 mL×1, store at 4°C.

Reagent I: Liquid 15 mL×1, store at 4°C.

Reagent II: Powder×2, store at 4°C. The day before it is to be used, add 2 mL of distilled water to Reagent II, fully stir for dissolving, then add 0.5 mL of phosphoric acid, mix thoroughly. Incubate at boiling water for 2 hours and add 8 mL of distilled water after cooling. The reagent can be stored for two weeks at 4°C.

Standard: Powder×1, 10 mg cysteine, store at 4°C. Dissolve in 4.13 mL of distilled water prepare as 20 $\mu\text{mol/mL}$ standard solution before use. The solution could be stored at 4°C for 4 weeks.

Description:

Protein contains three kinds of sulfur-containing amino acids: methionine, cystine and cysteine(Cys). Cys is the only sulfur-containing amino acid containing sulfhydryl groups, which derived from methionine and could be transformed with cystine. Cys participates in the formation of protein disulfide bonds, which usually is a component of active centers of protein and can provide mercapto groups for other physiological and biochemical reactions. Besides, a large amount of Cys accumulates on skin and mucosal surfaces to maintain elasticity and texture of skin and keep the activity of thiolase in the process of keratin production. It has the functions of whitening, detoxification, inflammation improvement and so on.

The phosphotungstic acid is reduced to tungsten blue by Cys, and the tungsten blue has absorption peak at 600 nm. In this kit, the content of Cys is calculated by measuring the absorbance at 600 nm.

Required but not provided:

Refrigerated centrifuge, transferpettor, spectrophotometer/microplate reader, micro glass cuvette/96 well flat-bottom plate, mortar/ homogenizer, phosphoric acid and distilled water.

Protocol:

I. Sample preparation

1. Liquid sample:

Add 0.3 mL of Extract solution to 0.2 mL of liquid sample, mix thoroughly. Centrifuge at 11000 rpm for 10 minutes at 4°C to remove insoluble materials, and take the supernatant on ice for test.

2. Tissue sample:

Add 0.5 mL of Extract solution to 0.2 g of tissue, mix thoroughly on ice. Centrifuge at 11000 rpm for 10 minutes at 4°C to remove insoluble materials, and take the supernatant on ice for test.

II. Detection

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 600 nm and set zero with distilled water.
2. Standard: Dilute the standard solution with 4.13 mL of distilled water to generate a 20 $\mu\text{mol/mL}$ standard. Then diluted to 2, 1, 0.5, 0.25, 0.125, 0.0625 $\mu\text{mol/mL}$ standard with distilled water.
3. Add reagents as the following table.

Reagent (μL)	Test tube (T)	Standard tube (S)	Blank tube (B)
Sample	40	-	-
Standard	-	40	-
Distilled water	-	-	40
Reagent I	100	100	100
Reagent II	60	60	60

Mix and keep at room temperature for 15 minutes, detect the absorbance at 600 nm.

III. Calculation

1. Standard curve

The concentration of standard solution as x-axis, $\Delta A(A_S - A_B)$ as y-axis, obtain the equation $y = kx + b$. Take $(A_T - A_B)$ to the equation to acquire x value.

2. Calculate

- 1) Liquid sample

$$\text{Cys } (\mu\text{mol/mL}) = x \times V_{ST} \div V_{S1} = 2.5x$$

- 2) Sample weight

$$\text{Cys } (\mu\text{mol/g FW}) = x \times V_{S2} \div (W \times V_{S2} \div V_{ST}) = 0.5x \div W$$

V_{S1} : Liquid volume, 0.2 mL;

V_{S2} : Sample volume, 0.04 mL;

V_{ST} : Extract solution volume, 0.5 mL;

W: Sample weight, g.

Note:

If the absorbance value exceeds the linear range, the sample size can be increased or diluted before the determination.

Recent Product Citations:

[1] Huang Q, Wang M, Xia Z. The SULTR gene family in maize (*Zea mays* L.): gene cloning and expression analyses under sulfate starvation and abiotic stress[J]. Journal of plant physiology, 2018, 220: 24-33.

[2] Yansha Han, Mengyang Wu, Lihong Hao, et al. Sulfur dioxide derivatives alleviate cadmium toxicity by enhancing antioxidant defence and reducing Cd^{2+} uptake and translocation in foxtail millet seedlings. Ecotoxicology and Environmental Safety. August 2018;(IF4.527)

Related Products:

- NA0744/NA0502 Glutamic-pyruvic Transaminase(GPT) Activity Assay Kit
- NA0743/NA0501 Glutamic-oxalacetic Transaminase(GOT) Activity Assay Kit
- NA0845/NA0603 Proline(PRO) Content Assay Kit
- NA0742/NA0500 Amino Acid(AA) Content Assay Kit
- NA0741/NA0499 Glutamic Acid(Glu) Content Assay Kit

Technical Specification:

Detection Limit: 0.0066 $\mu\text{mol/mL}$

Linear range: 0.03125-3 $\mu\text{mol/mL}$