Cysteine (Cys) Content Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Detection equipment: Spectrophotometer/Microplate reader

Cat No: BC0185

Size: 100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 50 mL×1	2-8℃
Reagent I	Liquid 15 mL×1	2-8℃
Reagent II	Powder ×2	2-8℃
Standard	Powder ×1	2-8℃

Solution Preparation:

1. Reagent II: The day before it is to be used, add 2 mL of distilled water to one Reagent II, fully stir for dissolving, then add 0.5 mL phosphoric acid, mix thoroughly. Incubate at boiling water for 2 hours (Wrap the sealing film to prevent bursting) and add 8 mL distilled water after cooling. The reagent can be stored for two weeks at 2-8℃. (One bottle of reagent is enough for 100T, another is to prolong the stable storage time of the reagent)
2. Standard: 10 mg of cysteine. Dissolve in 4.13 mL of distilled water to prepare as 20 μmol/mL standard solution before use. The solution could be stored at 2-8℃ 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:

Spectrophotometer/microplate reader, refrigerated centrifuge, transferpettor, micro glass cuvette/96 well flat-bottom plate, mortar/ homogenizer, concentrated phosphoric acid (85%, AR) 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℃ 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℃ 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 to 2, 1, 0.5, 0.25, 0.125, 0.0625 μmol/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. Note as A_T, A_S, A_B. calculate $\Delta A_T=A_T-A_B$, $\Delta A_S=A_S-A_B$. The blank and standard curve only need to be measured 1-2 times.

III. Calculation

1. Standard curve

The concentration of standard solution as x-axis, ΔA_S as y-axis, obtain the equation y=kx+b. Take ΔA_T to the equation to acquire x value.

2. Calculate

1) Liquid sample

Cys content (μmol/mL)=$x\times V_{ST}\div V_{S1}=2.5x$

2) Sample weight

Cys content (μmol/g weight) =$x\times V_{S2}\div(W\times V_{S2}\div V_{ST})=0.5x\div W$

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] Jing Y, Luo L, Chen Y, Westerberg LS, Zhou P, Xu Z, Herrada AA, Park CS, Kubo M, Mei H, Hu Y, Lee PP, Zheng B, Sui Z, Xiao W, Gong Q, Lu Z, Liu C. SARS-CoV-2 infection causes immunodeficiency in recovered patients by downregulating CD19 expression in B cells via enhancing B-cell metabolism. Signal Transduct Target Ther. 2021 Sep 22;6(1):345. doi: 10.1038/s41392-021-00749-3. PMID: 34552055; PMCID: PMC8456405.

[2] Li K, Lin C, Li M, Xu K, He Y, Mao Y, Lu L, Geng W, Li X, Luo Z, Cai K. Multienzyme-like Reactivity Cooperatively Impairs Glutathione Peroxidase 4 and Ferroptosis Suppressor Protein 1 Pathways in Triple-Negative Breast Cancer for Sensitized Ferroptosis Therapy. ACS Nano. 2022 Feb 22;16(2):2381-2398. doi: 10.1021/acsnano.1c08664. Epub 2022 Jan 18. PMID: 35041395.

[3] Mao HT, Chen LX, Zhang MY, Shi QY, Xu H, Zhang DY, Zhang ZW, Yuan M, Yuan S, Zhang HY, Su YQ, Chen YE. Melatonin improves the removal and the reduction of Cr (VI) and alleviates the chromium toxicity by antioxidative machinery in Rhodobacter sphaeroides. Environ Pollut. 2023 Feb 15; 319:120973. doi: 10.1016/j.envpol. 2022.120973. Epub 2022 Dec 28. PMID: 36584859.

[4] Zeng YY, Luo YB, Ju XD, Zhang B, Cui YJ, Pan YB, Tian JH, Teng WJ, Wu J, Li Y. Solasonine Causes Redox Imbalance and Mitochondrial Oxidative Stress of Ferroptosis in Lung Adenocarcinoma. Front Oncol. 2022 May 18; 12:874900. doi: 10.3389/fonc.2022.874900. PMID: 35664792; PMCID: PMC9158126.

[5] Luo Y, Pang J, Peng C, Ye J, Long B, Tong J, Shi J. Cr (VI) Reduction and Fe (II) Regeneration by Penicillium oxalicum SL2-Enhanced Nanoscale Zero-Valent Iron. Environ Sci Technol. 2023 Aug 1;57(30):11313-11324. doi: 10.1021/acs.est.3c01390. Epub 2023 Jul 20. PMID: 37474249

Related Products:

BC1550/BC1555	Glutamic-pyruvic Transaminase(GPT) Activity Assay Kit
BC1560/BC1565	Glutamic-oxalacetic Transaminase(GOT) Activity Assay Kit
BC0290/BC0295	Proline(Pro) Content Assay Kit
BC1570/BC1575	Amino Acid(AA) Content Assay Kit

Technical Specification:

Detection Limit: 0.0066 μmol/mL

Linear range: 0.03125-3 μmol/mL