Cysteine (Cys) Assay Kit

Catalog No.: abx298974

Size: 96 tests

Detection Range: 0.07 mmol/L – 2.0 mmol/L

Sensitivity: 0.03 mmol/L

Storage: Store all components at 4°C in the dark for up to 12 months.

Application: For detection and quantification of Cys content in serum, plasma, tissue homogenates and cell lysates.

Introduction

Cysteine is an amino acid used in the biosynthesis of proteins. Cysteine is produced in the body from the amino acid serine, and is obtained in the diet through high protein foods. A nucleophile in many enzymatic reactions, Cysteine plays crucial roles in metabolism and as an antioxidant. In industry, Cysteine is used as a precursor, for example, as a precursor to meat flavoring produced in the Maillard reaction.

Abbexa's Cysteine Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Cysteine content. The absorbance should be measured at 600 nm. The intensity of the color is proportional to the content of Cysteine, which can then be calculated.

Kit components

1. 96-well microplate
2. Assay buffer: 15 ml
3. Acid reagent: 2 × 60 ml
4. Chromogenic Reagent: 12 ml
5. Standard: 1 vial
6. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (600 nm)
2. Double distilled water
3. Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. Vials/tubes
6. Sonicating water bath
7. Centrifuge
8. Vortex mixer

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Add 0.05 ml of the supernatant to 0.45 ml of Acid Reagent and mix fully. Centrifuge at 10,000 × g for 10 minutes at 4°C. Take the supernatant immediately on ice for assay.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge for 10 mins at 1000-2000 × g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets). Add 0.05 ml of the supernatant to 0.45 ml of Acid Reagent and mix fully. Centrifuge at 10,000 × g for 10 minutes at 4°C. Take the supernatant on ice immediately for assay.
- **Tissue Homogenates:** Weigh the tissue homogenate. For each 1 g of homogenate, add 9 ml Acid Reagent. Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 10,000 x g at 4°C for 10 min. Collect the supernatant and assay immediately.
- **Cell lysates:** Collect cells into a centrifuge tube and wash with PBS. Centrifuge at 1000 × g for 10 min and discard the supernatant. Add 300-500 µl Acid Reagent per 1 × 10^6 cells, then sonicate in an ice water bath. Centrifuge at 10,000 × g at 4 °C for 10 min. Take the supernatant into a new centrifuge tube (while kept on ice) and analyze immediately. The protein concentration in the supernatant should be determined separately.

Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

We recommend carrying out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment.

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.
- If sample dilutions are required, the Acid reagent should be used as the diluent.

2. Reagents

- **10 mmol/L Standard solution:** Dissolve a vial of standard with 10 ml double distilled water and mix fully. Do not vortex. The reconstituted standard can be stored at 2-8°C in the dark for up to 4 days.

B. Assay Procedure

1. **Standard curve preparation:** Label 8 tubes with 2.0, 1.5, 1.0, 0.75, 0.5, 0.25 and 0.125 and 0 mmol/L. Dilute the 10 mmol/L standard solution with double distilled water according to the table below.

Concentration (mmol/L)	Volume of 10 mmol/L standard (µl)	Volume of double distilled water (µl)
2.000	400	1600
1.500	300	1700
1.000	200	1800
0.750	150	1850
0.500	100	1900
0.250	50	1950
0.125	25	1975
0	0	2000

2. Sample detection

2.1. Set the Standard, Sample and Blank wells on the well-plate and record their positions.
2.2. Add 20 µl of prepared standards to the Standard wells.
2.3. Add 20 µl of each sample to the sample wells.
2.4. Add 100 µl of Assay Buffer to all wells.
2.5. Add 100 µl of Chromogenic Reagent to all wells.
2.6. Tap the plate gently to mix. Allow to stand at room temperature for 10 minutes.
2.7. Measure the OD values at 600 nm with a microplate reader.

C. Calculation of Results

The standard curve can be plotted as the absolute OD_{600} of each standard solution (*y*) vs. the respective concentration of the standard solution (*x*). A linear fit is recommended for the standard curve (*y* = a*x* + b). The Cysteine concentration of the samples can be interpolated from the standard curve.

1. Serum and plasma samples:

$$\text{Cys (mmol/L)} = \frac{\Delta A_{600} - b}{a} \times f \times 10$$

2. Tissues samples:

$$\text{Cys (mmol/kg)} = \frac{\Delta A_{600} - b}{a} \times f \times \frac{V_1}{m}$$

3. Cell lysate samples:

$$\text{Cys (mmol/}10^9\text{ cells)} = \frac{\Delta A_{600} - b}{a} \times f \times \frac{V_2}{n}$$

where:

Cys	concentration of cysteine
ΔA_{600}	OD value of the sample ($OD_{Sample} - OD_{Blank}$)
a	gradient of the standard curve (linear fit)
b	y-intercept of the standard curve (linear fit)
f	dilution factor of the sample
n	number of cells × 10^6. For example, for 8 × 10^6 cells, n = 8
m	mass of tissue homogenate sample
10	dilution factor for serum/plasma samples
V_1	volume of Acid reagent added in the sample preparation for tissue samples
V_2	volume of Acid reagent added in the sample preparation for cell lysate samples