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.

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

Introduction

Abbexa's Cysteine Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Cysteine content. Phosphotungstic acid is reduced by Cys to form tungsten blue, which has an absorbance maxima 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. Acidic 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. Pipette and pipette tips
4. Sonicator
5. Centrifuge and centrifuge tubes
6. Vortex mixer
7. Manual homogenizer

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Protocol

A. Preparation of samples and reagents

1. Reagents

- **10 mmol/L Standard:** Reconstitute 1 vial of standard with 10 ml double-distilled water and mix thoroughly. Unused 10 mmol/L standard can be stored at 2-8°C in the dark for up to 4 days.
- **Standards:** Label 7 tubes with 0.125 nmol/L, 0.25 nmol/L, 0.5 nmol/L, 0.75 nmol/L, 1 nmol/L, 1.5 nmol/L, and 2 nmol/L. Dilute the 10 mmol/L Standard with double-distilled water according to the following dilution scheme:

Standard Dilution (nmol/L)	0.125	0.25	0.5	0.75	1	1.5	2
10 nmol/L Standard (µl)	25	50	100	150	200	300	400
Double-distilled water (µl)	1975	1950	1900	1850	1800	1700	1600

For the blank, use pure double-distilled water. The volume of each standard dilution will be 2000 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.

2. 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 and Plasma:** Per 50 µl of serum or plasma, add 450 µl of Acidic Reagent and mix thoroughly. Centrifuge at 10,000 × g for 10 minutes at 4°C. Collect the supernatant, keep on ice, and assay immediately.
- **Tissue Homogenates:** Weigh at least 0.1 g tissue homogenate. Per 0.1 g of tissue, add 9 ml Acidic Reagent. Homogenize manually, using a mechanical homogenizer at 4°C. Centrifuge at 10,000 × g at 4°C for 10 minutes. Collect the supernatant and assay immediately.
- **Cell lysates:** Collect at least 1 × 10^6 cells into a centrifuge tube. Centrifuge at 1000 × g for 10 minutes, then discard the supernatant. Per 1 × 10^6 cells, add 200 µl Acidic Reagent. Homogenize manually, using a mechanical homogenizer or by sonication. Centrifuge at 10,000 × g at 4 °C for 10 minutes. Collect the supernatant, keep on ice, and assay immediately.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with Acidic Reagent, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human serum	1
Mouse serum	1
10% Rat lung tissue homogenate	1
10 Mouse heart tissue homogenate	1
10% Rat brain tissue homogenate	1

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.

B. Assay Procedure

1. Mark positions on the 96-well microplate for each standard, blank, sample, and control. *Each sample requires a corresponding control. It is strongly recommended to test all wells in duplicate*.
1. Add 20 µl of prepared Standard Dilutions to the Standard wells.
2. Add 20 µl of double-distilled water to the blank wells.
3. Add 20 µl of sample to the sample wells.
4. Add 100 µl of Assay Buffer to each well.
5. Add 100 µl of Chromogenic Reagent to each well.
6. Mix the well contents by tapping the plate, or shaking with a microplate shaker, for at least 5 seconds.
7. Stand at room temperature for 10 minutes.
8. Measure the OD values of each well with a microplate reader at 600 nm.

C. Calculation of Results

Average the duplicate readings for each standard dilution. Subtract the mean OD value of the control well from each standard dilution to get the absolute OD values. Plot the standard curve, using the OD of the standard dilutions on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the concentration of Cysteine in each sample well can be derived with the following formulae:

1. Serum and plasma samples:

$$\mathbf{Cys\ (mmol/L)} = \frac{(\Delta \mathbf{A} - \mathbf{b})}{\mathbf{a}} \times \mathbf{10} \times \mathbf{F}$$

2. Tissues samples:

$$\mathbf{Cys\ (mmol/kg)} = \frac{(\Delta \mathbf{A} - \mathbf{b})}{\mathbf{a}} \times \frac{\mathbf{V_1}}{\mathbf{W}} \times \mathbf{F}$$

3. Cell lysate samples:

$$\mathbf{Cys\ (mmol/10^9\ cells)} = \frac{(\Delta \mathbf{A} - \mathbf{b})}{\mathbf{a}} \times \frac{\mathbf{V_2}}{\mathbf{n}} \times \mathbf{F}$$

where:

ΔA	OD value of sample – OD value of blank
a	Gradient of the standard curve ($y = ax + b$)
b	Y-intercept of the standard curve ($y = ax + b$)
F	Dilution factor of the sample
n	Number of cells $\times 10^6$ (e.g. 5×10^6 cells, n = 5)
W	Weight of the tissue sample (kg)
10	Dilution factor for serum and plasma samples
V_1	Volume of Acidic reagent added in the sample preparation for tissue samples
V_2	volume of Acidic reagent added in the sample preparation for cell lysate samples

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.