Technical Manual

Cysteine (Cys) Colorimetric Assay Kit

- **Catalogue Code: MAES0181**
- **Size: 96T**
- **Research Use Only**

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.07-2.0 mmol/L

Sensitivity:

0.03 mmol/L

Storage:

2-8°C for 3 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 3 months.

Do not use components from different batches of kit.

2. Background

Cysteine (Cys) is one of the most widely used molecules in biology, with different functions such as catalysis, structure, regulation and electron transport and it is the donor of sulfides in all cells. Cysteine is the most abundant thiol in plasma and can be used as an extracellular regulatory factor of thiols and disulfide bonds to maintain proper redox state. The concentration of total cysteine in serum or plasma is related to the risk of vascular disease.

3. Intended Use

This kit can be used to measure cysteine (Cys) content in serum, plasma, animal tissue and cells samples.

4. Detection Principle

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.

5. Kit Components & Storage

Item	Specification	Storage
Acid Reagent	60 mL × 2 vials	2-8°C, 3 months, avoid direct sunlight
Buffer Solution	15 mL × 1 vial	2-8°C, 3 months
Chromogenic Agent	12 mL × 1 vial	2-8°C, 3 months, avoid direct sunlight
Standard	Lyophilized × 1 vial	2-8°C, 3 months, avoid direct sunlight
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Water bath
- Vortex mixer
- centrifuge
- Microplate reader (600-620 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL, 5 mL)
- Double distilled water

6. Assay Notes:

It is recommended to take fresh samples for detection.

7. Reagent Preparation:

Preparation of **standard solution (10 mmol/L):** Dissolve a vial of standard powder with 10 mL distilled water fully. The prepared standard solution can be stored at 2-8°C and avoid direct sunlight for 4 days.

8. Sample Preparation

1. Serum sample:
Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) and preserve on ice before detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) and preserve on ice before detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. Tissue sample:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Use filter paper to absorb excess water and weigh. Homogenize at the ratio of the volume of acid reagent (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

4. Cell sample:
Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (10^6): acid reagent (μL) =1: 300-500. Sonicate the sample on an ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve on ice before detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.07-2.0 mmol/L).

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: The diluent is acid reagent.

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 600 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

10. Operation Steps

The preparation of standard curve

Dilute 10 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.125, 0.25, 0.5, 0.75, 1, 1.5, 2 mmol/L.

Extraction of Cys in samples

Extraction of Cys in serum (plasma) sample: take 0.05 mL of serum (plasma) sample, add 0.45 mL of acid reagent and mix fully. Centrifuge at 10000 g for 10 min at 4°C, then take the supernatant for measurement.

Extraction of Cys in tissue sample: add the appropriate volume of acid reagent according to the ratio of Weight (g): Volume (mL) =1: 9 (It is recommended to weigh 0.1 g of tissue, and add 0.9 mL of acid reagent). Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min at 4°C, then take the supernatant for measurement.

Extraction of Cys in culture cells: collect the cells into the centrifuge tube, centrifuge and discard the supernatant. Add acid reagent into the sediment according to the ratio of cells number (10^6): acid reagent (mL) =1: 0.3-0.5, then treat the sample with sonication or homogenization (there is no obvious cell sediment under the microscope). Centrifuge at 10000 g for 10 min at 4°C. Take the supernatant and preserve on ice before detection.

The measurement of samples

1. **Standard well:** Take 20 µL of standard solution with different concentrations to the wells.
   Sample well: Take 20 µL of sample to the wells.

2. Add 100 µL of buffer solution into each well.

3. Add 100 µL of chromogenic agent into each well.

4. Mix fully with microplate reader for 5 s and stand for 10 min at room temperature.

5. Measure the OD value at 600 nm with microplate reader.

Operation Table

	Standard well	Sample well
Standard solution of different concentrations (µL)	20	
Sample (µL)		20
Buffer solution (µL)	100	100
Chromogenic agent (µL)	100	100
Mix fully with microplate reader for 5 s and stand for 10 min at room temperature. Measure the OD value at 600 nm with microplate reader.		

11. Calculations

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

1. Serum (plasma) sample:

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

2. Tissue sample:

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

3. Cell sample:

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

y: $OD_{Standard}$ – OD_{Blank} (OD_{Blank} is the OD value when the standard concentration is 0);
x: The concentration of standard;
a: The slope of standard curve;
b: The intercept of standard curve;
Δ_{A600}: OD_{Sample} – OD_{Blankl};
10*: Dilution factor of serum (plasma) sample in extraction of Cys.
m: The weight of tissue sample.
n*: The number of the cells, when the number of cells is 5×10^6, that "n" is 5.
V_1: The volume of acid reagent added in the extraction step of tissue sample.
V_2: The volume of acid reagent added in the extraction step of cell sample.

12. Performance Characteristics

Detection Range	0.07-2.0 mmol/L
Sensitivity	0.03 mmol/L
Average recovery rate (%)	94
Average inter-assay CV (%)	1.3
Average intra-assay CV (%)	1.1

Analysis

Take 0.05 mL of mouse serum sample and carry the assay according to the operation table.

The results are as follows:

Standard curve: y = 0.1074 x – 0.0046, the average OD value of the sample is 0.046, the average OD value of the blank is 0.041, and the calculation result is:

$$\textbf{Cys content (mmol/L)} = \frac{(0.046 - 0.041 + 0.0046)}{0.1074} \times 10$$

$$= 0.89 \text{ mmol/L}$$

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Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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