（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K352-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（600-620 nm）

Detection range：0.07-2.0 mmol/L

Elabscience® Cysteine (Cys) Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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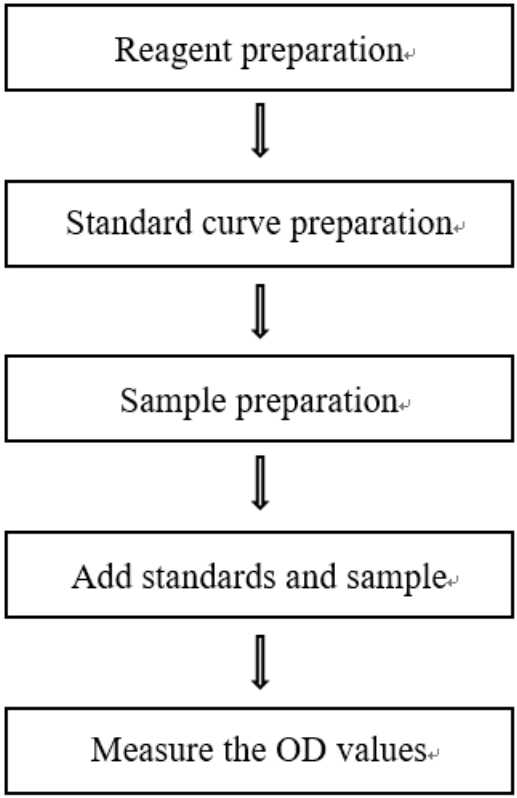
Assay summary



Intended use

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

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.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Acid Reagent	60 mL ×1 vial	60 mL ×2 vials	2-8℃, 12 months shading light
Reagent 2	Buffer Solution	7 mL ×1 vial	15 mL ×1 vial	2-8℃, 12 months
Reagent 3	Chromogenic Agent	6 mL ×1 vial	12 mL ×1 vial	2-8℃, 12 months shading light
Reagent 4	Standard	Powder ×1 vial	Powder ×1 vial	2-8℃, 12 months shading light
	Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (600-620 nm), Micropipettor, Centrifuge, Water bath, Incubator, Vortex mixer

Reagents:

Double distilled water

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of 10 mmol/L standard solution：

Dissolve a vial of standard powder with 10 mL distilled water and mix well. Store at 2~8 ℃ for 4 days protected from light.

③ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

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. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.125**	**0.250**	**0.500**	**0.750**	**1.000**	**1.500**	**2.000**
10 mmol/L standard (μL)	0	25	50	100	150	200	300	400
Double distilled water (μL)	2000	1975	1950	1900	1850	1800	1700	1600

Sample preparation

① Sample preparation

Extraction of Cys in serum (plasma) sample:

① Take 0.05 mL of serum (plasma) sample, add 0.45 mL of acid reagent and mix fully.

② Centrifuge at 10000 g for 10 min at 4℃, then take the supernatant and stand on ice for measurement.

Extraction of Cys in tissue sample:

① 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.

② Centrifuge at 10000 g for 10 min at 4℃, then take the supernatant for measurement.

Extraction of Cys in culture cells:

① Collect the cells into the centrifuge tube, centrifuge and discard the supernatant.

② Add acid reagent into the sediment according to the ratio of cells number (10^6): acid reagent (mL) =1: 0.2, then treat the sample with sonication or homogenization (there is no obvious cell sediment under the microscope).

③ Centrifuge at 10000 g for 10 min at 4℃. Take the supernatant and preserve it on ice for detection.

② Dilution of sample

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. For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

It is recommended to take fresh samples for detection.

Operating steps

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 to each well.

③ Add 100 μL of chromogenic agent to 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.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

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

2. Tissue sample:

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

3. Cell sample:

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

[Note]

ΔA_{600}: $OD_{Sample} - OD_{Blank}$.

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.

f: Dilution factor of sample before tested.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.42	1.05	1.50
%CV	1.4	1.2	0.7

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.42	1.05	1.50
%CV	0.9	1.4	1.6

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 94%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.185	0.62	1.3
Observed Conc. (mmol/L)	0.2	0.6	1.2
Recovery rate (%)	95	92	95

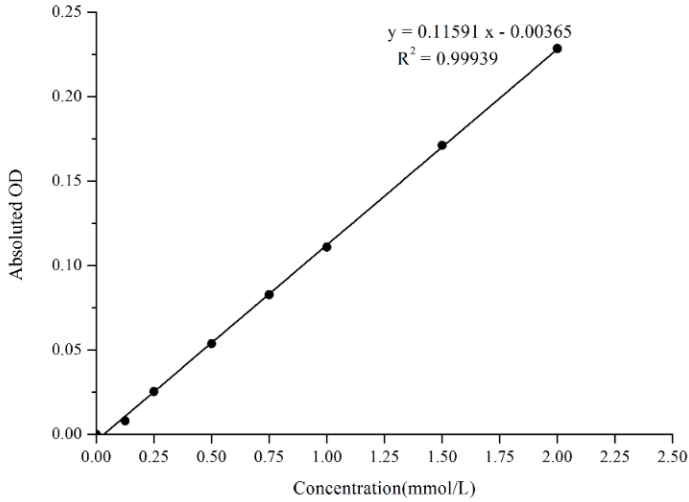
Sensitivity

The analytical sensitivity of the assay is 0.03 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (mmol/L)	**0.00**	**0.125**	**0.25**	**0.50**	**0.75**	**1.00**	**1.50**	**2.00**
OD value	0.038	0.046	0.066	0.092	0.122	0.149	0.206	0.266
	0.038	0.046	0.061	0.091	0.120	0.149	0.212	0.268
Average OD	0.038	0.046	0.064	0.092	0.121	0.149	0.209	0.267
Absoluted OD	0.000	0.008	0.026	0.054	0.083	0.111	0.171	0.229



Appendix II Example Analysis

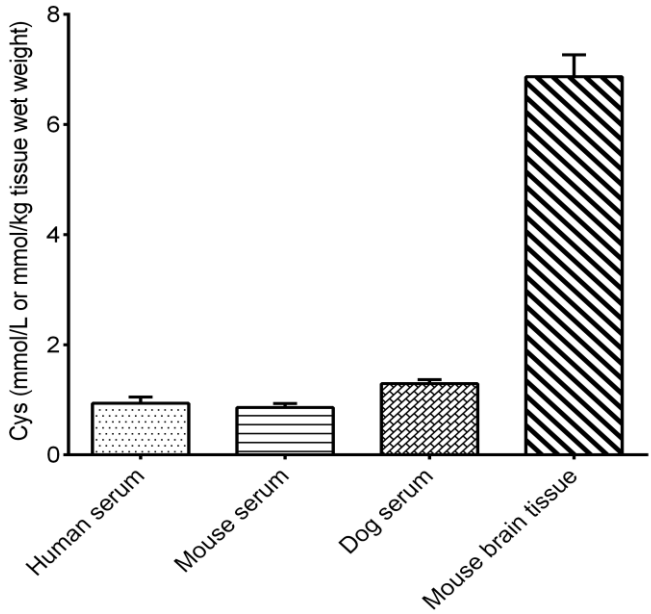
Example analysis:

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

$$\underset{(\text{mmol/L})}{\text{Cys content}} = (0.046 - 0.041 + 0.0046) \div 0.1074 \times 10 = 0.89 \text{ mmol/L}$$

Detect human serum, mouse serum, dog serum and 10% mouse brain tissue homogenate (the wet weight content is 0.11 g/mL) according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.