PRODUCT INFORMATION & MANUAL

Cysteine Assay Kit (Colorimetric)

NBP3-25785

For research use only.
Not for diagnostic or therapeutic procedures.

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Cysteine Assay Kit (Colorimetric)

Catalog No: NBP3-25785

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.03 mmol/L

Detection range: 0.07-2.0 mmol/L

Average intra-assay CV (%): 1.1

Average inter-assay CV (%): 1.3

Average recovery rate (%): 94

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

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

▲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.

▲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	Specification	Storage
Reagent 1	Acid Reagent	60 mL × 2 vials	2-8℃ , 12 months, shading light
Reagent 2	Buffer Solution	15 mL × 1 vial	2-8℃ , 12 months
Reagent 3	Chromogenic Agent	12 mL × 1 vial	2-8℃ , 12 months, shading light
Reagent 4	Standard	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.			

▲Materials prepared by users

Instruments

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

Reagents:

Double distilled water

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

⚠ Precautions

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

▲ The key points of the assay

1. It is recommended to take fresh samples for detection.

Pre-assay preparation

▲Reagent preparation

Preparation of 10 mmol/L standard solution

Dissolve a vial of standard powder with 10 mL distilled water fully. The prepared standard solution can be stored at 2-8℃ with shading light for 4 days.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and 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 reagent 1..

Assay protocol

▲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.

▲Detailed operating steps

1. 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. Reference is as follows:

Number	Standard concentrations (mmol/L)	10 mmol/L Standard(μL)	Double distilled water (μL)
A	0	0	2000
B	0.125	25	1975
C	0.250	50	1950
D	0.500	100	1900
E	0.750	150	1850
F	1.000	200	1800
G	1.500	300	1700
H	2.000	400	1600

2. Extraction of Cys in samples

1) Extraction of Cys in serum (plasma) sample: take 0.05 mL of serum (plasma) sample, add 0.45 mL of reagent 1 and mix fully. Centrifuge at 10000 g for 10 min at 4℃ , then take the supernatant for measurement.

2) Extraction of Cys in tissue sample: add the appropriate volume of reagent 1 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 reagent 1). Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min at 4℃ , then take the supernatant for measurement.

3) Extraction of Cys in culture cells: collect the cells into the centrifuge tube, centrifuge and discard the supernatant. Add reagent 1 into the sediment according to the ratio of cells number (10^6): reagent 1 (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℃ . Take the supernatant and preserve it on ice for detection.

3. 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 reagent 2 into each well.

3) Add 100 μL of reagent 3 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.

▲Summary operation table

	Standard well	Sample well
Standard solution of different concentrations (μL)	20	
Sample (μL)		20
Reagent 2 (μL)	100	100
Reagent 3 (μL)	100	100
Mix fully and stand for 10 min at room temperature. Measure the OD value at 600 nm.		

▲Calculation

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 samples:

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

2.Tissue samples:

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

3.Cell samples:

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

Note:

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.

f: Dilution factor of sample before tested.

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 reagent 1 added in the extraction step of tissue sample.

V_2: The volume of reagent 1 added in the extraction step of cell sample.

Appendix I Data

▲Example 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:

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

Appendix II References

1. Hell R, Wirtz M. Molecular Biology, Biochemistry and Cellular Physiology of Cysteine Metabolism in Arabidopsis thaliana[J]. Arabidopsis Book, 2011, 9: 1-19.
2. Özkan Y, Özkan E, Şimşek B. Plasma total homocysteine and cysteine levels as cardiovascular risk factors in coronary heart disease[J]. International Journal of Cardiology, 82(3): 269-277.
3. El-Khairy L, Vollset S E, Refsum H, et al. Plasma total cysteine, pregnancy complications, and adverse pregnancy outcomes: the Hordaland Homocysteine Study 1, 2[J]. 2003, 77(2): 467-472.