TECHNICAL MANUAL

Cysteine (Cys) Colorimetric Assay Kit

- **SKU CODE:** MAES0181
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Measure the OD value

2. Intended use

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

3. Detection principle

Phosphotungstic acid can be reduced by cysteine (Cys) to form tungsten blue, which has an absorption peak at 600 nm. The Cys content can be calculated using the absorbance at 600 nm.

4. Kit components & storage

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

5. Materials prepared by users

Instruments: Microplate reader (600-620 nm), micropipettor, centrifuge, water bath, incubator, vortex mixer

Reagents: Double distilled water

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of 10 mmol/L standard solution:** Dissolve one vial of standard powder with 10 mL distilled water and mix well. Store at 2-8 °C for up to 4 days, protected from light.
3. **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 serial concentrations. The recommended dilution gradient is: 0, 0.125, 0.25, 0.5, 0.75, 1, 1.5, 2 mmol/L.

7. Standard dilution table

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

8. 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 thoroughly.

2. Centrifuge at 10,000 xg for 10 min at 4 °C, then take the supernatant and keep 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). Mechanically homogenize the sample in an ice water bath.

2. Centrifuge at 10,000 xg for 10 min at 4 °C, then take the supernatant for measurement.

Extraction of Cys in cultured cells:

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

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

3. Centrifuge at 10,000 xg for 10 min at 4 °C. Take the supernatant and preserve on ice for detection.

9. Sample dilution

The recommended dilution factors for different samples are as follows (for reference only):

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 dilution of other sample types, please perform a pre-test to confirm the appropriate dilution factor.

10. Key points of the assay

It is recommended to use fresh samples for detection.

11. Operating steps

1. Standard wells: Add 20 µL of standard solution at different concentrations to the wells. Sample wells: Add 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 thoroughly using the microplate reader for 5 s and incubate for 10 min at room temperature.
5. Measure the OD value at 600 nm using a microplate reader.

12. Calculation

Standard curve:

1. Average the duplicate readings for each standard.

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

3. Plot the standard curve using the absolute OD value of standards as the y-axis and the corresponding concentration as the x-axis. Create the standard curve (y = ax + b) using graph software (or Excel).

Sample calculations:

1. Serum (plasma) sample:

Cys content (mmol/L) = (ΔA_600 - b) ÷ a × 10* × f

2. Tissue sample:

Cys content (mmol/kg fresh weight) = (ΔA_600 - b) ÷ a × f ÷ m × V_1

3. Cell sample:

Cys content (mmol/10^9) = (ΔA_600 - b) ÷ a × f ÷ 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 cells (when the number of cells is 5×10^6, "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 testing

13. Appendix I Performance Characteristics

Parameters:

Intra-assay Precision

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

Intra-assay Precision Results

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.

Inter-assay Precision Results

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

Recovery

Three samples of high, middle, and low concentrations were tested with 6 replicates each to obtain an average recovery rate of 94%.

Recovery Results

Parameters	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

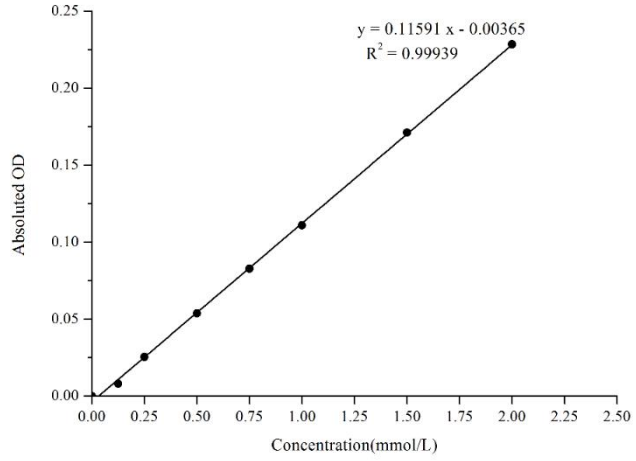
Parameters	Standard 1	Standard 2	Standard 3
Recovery rate (%)	95	92	95

Sensitivity

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

Standard curve

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



Standard curve data

Concentration (mmol/L)	OD value 1	OD value 2	Average OD	Absolute OD
0.00	0.038	0.038	0.038	0.000
0.125	0.046	0.046	0.046	0.008
0.25	0.066	0.061	0.064	0.026
0.50	0.092	0.091	0.092	0.054
0.75	0.122	0.120	0.121	0.083

Concentration (mmol/L)	OD value 1	OD value 2	Average OD	Absolute OD
1.00	0.149	0.149	0.149	0.111
1.50	0.206	0.212	0.209	0.171
2.00	0.266	0.268	0.267	0.229

14. Appendix II Example Analysis

Example analysis:

Take 0.05 mL of mouse serum sample and perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 0.1074x - 0.0046

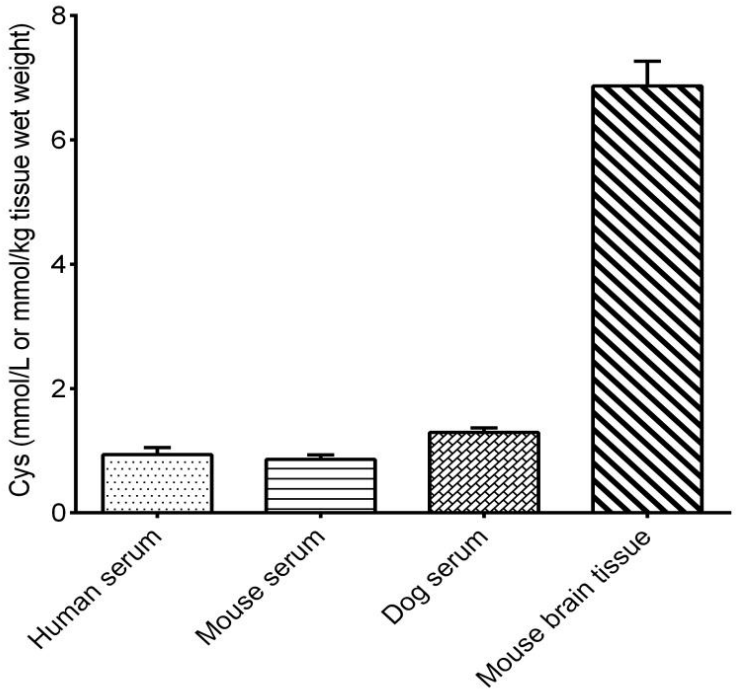
Average OD value of the sample: 0.046

Average OD value of the blank: 0.041

Calculation result:

Cys content (mmol/L) = (0.046 - 0.041 + 0.0046) ÷ 0.1074 × 10 = 0.89 mmol/L

Detection of human serum, mouse serum, dog serum, and 10% mouse brain tissue homogenate (wet weight content is 0.11 g/mL) according to the protocol yields the following results:



15. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.