TECHNICAL MANUAL

Total Sulfhydryl Group/Total Thiol (-SH) Colorimetric Assay Kit

- **SKU CODE:** MAES0167
- **SIZE:** 96 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
- Add buffer solution and chromogenic agent
- Measure absorbance at 412 nm

2. Intended use

This kit can be used to measure total sulfhydryl group (-SH) content in serum, plasma, and animal tissue samples.

3. Detection principle

Sulfhydryl compounds react with 5,5'-dithiobis (2-nitrobenzoic acid) under neutral or alkaline conditions to produce a yellow product which has a maximum absorption peak at 412 nm. The absorbance is measured and used to calculate the total sulfhydryl content indirectly.

4. Kit components & storage

Item	Component	Size (96 T)	Size2 (500 Assays)	Storage
Reagent 1	Buffer Solution	20 mL x 1 vial	20 mL x 5 vials	2-8 °C, 12 months
Reagent 2	Chromogenic Agent	1.3 mL x 1 vial	1.3 mL x 5 vials	2-8 °C, 12 months, shading light
Reagent 3	Standard Powder	Powder x 2 vials	Powder x 10 vials	2-8 °C, 12 months
	Microplate	96 wells	/	No requirement
	Plate Sealer	2 pieces		

5. Materials prepared by users

Instruments:

Microplate reader (410-420 nm), Micropipette, Water bath, Incubator, Vortex mixer, Centrifuge

Reagents:

Double distilled water, Absolute ethyl alcohol

6. Reagent preparation

1. Equilibrate other reagents to room temperature before use.
2. **Preparation of 5 mmol/L standard solution:** Dilute one vial of standard powder with 10 mL of normal saline, mix well. Store at 2-8 °C for 1 day.
3. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 5 mmol/L standard solution with normal saline to a serial concentration. The recommended dilution gradient is as follows: 0, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1 mmol/L.

7. Sample preparation

1. **Sample preparation:** Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80 °C for a month. Tissue sample: 1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg). 2. Wash tissue in cold normal saline (0.9% NaCl). 3. Homogenize 20 mg tissue in 180 µL normal saline (0.9% NaCl) with a dounce homogenizer at 4 °C. 4. Centrifuge at 10,000 xg for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.
2. **Dilution of sample:** The recommended dilution factor for different samples is as follows (for reference only): Human serum: 4-6; Human plasma: 4-6; Human urine: 1; Rabbit serum: 3-5; Porcine serum: 4-6; 10% Rat liver tissue homogenate: 4-6; 10% Rat kidney tissue homogenate: 4-6; 10% Rat spleen tissue homogenate: 4-6. The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. The kit contains reagents with pungent odor. Please carry out the assay in a fume hood.
2. In the pretreatment step of sample supernatant, the supernatant after centrifugation should be clear.

3. When the protein concentration of the sample is too high, the reaction system will become turbid after addition of the chromogenic agent. The sample should be diluted and tested again.

9. Operating steps

1. Standard well: add 40 μL of standard solution with different concentrations to the corresponding wells. Sample well: add 40 μL of sample to the corresponding wells. Control well: add 40 μL of sample to the corresponding wells.
2. Add 150 μL of buffer solution to each well.
3. Add 10 μL of chromogenic agent to standard wells and sample wells.
4. Add 10 μL of absolute ethyl alcohol (self-prepared) to control wells.
5. Mix fully and stand for 10 minutes at room temperature. Then measure the absorbance value of each well at 412 nm with microplate reader.

10. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve using absolute absorbance 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:

Total (-SH) content (μmol/L) = (ΔA_412 - b) ÷ a × 1000* × f

2. Tissue sample:

Total (-SH) content (μmol/g fresh weight) = (ΔA_412 - b) ÷ a ÷ m × V × f

[Note]

f: Dilution factor of sample before tested.

ΔA_412: $Absorbance_{Sample}$ - $Absorbance_{Control}$.

1000*: 1 mmol/L = 1000 μmol/L.

m: The fresh weight of sample, g.

V: The volume of normal saline in preparation step of tissue sample, mL.

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (µmol/L)	25.60	254.00	603.50
%CV	2.8	2.4	2.3

Inter-assay Precision

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

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (µmol/L)	25.60	254.00	603.50
%CV	3.0	2.6	3.1

Recovery

Three samples of high, medium, and low concentrations were tested in parallel 6 times each to obtain an average recovery rate of 104%.

Recovery

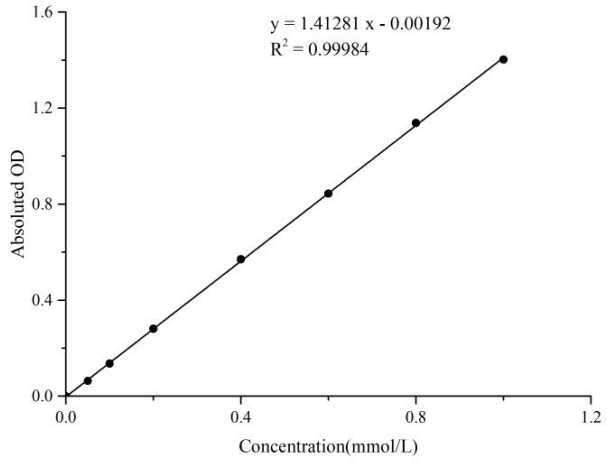
Parameters	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.08	0.35	0.74
Observed Conc. (mmol/L)	0.1	0.4	0.8
Recovery rate (%)	101	105	106

Sensitivity

The analytical sensitivity of the assay is 9.91 µmol/L. This was determined by adding two standard deviations to the mean absorbance obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

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



Standard curve data

Concentration (mmol/L)	**0**	**0.05**	**0.1**	**0.2**	**0.4**	**0.6**	**0.8**	**1.0**
OD value	0.078	0.141	0.216	0.358	0.647	0.922	1.219	1.486
	0.077	0.142	0.213	0.358	0.649	0.921	1.214	1.474
Average OD	0.078	0.142	0.214	0.358	0.648	0.922	1.216	1.480
Absolute OD	0.000	0.064	0.136	0.281	0.570	0.844	1.138	1.402

11. Appendix II Example Analysis

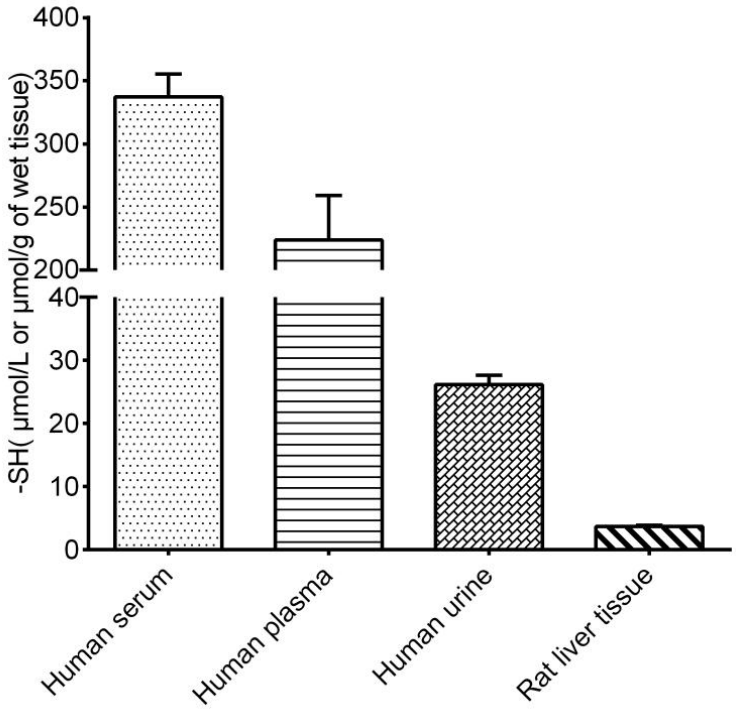
Example analysis:

Dilute the human serum sample with normal saline 5-fold and carry out the assay according to the operating steps. The results are as follows:

Standard curve: y = 1.5351x - 0.0023, the average absorbance value of the sample is 0.216, the average absorbance value of the control is 0.115, and the calculation result is:

Total (-SH) content (μmol/L) = (0.216 - 0.115 + 0.0023) ÷ 1.5351 × 1000 × 5 = 336.46 μmol/L

Detect human serum (diluted 5-fold), human plasma (diluted 5-fold), human urine (diluted 5-fold) and 10% rat liver tissue homogenate (diluted 5-fold) according to the protocol. The results are as follows:



12. 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 pretest 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.