TECHNICAL MANUAL

Hydrogen sulfide (H2S) Colorimetric Assay Kit (Direct)

- **SKU CODE:** MAES0485
- **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 values

2. Intended use

This kit can be used to measure H2S content in serum (plasma) and animal tissue samples.

3. Detection principle

H2S can form methylene blue in the presence of Fe3+ and chromogenic agent. Methylene blue has a maximum absorption peak at 680 nm. H2S content can be calculated indirectly by measuring the OD value at 680 nm.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	9 mL x 1 vial	18 mL x 1 vial	2-8 °C, 12 months
Reagent 2	Chromogenic Agent	3 mL x 1 vial	6 mL x 1 vial	2-8 °C, 12 months, shading light
Reagent 3	Ferric Salt Reagent	3 mL x 1 vial	6 mL x 1 vial	2-8 °C, 12 months, shading light
Reagent 4	Standard	7.8 mg x 1 vial	7.8 mg x 1 vial	2-8 °C, 12 months, shading light
Reagent 5	Standard Diluent	60 mL x 2 vials	60 mL x 2 vials	2-8 °C, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments: Micropipettor, Vortex mixer, Centrifuge, Microplate reader (670-690 nm, optimum wavelength: 680 nm)

Reagents: Double distilled water, Normal Saline (0.9% NaCl)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of 1 mmol/L standard solution:** Dissolve one vial of standard with 100 mL of standard diluent, mix well to dissolve. Store at 2-8°C for 1 day protected from light.
3. **The preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 1 mmol/L standard solution with standard diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 30, 40, 60, 80, 100 μmol/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 PBS (0.01 M, pH 7.4). 3. Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C. 4. Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection. 5. Meanwhile, determine the protein concentration of supernatant (MAES0177).
2. **Dilution of sample:** The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For the dilution of other sample types, please perform pretest to confirm the dilution factor.

8. The key points of the assay

1. Prepare the standard solution and carry out standard curve experiment in the fume hood.
2. Sample hemolysis should be avoided as it may affect the measurement results.
3. If the sample well is turbid during testing, dilute the sample before testing.

4. For mouse serum sample, it is recommended to increase the volume of sample and different concentrations of standards to 100 µL, and reduce the volume of buffer solution to 100 µL.

9. Operating steps

1. Standard well: add 150 µL of buffer solution into the standard wells. Sample well: add 150 µL of buffer solution into the sample wells.
2. Standard well: add 50 µL of standards with different concentrations into the standard wells. Sample well: add 50 µL of sample into the sample wells.
3. Add 50 µL of chromogenic agent to each well.
4. Add 50 µL of ferric salt reagent to each well.
5. Mix fully for 10 s with microplate reader and stand at room temperature protected from light for 20 min. Measure the OD values of each well at 680 nm with microplate reader.

10. 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 absolute OD value.

3. Plot the standard curve by using absolute 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:

H2S content (µmol/L) = (ΔA_680 - b) ÷ a × f

2. Tissue sample:

H2S content (µmol/gprot) = (ΔA_680 - b) ÷ a × f ÷ C_{pr}

[Note]

ΔA_680: OD_{Sample} – OD_{Blank}

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, gprot/L

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (µmol/L)	25.00	50.00	75.00
%CV	5.0	2.0	3.0

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (µmol/L)	25.00	50.00	75.00
%CV	8.1	6.2	7.9

Recovery

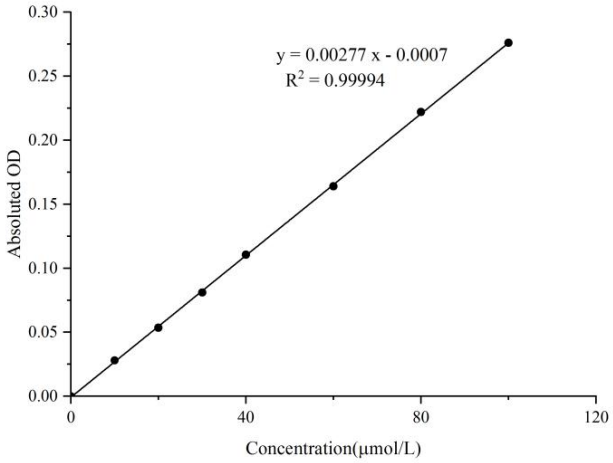
	Standard 1	Standard 2	Standard 3
Expected Conc.(µmol/L)	16.5	33.2	75.6
Observed Conc. (µmol/L)	16.7	31.5	74.1
Recovery rate (%)	101	95	98

Sensitivity

The analytical sensitivity of the assay is 1.97 µmol/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.

Standard curve data

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:



11. Appendix II Example Analysis

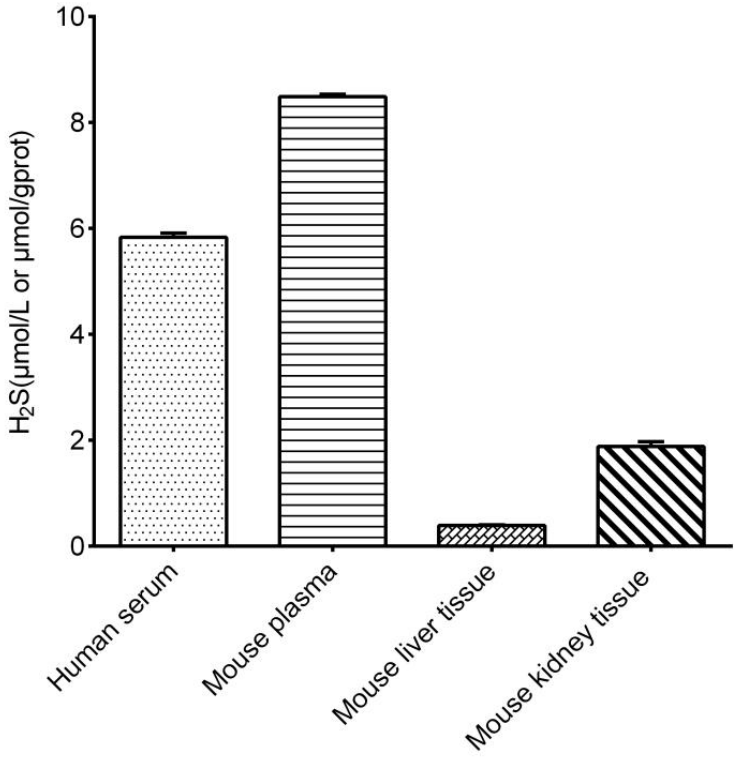
Example analysis:

For human serum, take 50 μL of sample and carry the assay according to the operation table. The results are as follows:

standard curve: y = 0.00277x – 0.0007, the average OD value of the sample is 0.097, the average OD value of the blank is 0.082, and the calculation result is:

H2S content (μmol/L) = (0.097 - 0.082 + 0.0007) ÷ 0.00277 = 5.67 μmol/L

Detect human serum, mouse plasma, 10% mouse liver tissue homogenate (the concentration of protein is 13.44 gprot/L) and 10% mouse kidney tissue homogenate (the concentration of protein is 7.97 gprot/L) according to the protocol, the result is 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 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.