TECHNICAL MANUAL

H2S Colorimetric Assay Kit

- **SKU CODE:** MAES0185
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Measurement of samples
- Calculation

2. Intended use

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

3. Detection principle

In the presence of Fe3+, H2S reacts with the chromogenic agent to form stable methylene blue, which has a maximum absorption peak at 665 nm. The H2S content can be calculated by measuring the OD value at 665 nm.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	13 mL × 1 vial	25 mL × 1 vial	2-8°C, 12 months
Reagent 2	Alkali Reagent	6 mL × 1 vial	12 mL × 1 vial	2-8°C, 12 months
Reagent 3	Chromogenic Agent	6 mL × 1 vial	12 mL × 1 vial	2-8°C, 12 months shading light
Reagent 4	Protein Precipitator	6 mL × 1 vial	12 mL × 1 vial	2-8°C, 12 months
Reagent 5	Ferric Salt Reagent	2.5 mL × 1 vial	2.5 mL × 1 vial	2-8°C, 12 months shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		

5. Materials prepared by users

Instruments:

Microplate reader (660-670 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.

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 one 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 minutes 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 recommended dilution factor for different samples is as follows (for reference only): Human plasma: 1 Mouse plasma: 1 10% Rat spleen tissue homogenate: 1 10% Rat kidney tissue homogenate: 1 10% Rat brain tissue homogenate: 1 Note: 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

Do not take the sediment when adding the supernatant to microplate, otherwise the result will be affected.

9. Operating steps

1. **The measurement of samples:** Control tube: Take 100 µL of buffer solution to the control tubes. Sample tube: Take 100 µL of buffer solution to the sample tubes.
2. Control tube: Add 100 µL of double distilled water to the control tubes. Sample tube: Add 100 µL of sample to the sample tubes.
3. Add 100 µL of alkali reagent to each tube and mix fully with vortex mixer for 3 s.
4. Centrifuge at 12000×g for 10 min at 4°C, discard the supernatant and keep the sediment.

5. Add 150 μL of double distilled water and vortex with vortex mixer for 3 s.

6. Centrifuge at 12000×g for 10 min at 4°C, discard the supernatant and keep the sediment.

7. Add 100 μL of buffer solution to each tube.

8. Add 100 μL of chromogenic agent to each tube and mix fully with vortex mixer for 10 s.

9. Add 20 μL of Ferric Salt Reagent to each tube. Stand for 30 s, then mix fully with vortex mixer for 5 s.

10. Add 100 μL of protein precipitator to each tube and mix fully with vortex mixer for 3 s.

11. Centrifuge at 12000×g for 10 min at 4°C, then take 250 μL of supernatant to corresponding wells of microplate respectively.

12. Stand for 10 min at room temperature and measure the OD value of each well with microplate reader at 665 nm.

10. Calculation

The sample:

1. Serum (plasma) sample:

H2S (μmol/L) = ΔA_665 / (ε × b) × f

2. Tissue sample:

H2S (μmol/gprot) = ΔA_665 / (ε × b) × f ÷ C_{pr}

[Note]

f: Dilution factor of sample before tested.

ε: The molar extinction coefficient of Methylene blue at 665 nm, 9.4×10^{-3} L/μmol/cm.

b: Optical path, 0.7 cm.

ΔA_665: OD_{Sample} - $OD_{Control}$.

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

11. Appendix I Performance Characteristics

Parameter:

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)	12.50	35.60	84.20
%CV	3.5	3.2	2.6

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)	12.50	35.60	84.20
%CV	9.5	10.4	9.8

Recovery

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

Recovery

Standard 1	Standard 2	Standard 3
18.6	37.3	75
18.4	35.1	66.8
99	94	89

Sensitivity

The analytical sensitivity of the assay is 0.15 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.

12. Appendix II Example Analysis

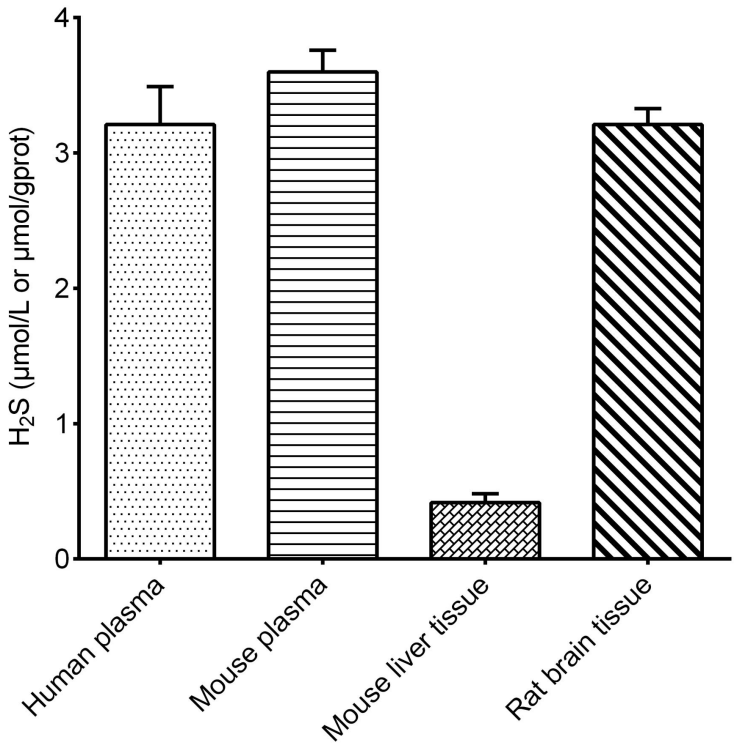
Example analysis:

Take 100 μL of 10% rat brain tissue homogenate and carry the assay according to the operation steps. The results are as follows:

The average OD value of the sample is 0.291, the average OD value of the control is 0.271, the concentration of protein in sample is 7.84 gprot/L, and the calculation result is:

H2S (μmol/gprot) = (0.291 - 0.271) ÷ 9.4 ÷ 0.7 × 1000 ÷ 7.84 gprot/L = 0.39 μmol/gprot

Detect human plasma, mouse plasma, 10% mouse liver tissue homogenate (the concentration of protein in sample is 12.57 gprot/L) and 10% rat brain tissue homogenate (the concentration of protein in sample is 2.82 gprot/L) according to the protocol, the result is as follows:



13. 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.