（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K355-M

Specification：48T(46 samples)/96T(94 samples)

Measuring instrument：Microplate reader（660-670 nm）

Detection range：0.76-100 μmol/L

Elabscience® Hydrogen Sulfide (H_2S) Colorimetric Assay Kit(Indirect Method)

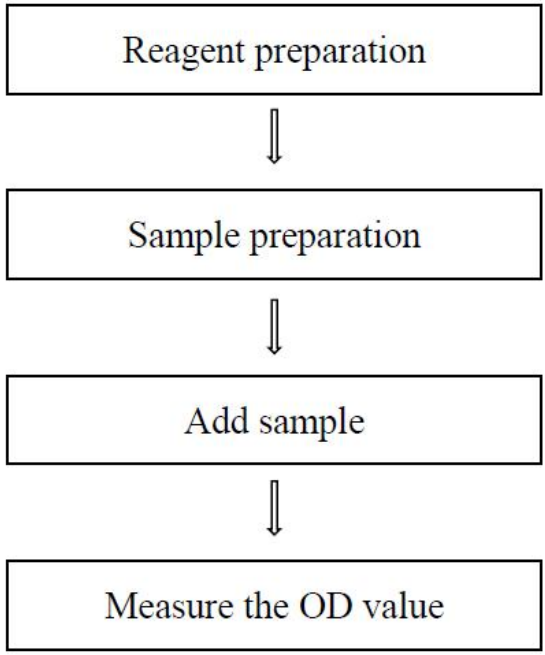
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary



Intended use

This kit can be used to measure H_2S content in serum, plasma, animal tissue samples.

Detection principle

In the presence of Fe^{3+}, H_2S reacts with the chromogenic agent to form stable methylene blue, which has a maximum absorption peak at 665 nm, and the H_2S content can be calculated by measuring the OD value at 665 nm.

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		

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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

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 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
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 do pretest to confirm the dilution factor

The key points of the assay

① Do not take the sediment when adding the supernatant to microplate, otherwise the result will be affect.

Operating steps

The measurement of samples

① 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.

Calculation

The sample:

1. Serum (plasma) sample:

$$H_2S\ (\mu mol/L) = \frac{\Delta A_{665}}{\varepsilon \times b} \times f$$

2. Tissue sample:

$$H_2S\ (\mu mol/gprot) = \frac{\Delta A_{665}}{\varepsilon \times b} \times f \div C_{pr}$$

[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.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

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

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.

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 parallelly to get the average recovery rate of 94%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/L)	18.6	37.3	75
Observed Conc. (μmol/L)	18.4	35.1	66.8
Recovery rate (%)	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.

Appendix II Example Analysis

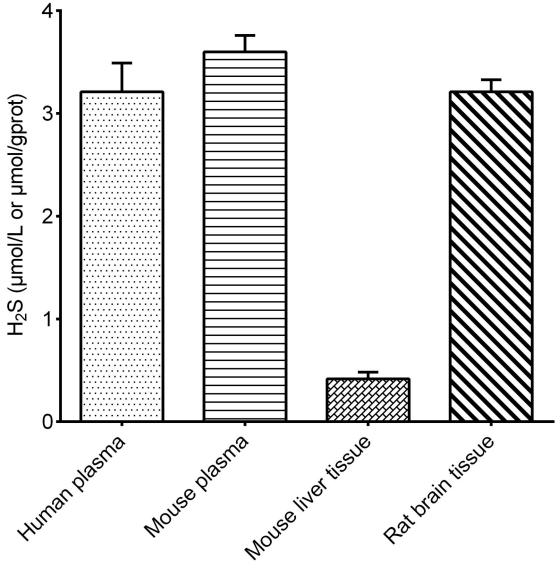
Example analysis:

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:

$$\underset{(\mu mol/gprot)}{H_2S} = (0.291 - 0.271) \div 9.4 \div 0.7 \times 1000 \div 7.84\ gprot/L = 0.39\ \mu mol/gprot$$

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:



Appendix III Publications

1. Zong Q , Li J , Xu Q ,et al.Self-immolative poly(thiocarbamate) with localized H2S signal amplification for precise cancer imaging and therapy[J].Nature Communications, 15(1):1-14[2025-04-27].DOI:10.1038/s41467-024-52006-0.
2. Liang J , Ling J , Sun D ,et al.Dextran‐Based Antibacterial Hydrogel Dressings for Accelerating Infected Wound Healing by Reducing Inflammation Levels[J].Advanced Healthcare Materials, 2024, 13(22).DOI:10.1002/adhm.202400494.
3. Stachowicz A , Czepiel K , Winiewska A ,et al.Mitochondria-targeted hydrogen sulfide donor reduces fatty liver and obesity in mice fed a high fat diet by inhibiting de novo lipogenesis and inflammation via mTOR/SREBP-1 and NF-κB signaling pathways[J].Pharmacological Research, 2024, 209(000):16.DOI:10.1016/j.phrs.2024.107428.
4. Yu S , Wang X , Li Z ,et al.Solobacterium moorei promotes the progression of adenomatous polyps by causing inflammation and disrupting the intestinal barrier[J].Journal of Translational Medicine, 2024, 22(1):17.DOI:10.1186/s12967-024-04977-3.
5. Zhang Y , Fang J , Ye S ,et al.A hydrogen sulphide-responsive and depleting nanoplatform for cancer photodynamic therapy[J].Nature Communications, 2022, 13.DOI:10.1038/s41467-022-29284-7.
6. Griffiths K K , Wang A , Levy J R J .Sulfide quinone oxidoreductase contributes to voltage sensing of the mitochondrial permeability transition pore[J].The FASEB Journal, 2024, 38(4):e23494-e23494.DOI:10.1096/fj.202301280R.

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.