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

Catalog No：E-BC-K894-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（670-690 nm）

Detection range：1.97-100 μmol/L

Elabscience® Hydrogen Sulfide (H_2S) Colorimetric Assay Kit (Direct 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

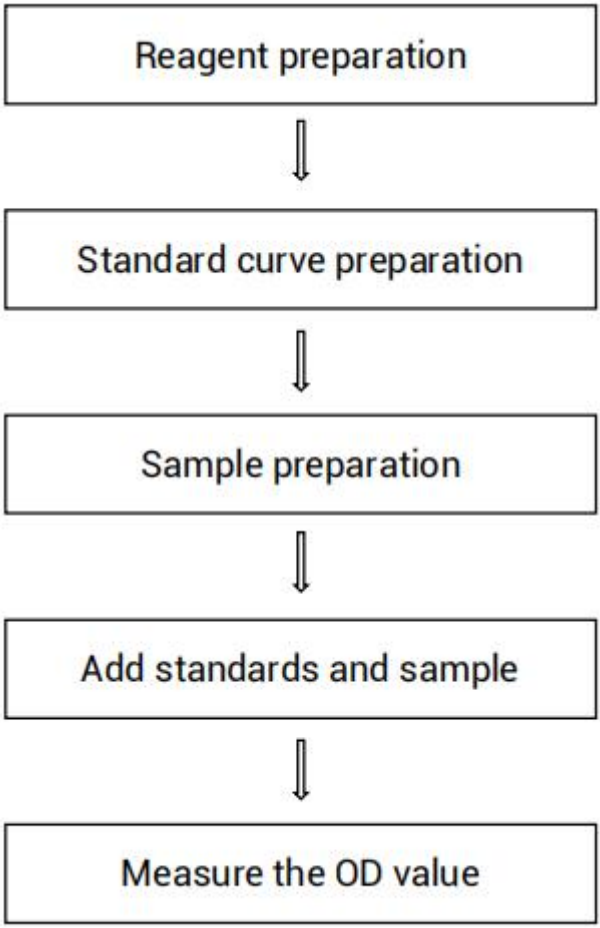
Email: techsupport@elabscience.com

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) and animal tissue samples.

Detection principle

H_2S can form methylene blue in the presence of Fe^{3+} and chromogenic agent. Methylene blue has a maximum absorption peak at 680 nm. H_2S content can be calculated indirectly by measuring the OD value at 680 nm.

Kit components & storage

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

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:

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

Reagents:

Double distilled water, Normal Saline (0.9% NaCl)

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. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**10**	**20**	**30**	**40**	**60**	**80**	**100**
1 mmol/L standard (μL)	0	10	20	30	40	60	80	100
Standard diluent (μL)	1000	990	980	970	960	940	920	900

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℃ 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 (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 kidney tissue homogenate	1
10% Rat brain tissue homogenate	1
10% Rat spleen tissue homogenate	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor

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.

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.

③ Add 50 μL of ferric salt reagent to each well.

④ 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.

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 absoluted OD value.

3. Plot the standard curve by using absoluted 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:

$$H_2S \text{ content } (\mu mol/L) = (\Delta A_{680} - b) \div a \times f$$

2. Tissue sample:

$$H_2S \text{ content } (\mu mol/gprot) = (\Delta A_{680} - b) \div a \times f \div C_{pr}$$

[Note]

ΔA_{680}: $OD_{Sample} - OD_{Blank}$

f: Dilution factor of sample before test.

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

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)	25.00	50.00	75.00
%CV	5.0	2.0	3.0

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

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

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 98%.

	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

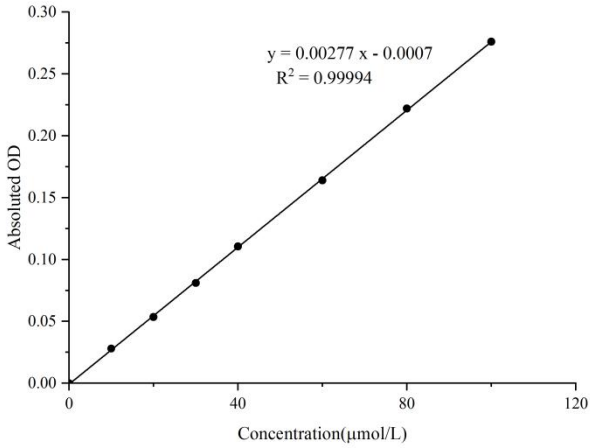
The analytical sensitivity of the assay is 1.97 μmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the

corresponding concentration.

2. Standard curve:

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：

Concentration (μmol/L)	0	10	20	30	40	60	80	100
OD value	0.075	0.101	0.128	0.155	0.185	0.238	0.296	0.351
	0.073	0.103	0.127	0.155	0.184	0.238	0.296	0.349
Average OD	0.074	0.102	0.128	0.155	0.184	0.238	0.296	0.350
Absoluted OD	0.000	0.028	0.054	0.081	0.110	0.164	0.222	0.276



Appendix Π Example Analysis

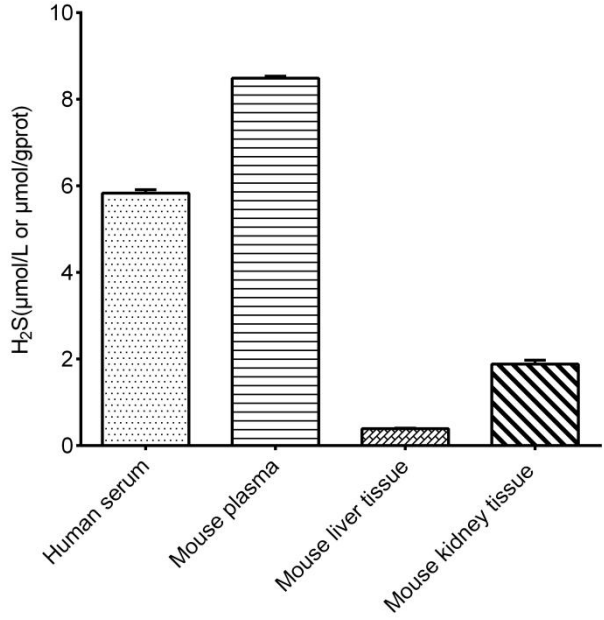
Example analysis：

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 = 0.00277 x – 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:

$$H_2S \text{ content } (\mu mol/L) = (0.097 - 0.082 + 0.0007) \div 0.00277 = 5.67\ \mu mol/L$$

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：



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.