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

Catalog No：E-BC-K355-M

Specification：48T(31 samples)/96T(79 samples)

Measuring instrument：Microplate reader（660-670 nm）

Detection range：4.86-100 μmol/L

Elabscience® H_2S Colorimetric Assay Kit

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.

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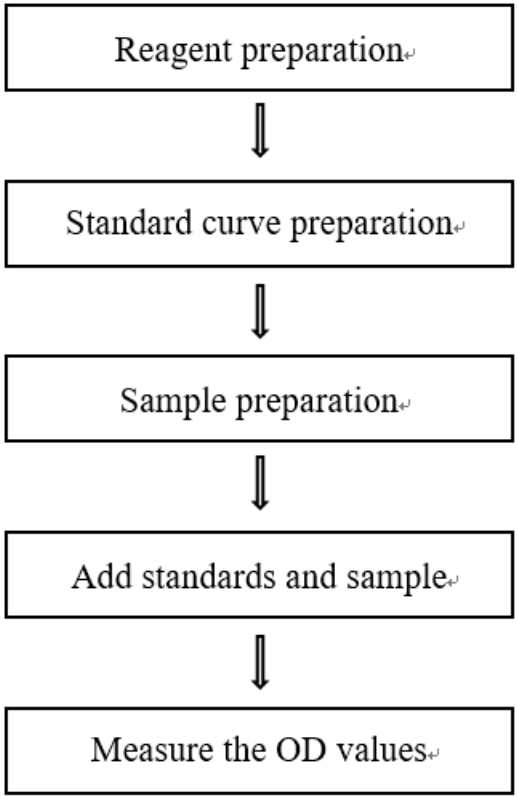
Assay summary



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 665nm, 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℃, 12 months
Reagent 2	Alkali Reagent	7 mL ×1 vial	13 mL ×1 vial	2-8℃, 12 months
Reagent 3	Chromogenic Agent	7 mL ×1 vial	13 mL ×1 vial	2-8℃, 12 months shading light
Reagent 4	Protein Precipitator	7 mL ×1 vial	13 mL ×1 vial	2-8℃, 12 months
Reagent 5	Ferric Salt Reagent	2 mL ×1 vial	2 mL ×1 vial	2-8℃, 12 months shading light
Reagent 6	Standard	7.8 mg ×1 vial	7.8 mg ×1 vial	2-8℃, 12 months shading light
Reagent 7	Standard Diluent	60 mL ×2 vials	60 mL ×2 vials	2-8℃, 12 months
	Microplate	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 The preparation of 1 mmol/L standard solution：

Dissolve a vial of standard with 100 mL standard diluent and mix fully. Store at 2~8 ℃ for 1 day protected from light.

② 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℃.

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 It should be performed in a fume hood when preparing standard solutions and standard curve.

② Do not take the sediment when adding the supernatant to microplate, otherwise the result will be affect.

Operating steps

The measurement of standard curve

① Add 100 μL of buffer solution to 1.5 mL EP tube marked from A to H with duplication.

② Add 100 μL of standard solution with different concentration to each tubes.

③ Add 100 μL of chromogenic agent to each tube.

④ Mix fully with vortex mixer for 5 s, then take 225 μL of mixed solution to corresponding wells of microplate respectively.

⑤ Add 15 μL of ferric salt reagent to each well (the multi-channel pipette is recommended).

⑥ Mix fully with microplate reader for 10 s, stand for 20 min at room temperature and measure the OD value of each well with microplate reader at 665 nm.

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

⑩ Centrifuge at 12000 g for 10 min at 4°C , then take 225 μL of supernatant to corresponding wells of microplate respectively.

⑪ Add 15 μL of ferric salt reagent to each well (the multi-channel pipette is recommended).

⑫ Mix fully with microplate reader for 10 s, stand for 20 min at room temperature and measure the OD value of each well with microplate reader at 665 nm.

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:

$$\underset{(\mu mol/L)}{H_2S} = (\Delta A_{665} - b) \div a \times f$$

2. Tissue sample:

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

[Note]

f: Dilution factor of sample before tested.

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

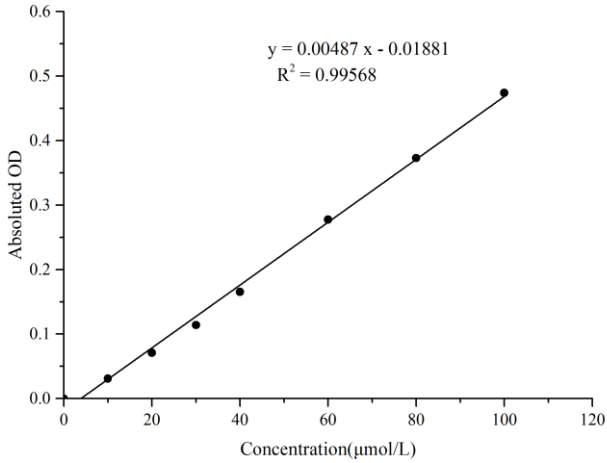
Sensitivity

The analytical sensitivity of the assay is 2.75 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	0.083	0.113	0.152	0.195	0.245	0.363	0.460	0.548
	0.084	0.116	0.157	0.200	0.253	0.359	0.453	0.567
Average OD	0.084	0.115	0.155	0.198	0.249	0.361	0.456	0.558
Absoluted OD	0.000	0.031	0.071	0.114	0.165	0.277	0.372	0.474



Appendix II Example Analysis

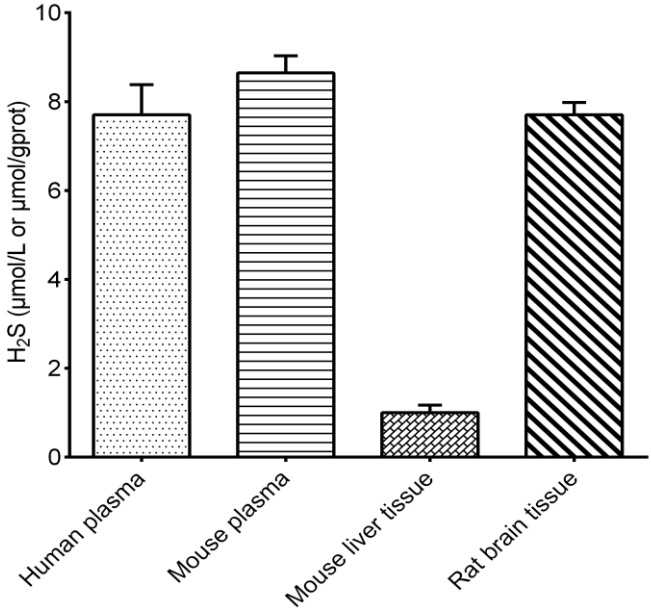
Example analysis:

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

standard curve: y = 0.0049 x – 0.0188, the average OD value of the sample is 0.096, the average OD value of the control is 0.078, the concentration of protein in sample is 3.81 gprot/L, and the calculation result is:

$$\underset{(\mu mol/gprot)}{H_2S} = (0.096 - 0.078 + 0.0188) \div 0.0049 \div 3.81\ gprot/L = 1.97\ \mu mol/gprot$$

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:



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.