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

Endogenous Hydrogen Sulfide (H_2S) Assay Kit

Catalog No.: BC00210

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Endogenous Hydrogen Sulfide (H_2S) Assay Kit
Detection Method	Colorimetric
Sample Type	Tissue, bacteria, cell, serum, plasma
Assay Type	Quantitative
Detection Instrument	Spectrophotometer (665 nm)

Product Introduction

Hydrogen sulfide (H_2S) is a novel gaseous signaling molecule and an endogenous neurotransmitter in the brain. Physiological concentrations of H_2S exert crucial regulatory effects on long-term potentiation in the hippocampus of the nervous system, and also play significant pathophysiological roles in the progression of spontaneous hypertension, hemorrhagic shock, liver cirrhosis and other diseases.

Principle

Hydrogen sulfide reacts with zinc acetate, N, N-dimethyl-p-phenylenediamine and ammonium ferric sulfate to form methylene blue. Methylene blue has a maximum absorption peak at 665 nm, and the content of hydrogen sulfide can be calculated by measuring its absorbance.

Components

No.	Components	Size	Note
Extraction solution	Liquid	50 mL	Store at 4°C.
Reagent 1	Liquid	50 mL	Store at 4°C.
Reagent 2	Liquid	32 mL	Store at 4°C.
Reagent 3	Liquid	16 mL	Store at 4°C away from light.

Reagent 4	Liquid	16 mL	Store at 4°C.
Reagent 5	Liquid	2.5 mL	Store at 4°C away from light.

Storage

The kit can be stored for 3 months under specified conditions.

Preparation

Balance, low-temperature centrifuge, visible spectrophotometer, 1 mL glass cuvette, distilled water.

- **Sample requirements**

1. Tissue: Homogenize tissue in ice bath at mass (g) to Extraction solution (mL) ratio of 1:5-10. Weigh 0.1 g tissue and add 1 mL extract as recommended. Centrifuge at 12000 rpm, 4°C for 10 min, take supernatant and keep on ice for test.
2. Bacteria and cell: Mix cells with extract at a ratio of 500-1000×10^4 cells per milliliter. Recommended ratio: 5 million cells in 1 mL extract. Lyse cells via ice-bath ultrasonication (power 300 W, 3 s sonication, 7 s interval, total 3 min). Centrifuge at 12000 rpm, 4°C for 10 min, collect supernatant and preserve on ice for detection.
3. Serum/plasma: Direct detection. Optimal samples are non-hemolytic and non-chylous serum collected from fasting subjects on the same day.

Operation process

	Blank tube	**Assay tube**
Sample (mL)		0.6
Distilled water (mL)	0.6	
Reagent 1 (mL)	0.6	0.6
Vortex thoroughly to mix evenly.		

Reagent 2 (mL)	0.6	0.6
Centrifuge at 10000g and 4°C for 10 min, discard supernatant and retain precipitate.		
Distilled water (mL)	0.6	0.6
Centrifuge at 10000g and 4°C for 10 min, discard supernatant and retain precipitate.		
Reagent 1 (mL)	0.3	0.3
Reagent 3 (mL)	0.3	0.3
Vortex thoroughly to mix evenly.		
Reagent 4 (mL)	0.3	0.3
Centrifuge at 10000g, 4°C for 10 min, transfer 0.8 mL supernatant into test tube.		
Reagent 5 (mL)	0.04	0.04
Mix well and stand at room temperature for 5 min. Measure absorbance at 665 nm using 1 mL glass cuvette, zero calibration with blank tube. Record measured absorbance and blank absorbance. Calculate $\triangle A = A_{Assay} - A_{Blank}$.		

Calculation

1. Tissue

Calculate based on tissue protein concentration

$$H_2S \text{ content (nmol/mg)} = \frac{\triangle A}{0.0044} \times \frac{V_{Total}}{V_{Sample} \times Cpr}$$

Calculate according to tissue sample weight

$$H_2S \text{ content (nmol/g)} = \frac{\triangle A}{0.0044} \times \frac{V_{Total}}{V_{Sample} \times W \div V}$$

2. Serum/plasma

$$H_2S\ \text{content}\ (\text{nmol/mL}) = \frac{\triangle A}{0.0044} \times \frac{V_{Total}}{V_{Sample}}$$

3. Cell

$$H_2S\ \text{content}\ (\text{nmol}/10^4\text{cell}) = \frac{\triangle A}{0.0044} \times \frac{V_{Total}}{V_{Sample} \times \text{cell number} \div V}$$

Annotation:

V: Total volume of extraction solution, 1mL

V_{Sample}: Volume of sample added, 0.6mL

V_{Total}: Total volume of reaction solution, 0.9 mL

W: Sample weight, g

Cpr: Sample protein concentration, mg/mL

Precautions

1. It is recommended to use disposable laboratory test tubes for operation.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.
3. The minimum detection limit is 1 nmol/mL.