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

H_2S Assay Kit

Catalog No: E-BC-K355
Method: Colorimetric method
Specification: 50 Assays

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.

Phone: 240-252-7368(USA) Fax: 240-252-7376(USA)
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.

It is recommended to take 2~3 samples which expected large difference to do pre-experiment before formal experiment.

Application

This kit can be used to measure H_2S content in animal serum, plasma, tissue, cells and other samples. This kit (50 Assays) can detect 48 samples.

Detection significance

H_2S is a new gaseous signal molecule, and is also a neurotransmitter in the brain. The H_2S of physiological concentration plays an important role in regulating the long term potentiation of the hippocampus of the nervous system, and plays an important pathophysiological effect on the process of spontaneous hypertension, hemorrhagic shock, liver cirrhosis and other diseases.

Detection principle

H_2S can react with zinc acetate, N, N-Dimethyl-p-phenylenediamine and ammonium ferric sulfate, and then produce methylthionine chloride, which has the maximum absorption peak at 665 nm. The content of H_2S can be calculated by measuring the absorbance value at 665 nm.

Kit composition

Extraction solution: Liquid, 50 mL ×1 vial. Store at 4℃.
Reagent 1: Liquid, 50 mL ×1 vial. Store at 4℃.
Reagent 2: Liquid, 32 mL ×1 vial. Store at 4℃.
Reagent 3: Liquid, 16 mL ×1 vial. Store at 4℃ with shading light.
Reagent 4: Liquid, 16 mL ×1 vial. Store at 4℃.
Reagent 5: Liquid, 2.5 mL ×1 vial. Store at 4℃ with shading light.

Experimental instruments

Refrigerated centrifuge, Spectrophotometer (665 nm), 1 cm diameter cuvette

Sample treatment

1. **Tissue:** Add the extraction solution at the ratio of Weight (g): Volume (mL) =1:5~10 (It is recommended to weigh 0.1 g tissue and add 1 mL of extraction solution). Then homogenize the sample in ice water bath. Centrifuge at 10000 g for 10 min at 4℃. Take the supernatant and preserve on ice for detection.
2. **Bacterium, fungus:** Add the extraction solution at the ratio of the number of cells (10^4): Volume (mL) =500~1000:1. (It is recommended to add 1 mL of extraction solution for 5×10^6 cells.) Ultrasonic breaking the cell in ice water bath (power: 300W, 3s/time, interval for 7s, the total process will be 3 min). Centrifuge at 10000 g for 10 min at 4℃. Take the supernatant and preserve on ice for detection.
3. **Serum (plasma):** Detect the sample directly.

Operation steps

Table 1: extraction process

	Blank tube	Sample tube
Sample (μL)		600
H_2O (μL)	600	
Reagent 1 (μL)	600	600
Oscillate and mix fully.		
Reagent 2 (μL)	600	600
Centrifuge at 10000g for 10 min at 4℃. Discard the supernatant and keep the sediment.		
H_2O (μL)	600	600
Centrifuge at 10000g for 10 min at 4℃. Discard the supernatant and keep the sediment.		

Table 2: reaction process

	Blank tube	Sample tube
Reagent 1 (μL)	300	300
Reagent 3 (μL)	300	300
Oscillate and mix fully.		
Reagent 4 (μL)	300	300
Centrifuge at 10000g for 10 min at 4℃. Take 800 μL of the supernatant to the 1 mL glass cuvette.		
Reagent 5 (μL)	40	40
Mix fully and stand for 5 min at 25℃. Set zero with the blank tube and measure the absorbance at 665 nm. Calculate the $\Delta A = \Delta A_{sample} - \Delta A_{blank}$.		

Calculation of results

The regression equation of standard curve: y = 0.0044x, R^2 = 0.9988.

1. **For tissue sample:**

   Calculate according to the protein concentration:

   H_2S content (*μmol/mg prot*)

   $= \Delta A \div 0.0044 \times V_{Total} \div (V_{Sample} \times Cpr)$

   $= 340.9 \times \Delta A \div Cpr$

   Calculate according to the weight of sample:

   H_2S content (*μmol/g*)

   $= \Delta A \div 0.0044 \times V_{Total} \div (V_{Sample} \div V_{Sample\ total} \times W)$

   $= 340.9 \times \Delta A \div W$

2. **For liquid sample:**

   H_2S content (*μmol/L*) $= \Delta A \div 0.0044 \times V_{Total} \div V_{Sample} = 340.9 \times \Delta A$

3. **For cells sample:**

   H_2S content (*μmol/10^4* cells)

   $= \Delta A \div 0.0044 \times V_{Total} \div [V_{Sample} \div V_{Sample\ total} \times$ Number of cells $(1 \times 10^4)]$

   $= 340.9 \times \Delta A \div$ Number of cells (1×10^4)

V_{Total}: Volume of the total reaction system (reagent 1, reagent 2, reagent 4) in table 2, 0.9 *mL*.

V_{Sample}: Volume of sample in the reaction system, 0.6 *mL*.

$V_{Sample\ total}$: Volume of added extraction solution, 1 *mL*.

W: Weight of sample, *g*.

Cpr: Concentration of protein, *mg/mL*.

Notes

1. The kit is for scientific research only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The valid of kit is 3 months.
4. Do not use components from different batches of kit.
5. The lowest detectable limit is 1 nmol/mL.