Hydrogen Sulfide (H2S) Test Kit

(Cat/No.:BC154 Size:50T/48S)

1. Reagent use

H2S is a novel gaseous signaling molecule and neurotransmitter present in the brain. Physiological concentrations of H2S have an important modulatory effect on the long-duration potentiation of the hippocampus of the nervous system and exert important pathophysiological effects on the course of diseases such as spontaneous hypertension, hemorrhagic shock and cirrhosis of the liver.

2. Principle of measurement

H2S reacts with zinc acetate, N,N-dimethyl-p-phenylenediamine and ferric ammonium sulfate to produce methylene blue, which has a maximum absorption peak at 665 nm, and the H2S content can be calculated by measuring its absorbance value.

3. Composition

Extract solution: liquid 50mL×1 bottle, stored at 4℃;

Reagent 1: Liquid 50mL×1 bottle, stored at 4℃;

Reagent 2: Liquid 32mL×1 bottle, stored at 4℃;

Reagent 3: Liquid 16mL×1 bottle, stored at 4℃ away from light;

Reagent 4: Liquid 16mL×1 bottle, stored at 4℃;

Reagent 5: Liquid 2.5mL×1 bottle, stored at 4℃ away from light.

4. Need to self-provide reagents and instruments

balance, cryogenic centrifuge, visible spectrophotometer, 1 mL glass cuvette, distilled water.

5. Pre-treatment

1. Tissue: homogenize in ice bath according to the ratio of tissue mass (g): volume of extraction solution (mL) of 1:5-10 (it is recommended to weigh about 0.1g of tissue and add 1mL of extraction solution), then centrifuge at 12,000rpm for 10min at 4℃, take the supernatant and put it on ice to be measured;

2. Bacteria, fungi: according to the number of cells (10^4): the volume of extraction solution (mL) for the ratio of 500-1000:1 (5 million cells are recommended to add 1mL of extraction solution), ice bath ultrasonic cell crushing (power of 300w, ultrasonic 3s, interval of 7s, the total time of 3min), and then 12000rpm, 4 ℃, centrifugation for 10min, the supernatant is placed on the ice to be measured;

3. Serum/plasma: direct measurement

Sample requirements: The most appropriate sample is a non-hemolyzed, celiac-free serum collected on

6. Testing steps

	Blank	Testing
Sample (mL)		0.6
Distilled water (mL)	0.6	
Reagent I (mL)	0.6	0.6
Shake well to mix		
Reagent II (mL)	0.6	0.6
10,000g, 4℃, centrifuge for 10min, remove supernatant, leave precipitate		
Distilled water	0.6	0.6
10,000g, 4℃, centrifuge for 10min, remove supernatant, leave precipitate		
Reagent I (mL)	0.3	0.3
Reagent III (mL)	0.3	0.3
Shake well to mix		
Reagent IV (mL)	0.3	0.3
10000g, 4℃, centrifuged for 10min, 0.8mL of supernatant was added into the test tube		
Reagent V (mL)	0.04	0.04
Mix well, let stand at room temperature for 5min, wavelength 665nm, 1mL glass cuvette, zero adjustment of the blank tube, determine the absorbance value of each tube at 665nm (A_{665}), recorded as A determination and A blank, $\Delta A = A_{determination} - A_{blank}$		

7. Concentration calculations

1. The standard curve regression equation is y = 0.0044x, r2 = 0.9988

2. Calculation formula

a. Organizational samples

① Calculated from tissue protein concentration

$$V_{H2S}\ (\text{nmol/mgprot}) = \frac{\Delta A}{0.0044} \times \frac{V_{Total\ reaction}}{(V_{sample} \times Cpr)} = 340.9 \times \Delta A \div Cpr$$

② Calculated from the weight of tissue samples

$$V_{H2S}\ (\text{nmol/g}\ V_{fresh\ sample}) = \frac{\Delta A}{0.0044} \times \frac{V_{Total\ Reaction}}{V_{Sample} \times W \div V_{Total\ Sample}} = 340.9 \times \Delta A \div W$$

b. Serum/plasma

$$V_{H2S}\ (\text{nmol/mL}) = \frac{\Delta A}{0.0044} \times \frac{V_{\text{Total Reaction}}}{V_{\text{sample}}} = 340.9 \times \Delta A$$

c. Cell samples

$$V_{H2S}\ (\text{nmol}/10^4\,\text{cell}) = \frac{\Delta A}{0.0044} \times \frac{V_{\text{Total Reaction}}}{V_{\text{Sample}} \times \text{Cell} \div V_{\text{Total } sample}} = 340.9 \times \Delta A \div Cell$$

V total sample :total reaction volume, 0.9 mL.

V sample :volume of sample in the reaction system, 0.6mL

V sample :volume of extract added, 1mL.

W: sample mass, g.

Cpr: sample protein concentration, mg/mL.

8. Precautions

The lowest detection limit was 1 nmol/mL.