

Hydrogen sulfide (H₂S) Content Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer

Catalog Number: NA0722

Size: 50T/48S

Components:

Reagent	Size	Storage
Extract solution I	Solution 60 mL×1	4°C
Extract solution II	Solution 10 mL×1	4°C
Reagent I	Solution 25 mL×1	4°C
Reagent II	Solution 25 mL×1	4°C

Product Description:

Hydrogen sulfide (H₂S) is a new type of gaseous signal molecule. It is a neurotransmitter that exists in the brain. The physiological concentration of H₂S has an important regulatory effect on the long-term enhancement of the hippocampus of the nervous system. It also plays an important pathophysiological effect on the process of spontaneous hypertension, hemorrhagic shock and liver cirrhosis.

H₂S can react with N, N-dimethyl-p-phenylenediamine and ferric ammonium sulfate to form methylene blue. Methylene blue has a maximum absorption peak at 665nm. The H₂S content can be calculated by measuring the absorbance value.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, desk centrifuge, pipette, 1mL glass cuvette, mortar/homogenizer, ice and distilled water.

Procedure

I. Sample preparation:

1. Bacteria or cells

Bacteria or cells: collecting bacteria or cells into the centrifuge tube, centrifugation and discard supernatant. Suggest add 1 mL of Extract solution I to 5 million of bacteria or cells. Use ultrasonication to splitting bacteria and cell (placed on ice, ultrasonic power 200W, ultrasonic 3 seconds, interval 7 seconds, total time 3 minutes). Centrifuge at 12000 ×g for 10 minutes at 4°C to remove insoluble materials. Then add 0.15 mL Extract solution II to 0.8 mL supernatant. Centrifuge at 12000 ×g for 10 minutes at 4°C to remove insoluble materials. Take the supernatant on ice for test.

2. Tissue: add 1 mL of Extract solution I into 0.1 g of tissue and fully grind on ice. Centrifuge at 12000 ×g for 10minutes at 4°C to remove insoluble materials. Then add 0.15 mL Extract solution II to 0.8 mL supernatant. Centrifuge at 12000 ×g for 10 minutes at 4°C to remove insoluble materials. Take the supernatant on ice for test.

3. Serum (plasma) or other liquid samples: add 1 mL of Extract solution I into 0.1 mL of serum (plasma). Centrifuge at 12000 ×g for 10 minutes at 4°C to remove insoluble materials. Then add 0.15 mL Extract solution II to 0.8 mL supernatant. Centrifuge at 12000 ×g for 10 minutes at 4°C to remove insoluble materials. Take the supernatant on ice for test.

II. Determination procedure:

1. Preheat spectrophotometer for 30min, adjust wavelength to 665 nm, set zero with distilled water.
2. Determination:

Reagent (μL)	Test tube	Blank tube
Sample	250	
Distilled water		250
Reagent I	375	375
Reagent II	375	375

Mix well. React at room temperature for 10 minutes. Measure the absorbance at 665 nm, record as A_T , A_B . Calculate the $\Delta A = A_T - A_B$.

Note: blank tube only need to be test one or two times.

III. Calculations:

Take the concentration of standard solution (nmol/mL) as x-axis, and the corresponding ΔA is y-axis. Then the linear regression equation $y = 0.0026x - 0.0268$, $R^2 = 0.9973$ is obtained. Bring ΔA into the equation to get x (nmol/mL).

1. Protein concentration:

$$\text{H}_2\text{S content (nmol/mg prot)} = x \times V_S \div (V_S \times \text{Cpr}) = x \div \text{Cpr}$$

2. Sample weight:

$$\text{H}_2\text{S content (nmol/g weight)} = x \times (V_{SP} + V_{EX2}) \div (W \times V_{SP} \div V_{EX1}) = 1.1875 \times x \div W$$

3. Cell amount:

$$\text{H}_2\text{S content (nmol/10}^4 \text{ cell)} = x \times (V_{SP} + V_{EX2}) \div (\text{cells} \times V_{SP} \div V_{EX1}) = 1.1875 \times x \div \text{cells}$$

4. Serum (plasma) sample:

$$\text{H}_2\text{S content (nmol/mL)} = x \times (V_{SP} + V_{EX2}) \div [(V_L \times V_{SP} \div (V_{EX1} + V_L))] = 13.0625 \times x$$

V_S : Sample volume in reaction, 0.05 mL;

V_{SP} : Supernatant volume in Extraction, 0.8 mL;

V_{EX1} : Extraction solution I volume, 1 mL;

V_{EX2} : Extraction solution II volume, 0.15 mL;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

cells: Total number of bacteria and cells, 10^4 ;

V_L : Liquid sample volume, 0.1 mL.

Note:

1. If the ΔA is lower, it is recommended to increase the sample size before determination; If $\Delta A > 0.6$, it is recommended to dilute the sample before determination. The calculation formula should be multiplied by the corresponding dilution factor.

Examples:

1. Take 0.1g of mouse liver to follow the determination procedure to operate. Determination with 1mL glass cuvette, and calculate $\Delta A = A_T - A_B = 0.090 - 0.026 = 0.064$. The calculated content is as follows:

H_2S content (nmol/g weight) = $x \times (V_{SP} + V_{EX2}) \div (W \times V_{SP} \div V_{EX1}) = 414.71$ nmol/g weight.

2. Take 0.1g of ginkgo leaf plum to follow the determination procedure to operate. Determination with 1mL glass cuvette, and calculate $\Delta A = A_T - A_B = 0.081 - 0.026 = 0.055$. The calculated content is as follows:

H_2S content (nmol/g weight) = $x \times (V_{SP} + V_{EX2}) \div (W \times V_{SP} \div V_{EX1}) = 373.61$ nmol/g weight.

Related products:

NA0781/ NA0540	Reduced Glutathione (GSH) Assay Kit
NA0780/ NA0539	Oxidized Glutathione (GSSG) Assay Kit
NA0779/ NA0538	Glutathione Peroxidase (GPX) Assay Kit
NA0783/ NA0542	Oxidized Thioredoxin Reductase (TrxR) Assay Kit
NA0752/ NA0510	Nitric oxide (NO) Assay Kit