

Hydrogen sulfide (H₂S) Content Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/ Microplate reader

Catalog Number: AK0617

Size: 100T/96S

Components:

Reagent	Size	Storage
Extract solution I	Solution 110 mL×1	2-8℃
Extract solution II	Solution 20 mL×1	2-8℃
Reagent I	Solution 8 mL×1	2-8℃
Reagent II	Solution 8 mL×1	2-8℃

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/microplate reader, desk centrifuge, pipette, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, ice and distilled water.

Procedure

I. Sample preparation:

- 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℃ 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℃ to remove insoluble materials. Take the supernatant on ice for test.
- 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℃ 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℃ 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 1 mL of serum (plasma). Centrifuge at 12000 ×g for 10 minutes at 4℃ 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℃ to remove insoluble materials. Take the supernatant on ice for test.

II. Determination procedure:

1. Preheat spectrophotometer/microplate reader for 30min, adjust wavelength to 665 nm, set spectrophotometer counter to zero with distilled water.

2. Determination:

Reagent (μL)	Test tube	Blank tube
Sample	50	-
Distilled water	-	50
Reagent I	75	75
Reagent II	75	75
Mix well. React at room temperature for 10 minutes. Measure the absorbance at 665 nm, record as A _T , A _B . Calculate the ΔA=A _T -A _B . Blank tube only need to be test one or two times.		

III. Calculations:

A. 96 well flat-bottom plate

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:

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

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;

C_{pr}: 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.

B . Micro glass cuvette

Take the concentration of standard solution(nmol/mL) as x-axis, and the corresponding ΔA is y-axis. Then the linear regression equation $y=0.0020x-0.0633$, $R^2=0.9951$ 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 $\Delta A > 1.2$, 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 96 well flat-bottom plate, and calculate $\Delta A = A_T - A_B = 0.150 - 0.121 = 0.029$. The calculated content is as follows:

$$\text{H}_2\text{S content (nmol/g weight)} = x \times (V_{SP} + V_{EX2}) \div (W \times V_{SP} \div V_{EX1}) = 548.03 \text{ nmol/g weight.}$$

2. Take 0. 1g of purple leaf plum to follow the determination procedure to operate. Determination with 96 well flat-bottom plate, and calculate $\Delta A = A_T - A_B = 0.270 - 0.121 = 0.149$. The calculated content is as follows:

$$\text{H}_2\text{S content (nmol/g weight)} = x \times (V_{SP} + V_{EX2}) \div (W \times V_{SP} \div V_{EX1}) = 1260.53 \text{ nmol/g weight.}$$

Related products:

AK0478/ AK0477 Reduced Glutathione (GSH) Assay Kit

AK0476/ AK0475 Oxidized Glutathione (GSSG) Assay Kit

AK0474/ AK0473 Glutathione Peroxidase (GPX) Assay Kit

AK0482/ AK0481 Oxidized Thioredoxin Reductase (TrxR) Assay Kit

AK0260/ AK0261 Nitric oxide (NO) Assay Kit