Hydrogen sulfide (H_2S) 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 (H_2S) is a new type of gaseous signal molecule. It is a neurotransmitter that exists in the brain. The physiological concentration of H_2S 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.

H_2S 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 H_2S 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:

1. **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.
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℃ 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 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.

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 $\Delta A=A_T-A_B$. 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:

H_2S content (nmol/mg prot) = $x\times V_S\div (V_S\times Cpr) = x\div Cpr$

2. Sample weight:

H_2S 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:

H_2S content (nmol/10^4 cell) = $x\times(V_{SP}+V_{EX2})\div (cells\times V_{SP}\div V_{EX1})=1.1875\times x\div cells$

4. Serum (plasma) sample:

H_2S content (nmol/mL) = $x\times(V_{SP}+V_{EX2})\div [(V_L\times V_{SP}\div(V_{EX1}+V_L)]=13.0625\times x$

Vs: 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.

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:

H_2S content (nmol/mg prot) = $x \times V_S \div (V_S \times Cpr) = x \div Cpr$

2. Sample weight:

H_2S 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:

H_2S content (nmol/10^4 cell) = $x \times (V_{SP}+V_{EX2}) \div (cells \times V_{SP} \div V_{EX1}) = 1.1875 \times x \div cells$

4. Serum (plasma) sample:

H_2S content (nmol/mL) = $x \times (V_{SP}+V_{EX2}) \div [(V_L \times V_{SP} \div (V_{EX1}+V_L)] = 13.0625 \times x$

Vs: 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 ΔA ＞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:

H_2S content (nmol/g weight) = $x \times (V_{SP}+V_{EX2}) \div (W \times V_{SP} \div V_{EX1})$=548.03 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: H_2S content (nmol/g weight) = $x \times (V_{SP}+V_{EX2}) \div (W \times V_{SP} \div V_{EX1})$=1260.53 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