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

Catalog Number: BC2050

Size: 50T/48S

Components:

Reagent	Size	Storage
Extract solution I	Solution 60 mL×1	2-8℃
Extract solution II	Solution 10 mL×1	2-8℃
Reagent I	Solution 20 mL×1	2-8℃
Reagent II	Solution 20 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, desk centrifuge, pipette, 1mL glass cuvette, 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 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 $\Delta A=A_T-A_B$. 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:

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, 0.25 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＞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：

BC1170/ BC1175	Reduced Glutathione（GSH）Assay Kit
BC1180/ BC1185	Oxidized Glutathione（GSSG）Assay Kit
BC1190/ BC1195	Glutathione Peroxidase （GPX）Assay Kit
BC1150/ BC1155	Oxidized Thioredoxin Reductase (TrxR) Assay Kit
BC1470/ BC1475	Nitric oxide (NO) Assay Kit

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