Technical Manual

H_2S Colorimetric Assay Kit

- **Catalogue Code: MAES0185**
- **Size: 96T**
- **Research Use Only**

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

4.86-100 μmol/L

Sensitivity:

2.75 μmol/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

H_2S is an important signal molecule involved in cellular signal transduction in the nervous system, circulatory system and many organs. H_2S is produced by three synthetic pathways: cysteine lyase, cysteine synthetase and 3-mercaptopyruvate sulfur transferase. It can regulate multiple biological processes from development, angiogenesis, nerve conduction to protein synthesis. It has the activity of vasodilating, angiogenic and anti-atherosclerotic, and can protect the heart, kidneys and other organs from ischemia or reperfusion injury.

3. Intended Use

This kit can be used to measure H_2S content in serum, plasma, animal tissue samples.

4. Detection Principle

H_2S can react with acetate solution to form ZnS which can be dissolved in alkaline solution. In the presence of Fe^{3+}, methylene blue can be formed. Methylene blue has a maximum absorption peak at 665 nm. H_2S content can be calculated indirectly by measuring the OD value at 665 nm.

5. Kit Components & Storage

Item	Specification	Storage
Buffer Solution	25 mL × 1 vial	2-8°C, 6 months
Alkali Reagent	13 mL × 1 vial	2-8°C, 6 months
Chromogenic Agent	13 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Protein Precipitator	13 mL × 1 vial	2-8°C, 6 months
Ferric Salt Reagent	2 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Standard	7.8 mg × 1 vial	2-8°C, 6 months, avoid direct sunlight
Standard Diluent	60 mL × 2 vials	2-8°C
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- incubator
- Vortex mixer
- centrifuge
- Microplate reader (660-670 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal saline (0.9% NaCl)

6. Assay Notes:

1. It should be performed in a fume hood when preparing standard solutions and standard curve.

2. Do not take the sediment when adding the supernatant to microplate, otherwise the result will be affect.

7. Reagent Preparation:

1. Bring all the reagents to room temperature before use.

2. Preparation of **standard solution (1 mmol/L):** Dissolve a vial of standard with 100 mL of standard diluent fully. The prepared standard solution can be stored at 2-8°C and avoid direct sunlight for a day.

8. Sample Preparation

1. Serum sample:
Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) and preserve on ice before detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) and preserve on ice before detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. Tissue sample:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Use filter paper to absorb excess water and weigh. Homogenize at the ratio of the volume of normal saline (0.9% NaCl) (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (6.82-100 μmol/L).

The recommended dilution factor for different samples is as follows (for reference only).

Sample Type:	Dilution Factor:
Human plasma	1
Mouse plasma	1
10% Rat spleen tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Rat brain tissue homogenate	1

Note: The diluent is normal saline (0.9% NaCl).

Email: info@assaygenie.com **Web:** www.assaygenie.com

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 665 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S0	S8	S16	S24	S32	S40	S48	S56	S64	S72
B	B	B	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
C	C	C	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
D	D	D	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
E	E	E	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
F	F	F	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
G	G	G	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
H	H	H	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79

Note: A-H, standard wells; S0, control well; S1-S79, sample wells.

10. Operation Steps

The preparation of standard curve

Dilute 1 mmol/L standard solution with standard diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 30, 40, 60, 80, 100 μmol/L.

The measurement of standard curve

1. Add 100 μL of standard solution with different concentration to 1.5 mL EP tubes marked from A to H with duplication.

2. Add 100 μL of buffer solution to each tube.

3. Add 100 μL of chromogenic agent to each tube.

4. Mix fully with vortex mixer for 5 s, then take 225 μL of mixed solution to corresponding wells of microplate respectively.

5. Add 15 μL of ferric salt reagent to each well (the multi-channel pipette is recommended).

6. Mix fully with microplate reader for 10 s, stand for 20 min at room temperature and measure the OD value of each well with microplate reader at 665 nm.

The measurement of samples

1. **Control tube:** Take 100 μL of double distilled water to the control tubes.
   Sample tube: Take 100 μL of sample to the sample tubes.

2. Add 100 μL of buffer solution to each tube and mix fully with vortex mixer for 10 s.

3. Add 100 μL of alkali reagent to each tube and mix fully with vortex mixer for 3 s.

4. Centrifuge at 12000 g for 10 min at 4°C, discard the supernatant and keep the sediment.

5. Add 150 μL of double distilled water and vortex with vortex mixer for 3 s.

6. Centrifuge at 12000 g for 10 min at 4°C, discard the supernatant and keep the sediment.

7. Add 100 μL of buffer solution to each tube.

8. Add 100 μL of chromogenic agent to each tube and mix fully with vortex mixer for 10 s.

9. Add 100 μL of protein precipitator to each tube and mix fully with vortex mixer for 3 s.

10. Centrifuge at 12000 g for 10 min at 4°C, then take 225 μL of supernatant to corresponding wells of microplate respectively.

11. Add 15 μL of ferric salt reagent to each well (the multi-channel pipette is recommended).

12. Mix fully with microplate reader for 10 s, stand for 20 min at room temperature and measure the OD value of each well with microplate reader at 665 nm.

The measurement of standard curve

	Standard tube
Standard solution with different concentrations (μL)	100
Buffer solution (μL)	100
Chromogenic agent (μL)	100
Mix fully with vortex mixer for 5 s, then take 225 μL of mixed solution to corresponding wells of microplate respectively.	
Ferric salt reagent (μL)	15
Mix fully with microplate reader for 10 s, stand for 20 min at room temperature and measure the OD value of each well with microplate reader at 665 nm.	

Email: info@assaygenie.com **Web:** www.assaygenie.com

The measurement of samples

	Control tube	Sample tube
Sample (μL)		100
Double distilled water (μL)	100	
Buffer solution (μL)	100	100
Mix fully with vortex mixer for 10 s.		
Alkali reagent (μL)	100	100
Mix fully with vortex mixer for 3 s. Centrifuge at 12000 g for 10 min at 4°C, discard the supernatant and keep the sediment.		
Double distilled water (μL)	150	150
Mix fully with vortex mixer for 3 s. Centrifuge at 12000 g for 10 min at 4°C, discard the supernatant and keep the sediment.		
Buffer solution (μL)	100	100
Chromogenic agent (μL)	100	100
Mix fully with vortex mixer for 10 s.		
Protein precipitator (μL)	100	100
Mix fully with vortex mixer for 3 s. Centrifuge at 12000 g for 10 min at 4°C, then take 225 μL of supernatant to corresponding wells of microplate respectively.		
Ferric salt reagent (μL)	15	15
Mix fully with microplate reader for 10 s, stand for 20 min at room temperature and measure the OD value of each well with microplate reader at 665 nm.		

11. Calculations

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b

1. Serum (plasma) sample:

$$H_2S\ (\mu mol/L) = \frac{\Delta A_{665} - b}{a} \times f$$

2. Tissue sample:

$$H_2S\ (\mu mol/gprot) = \frac{\Delta A_{665} - b}{a} \times f \div C_{pr}$$

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0)
x: The concentration of standard;
a: The slope of standard curve
b: The intercept of standard curve;
f: Dilution factor of sample before tested
ΔA_{665}: $OD_{Sample} - OD_{Control}$
C_{pr}: Concentration of protein in sample, gprot/L

12. Performance Characteristics

Detection Range	6.82-100 μmol/L
Sensitivity	6.73 μmol/L
Average recovery rate (%)	94
Average inter-assay CV (%)	9.9
Average intra-assay CV (%)	3.1

Analysis

Take 100 μL of 10% rat brain tissue homogenate and carry the assay according to the operation table.

The results are as follows:

Standard curve: y = 0.0049 x – 0.0188, the average OD value of the sample is 0.096, the average OD value of the control is 0.078, the concentration of protein in sample is 3.81 gprot/L, and the calculation result is:

$$\text{H}_2\text{S} \ (\mu mol/gprot) = \frac{(0.096 - 0.078 + 0.0188)}{0.0049} \div 3.81$$

$$= 1.97 \ \mu mol/gprot$$

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

Notes:

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Contact Details

Email: info@assaygenie.com

Web: www.assaygenie.com