H2S Assay Kit

Catalog No.: abx294158

Size: 96 tests

Detection Range: 4.86 µmol/L – 100 µmol/L

Sensitivity: 2.75 µmol/L

Storage: Store all components at 4°C. Store the Standard, Ferric Solution, and Chromogenic Reagent in the dark.

Application: For detection and quantification of H2S activity in serum, plasma, and tissue homogenates.

Introduction

Hydrogen sulfide (H2S, or H_2S) is a colorless, toxic, unpleasant-smelling gas well known as the cause of the distinct "rotten egg" smell encountered at active volcanic sites, swamps, and sewers. However, recent research has also found that H2S plays an important role as a signaling and protective molecule in numerous different organs. Produced via 3 main pathways, H2S is involved in regulating neurotransmitter receptors, angiogenesis, and vasodilation. In its protective role, H2S has been observed to reduce the risk of reperfusion injury, and it even protects against oxidative stress by scavenging reactive oxygen species in the heart and kidneys.

Abbexa's H2S Assay Kit is a quick, convenient, and sensitive method for measuring and calculating H2S concentration. In the presence of ferric iron, H2S combines with a chromogenic precursor to form methylene blue, with an absorbance maximum at 665 nm. The intensity of the color is proportional to the H2S concentration, which can then be calculated.

Kit components

1. 96-well microplate
2. Buffer Solution: 25 ml
3. Standard Diluent: 2 × 60 ml
4. Alkaline Reagent: 13 ml
5. Precipitating Reagent: 13 ml
6. Ferric Solution: 2 ml
7. Chromogenic Reagent: 13 ml
8. Standard: 1 vial
9. Plate sealer: 2

Materials required but not provided

1. Microplate reader (665 nm)
2. Double-distilled water
3. Normal saline (0.9% NaCl)
4. PBS (0.01 M, pH 7.4)
5. Pipette and pipette tips
6. 1.5 ml microcentrifuge tubes
7. Centrifuge
8. Vortex mixer
9. Incubator

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Collect fresh blood into a large centrifuge tube and stand for 30 minutes at 25°C, until clotted. Centrifuge at 2000 × g for 15 minutes at 4°C. Carefully take the upper liquid layer for detection. Keep on ice and assay immediately (within 24 hours of collection), or freeze at -80°C for long-term storage. Avoid freeze-thaw cycles, and discard any samples which show signs of hemolysis.
- **Plasma:** Collect fresh blood into a large centrifuge tube with an anticoagulant – heparin is recommended. Centrifuge at 1000 × g for 10 minutes at 4°C. Carefully take the uppermost liquid layer for detection. Keep on ice and assay immediately (within 24 hours of collection), or freeze at -80°C for up to 1 month.
- **Tissue Homogenates:** Take 0.1 g – 1 g of tissue, and wash with ice-cold PBS (0.01 M, pH 7.4). Absorb any excess liquid with absorbent filter paper, then weigh the sample carefully. Take the tissue into ice-cold normal saline (0.9% NaCl) in a ratio of 1:9 weight to volume (i.e. for 1 g of tissue, add 9 ml normal saline). Homogenize manually, keeping the homogenate on ice. Centrifuge the resulting homogenate at 10,000 × g for 10 minutes at 4°C. Take the resulting supernatant, keep on ice, and assay immediately.

  Note: To use the formula provided in **C. Calculation of Results** for tissue homogenates, the total protein concentration of the supernatant must be determined separately.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9% NaCl), then carry out the assay procedure. 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:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.
- Samples added to the microplate must be completely free of suspended, solid particles.

2. Reagents

- **Standard Solution (1 mmol/L):** Reconstitute the solid Standard in the vial with 100 ml Standard Diluent to create a 1 mmol/L Standard Solution. Mix fully to ensure it has fully dissolved. The Standard Solution can be stored for up to 1 day at 4°C in the dark. Keep out of direct light.

  CAUTION: The Standard and reconstituted Standard Solution may produce toxic fumes. These substances **must only be handled in a fume hood**.

- **Standards:** Label 7 tubes with 100 µmol/L, 80 µmol/L, 60 µmol/L, 40 µmol/L, 30 µmol/L, 20 µmol/L, 10 µmol/L. Add 100 µl, 80 µl, 60 µl, 40 µl, 30 µl, 20 µl, 10 µl of Standard Solution (1 mmol/L) to the 100 µmol/L, 80 µmol/L, 60 µmol/L, 40 µmol/L, 30 µmol/L, 20 µmol/L, 10 µmol/L tubes respectively, followed by 900 µl, 920 µl, 940 µl, 960 µl, 970 µl, 980 µl, and 990 µl of Standard Diluent, to prepare Standard Dilutions with concentrations 100 µmol/L, 80 µmol/L, 60 µmol/L, 40 µmol/L, 30 µmol/L, 20 µmol/L, 10 µmol/L. These volumes are summarized in the following table:

Standard Dilution (µmol/L)	**100**	**80**	**60**	**40**	**30**	**20**	**10**
Standard Solution (1 mmol/L) (µl)	100	80	60	40	30	20	10
Standard Diluent (µl)	900	920	940	960	970	980	990

For the blank, or 0 µmol/L standard, use pure Standard Diluent. The volume of each standard will be 1000 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.
- Do not remove the opened Standard or Standard Solution vials from the fume hood. Prepare the standard curve for this assay in the fume hood.

B. Assay Procedure

Mark microcentrifuge tubes for each standard, sample, and control. Only one control well is needed for this assay. *It is strongly recommended to prepare all the tubes in duplicate.*

Preparation and Measurement of the Standard Curve

1. Add 100 µl of Buffer Solution to each of the standard tubes.
2. Add 100 µl of each Standard Dilution to the corresponding standard tubes.
3. Add 100 µl of Chromogenic Reagent to all the standard tubes.
4. Vortex each tube for 5 seconds, to ensure the contents are mixed fully.
5. Take 225 µl of solution to the corresponding positions on the 96-well microplate.
6. Add 15 µl of Ferric Solution to all wells. *A multichannel pipette is recommended for this step. For best results, the time between adding the Ferric Solution to each well should be as short as possible.*
7. Mix the well contents fully using a microplate shaker for 10 seconds. Cover with a plate sealer, and leave to stand for 20 minutes at room temperature.
8. Measure the OD of each well with a microplate reader at 665 nm.

Preparation and Measurement of the Samples

1. Add 100 µl of Buffer Solution to the sample and control tubes.
2. Add 100 µl of sample to the corresponding sample tubes. Mix fully.
3. Add 100 µl double-distilled water to the control tubes. Mix fully.
4. Add 100 µl of Alkaline Reagent to each tube, and vortex for 3 seconds to mix fully.
5. Centrifuge at 12,000 × g for 10 minutes at 4°C. Carefully discard the supernatant and retain the sediment.
6. Add 150 µl double-distilled water to all tubes, and vortex for 3 seconds to mix fully.
7. Centrifuge at 12,000 × g for 10 minutes at 4°C. Carefully discard the supernatant and retain the sediment.
8. Add 100 µl Buffer Solution to each tube.
9. Add 100 µl Chromogenic Reagent to each tube, and vortex for 10 seconds to mix fully.
10. Add 100 µl of Precipitating Reagent to each tube, and vortex for 3 seconds to mix fully.
11. Centrifuge at 12,000 × g for 10 minutes at 4°C. Carefully discard the supernatant and retain the sediment.
12. Take 225 µl of the resulting supernatant, and transfer to the corresponding wells on the 96-well microplate.
13. Add 15 µl of Ferric Solution to all wells. *A multichannel pipette is recommended for this step. For best results, the time between adding the Ferric Solution to each well should be as short as possible.*
14. Mix the well contents fully using a microplate shaker for 10 seconds. Cover with a plate sealer, and leave to stand for 20 minutes at room temperature.
15. Measure the OD of each well with a microplate reader at 665 nm.

C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the concentration of H2S in each sample well can be derived with the following formulae:

1. Serum and Plasma samples:

$$\mathrm{H2S\ (\mu mol/L)} = \mathrm{F} \times \frac{(\mathrm{OD_{Sample}} - \mathrm{OD_{Control}} - \mathrm{b})}{\mathrm{a}}$$

2. Tissue samples:

$$\mathrm{H2S\ (\mu mol/g\ total\ protein)} = \mathrm{F} \times \frac{(\mathrm{OD_{Sample}} - \mathrm{OD_{Control}} - \mathrm{b})}{\mathrm{a} \times \mathrm{C_{Protein}}}$$

where:

$\mathrm{OD_{Sample}}$	OD value of sample
$\mathrm{OD_{Control}}$	OD value of control
$\mathrm{C_{Protein}}$	Concentration of protein in sample (g/L)
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
F	The dilution factor of sample