Product Manual

OxiSelect™ Free Hydrogen Sulfide Gas Assay Kit

Catalog Number

XAN-5084 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Hydrogen sulfide (H_2S) is a colorless gas containing a foul odor reminiscent of rotten eggs. High levels of H_2S are corrosive, flammable and poisonous. H_2S can be generated from the breakdown of organic matter by microbes when oxygen is not present (typically in swamps and sewers) by a process called anaerobic digestion. H_2S can also be found in natural gas, emissions from volcanoes, and in water from some wells. The human body produces minute levels of H_2S where the molecule functions as a gaseous messenger like carbon monoxide or nitric oxide. H_2S acts as a cerebrovascular dilator and is produced by the enzyme cystathione gamma lyase. Through activation of potassium ATP channels, H_2S can dilate arterioles. Furthermore, H_2S stimulates vascular smooth cell apoptosis and blocks proliferation-based vascular remodeling. Finally, H_2S has been shown to have neuroprotective and cardioprotective properties, lowering oxidative stress by increasing levels of glutathione by enhancing cystine and cysteine transport to neurons.

The OxiSelect™ Free Hydrogen Sulfide Gas Assay Kit is a novel technique for measurement of free H_2S gas emission. Liquid samples in a 96 well microplate release H_2S gas into the air which is captured in a gel spot containing Ag^+ ions on the underside of the microplate lid just above the sample (Figure 1). The resulting silver sulfide (Ag_2S) produced inside the spot creates a brownish color (Figure 4A) that can be measured colorimetrically on a microplate spectrophotometer. Each kit provides sufficient reagents to perform up to 100 assays, including positive control and unknown samples.

Assay Principle

The OxiSelect™ Free Hydrogen Sulfide Gas Assay Kit is a 96 well microplate based kit for simple detection of H_2S gas emission. First, a solution containing silver nitrate is coated on the underside of a microplate lid to form a thin polymer capture surface (Figure 2). Samples producing H_2S such as those containing an H_2S donor, enzyme, cells, or tissue are pipetted into the wells of the microplate. The coated lid is then placed over the microplate so that the polymer rests just above each sample. H_2S gas escapes from the sample in the well and enters the polymer coating. The Ag^+ ions in the polymer react with the H_2S gas to form Ag_2S nanoparticles, producing a brown discoloration of the polymer film. After one hour, the coated lid is transferred to another empty microplate and then OD is measured at 405 nm.

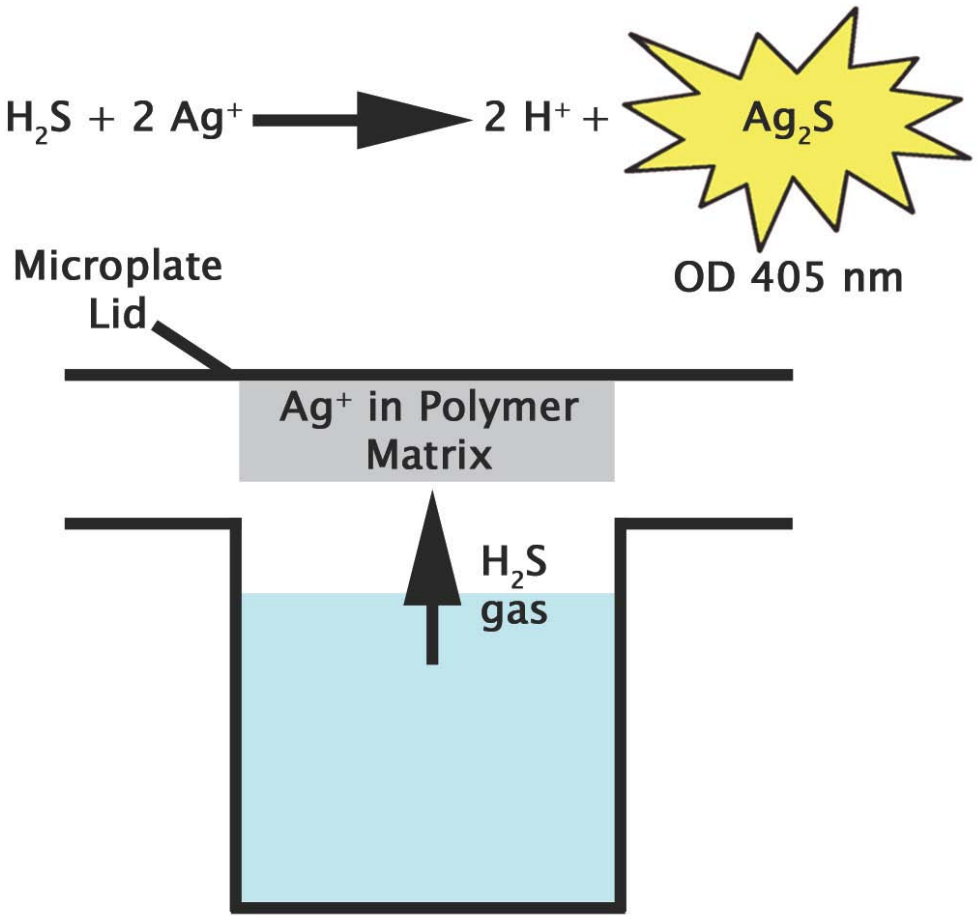


Figure 1: OxiSelect™ Free Hydrogen Sulfide Gas Assay Principle.

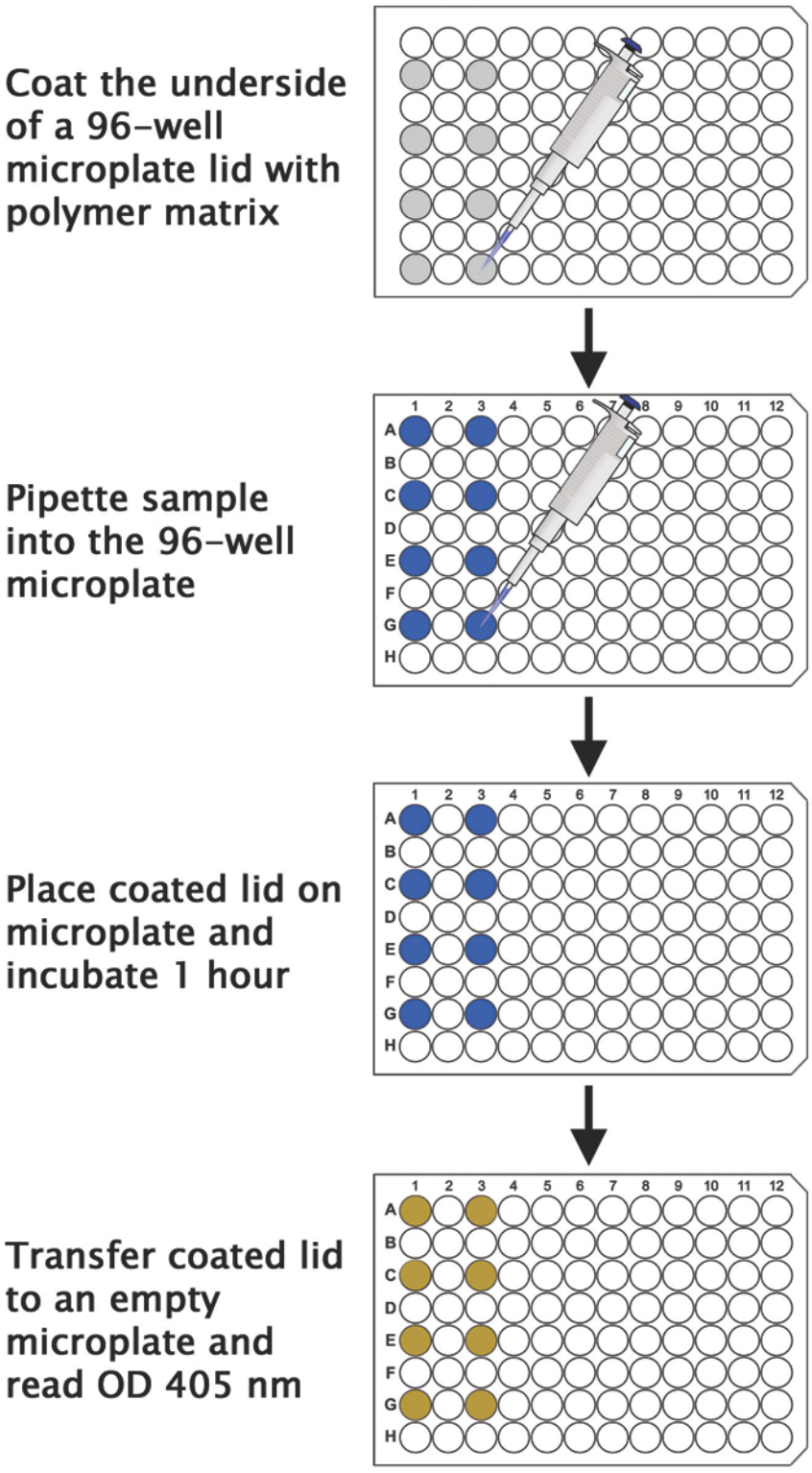


Figure 2: Schematic of Free Hydrogen Sulfide Gas Assay Protocol.

Related Products

1. STA-801: OxiSelect™ In Vitro Nitric Oxide (Nitrite/Nitrate) Assay Kit (Fluorometric)
2. STA-802: OxiSelect™ In Vitro Nitric Oxide (Nitrite/Nitrate) Assay Kit (Colorimetric)
3. XAN-5040: OxiSelect™ Trolox Equivalent Antioxidant Capacity (TEAC) Assay Kit (ABTS)

Kit Components

1. Sodium Sulfide (Part No. 50841B): One vial containing 100 mg of $Na_2S \cdot 9H_2O$
2. Polymer Matrix (Part No. 50842A): Two 1.5 mL vials.
3. 20X Silver Probe Solution (Part No. 50843A): One 150 µL vial.

Materials Not Supplied

1. 96 well microplate with lid (Costar 96-well plate Cat. No. 3370 recommended)
2. 1 µL to 20 µL adjustable single channel micropipettes with disposable tips
3. 20 µL to 200 µL adjustable single channel micropipettes with disposable tips
4. 200 µL to 1000 µL adjustable single channel micropipettes with disposable tips
5. Spectrophotometric microplate reader capable of reading 405-415 nm
6. Microcentrifuge tubes

Storage

Upon receipt, store the Sodium Sulfide at 4ºC. Store all other kit components at room temperature.

Preparation of Reagents

- Lid Coating Mix: Transfer the proper amount of Polymer Matrix to a microcentrifuge tube and dilute the 20X Silver Probe Solution 1:20 into the Polymer Matrix. For example add 45 µL of 20X Silver Probe Solution to 855 µL of Polymer Matrix and mix well. This Lid Coating Mix volume is enough for 10 lid spots. The Lid Coating Mix is stable for one day at room temperature in a tightly capped tube.

  Note: Prepare only enough for immediate use by scaling the above example proportionally.

- Sodium Sulfide Positive Control: Prepare a 10 mM solution of Sodium Sulfide by weighing out 5 milligrams of solid Sodium Sulfide and resuspending the solid at 2.4 mg/mL in deionized water. Then dilute the 10 mM Sodium Sulfide 1:50 into distilled water to a final concentration of 200 µM. For example, for 1 mL of Sodium Sulfide Positive Control, add 20 µL of 10 mM Sodium Sulfide to 980 µL of deionized water. While the 10 mM Sodium Sulfide solution is stable for up to 4 hours in a tightly capped tube, the 200 µM Sodium Sulfide Positive Control should be prepared immediately before use in the assay.

Preparation of Coated Lid

Notes:

- *Make sure that the coated circular areas will cover the wells of interest when the lid is properly applied to the microplate.*
- *To avoid cross contamination of H_2S gas between experimental wells, always arrange the experiment in the 96 well microplate so that a well containing a sample is bordered on every side by a blank well (see Figure 3).*

1. Place a 96 well lid upside down on a lab bench, or in a hood.
2. Draw up 30 µL of Lid Coating Mix (see Preparation of Reagents) with a micropipette and slowly apply about half of the Lid Coating Mix to the center of the circular area that covers one well. Use the pipette tip to evenly spread the Lid Coating Mix throughout the entire circular area. Then apply all but approximately 1-2 µL of the Lid Coating Mix remaining in the pipette tip to the circular area.

   Note: Leaving about 1-2 µL in the pipette tip ensures that no bubbles will be introduced into the coated circular area. If bubbles are accidently introduced into the circular area, use the pipette tip to gently aspirate away the bubbles.
3. Allow the inverted lid to dry undisturbed for 1 hour at room temperature. Do not touch or move the lid during this time.

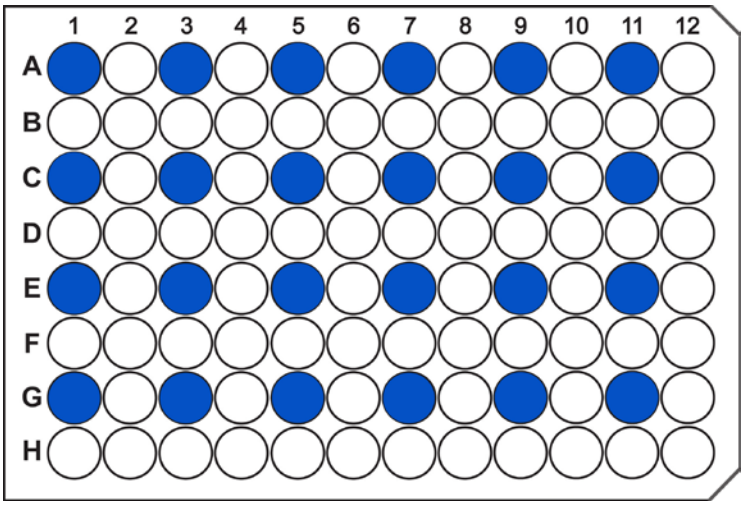


Figure 3. Schematic of a fully used microplate for the Free Hydrogen Sulfide Gas Assay Kit. Wells containing sample are labeled in blue.

Assay Protocol

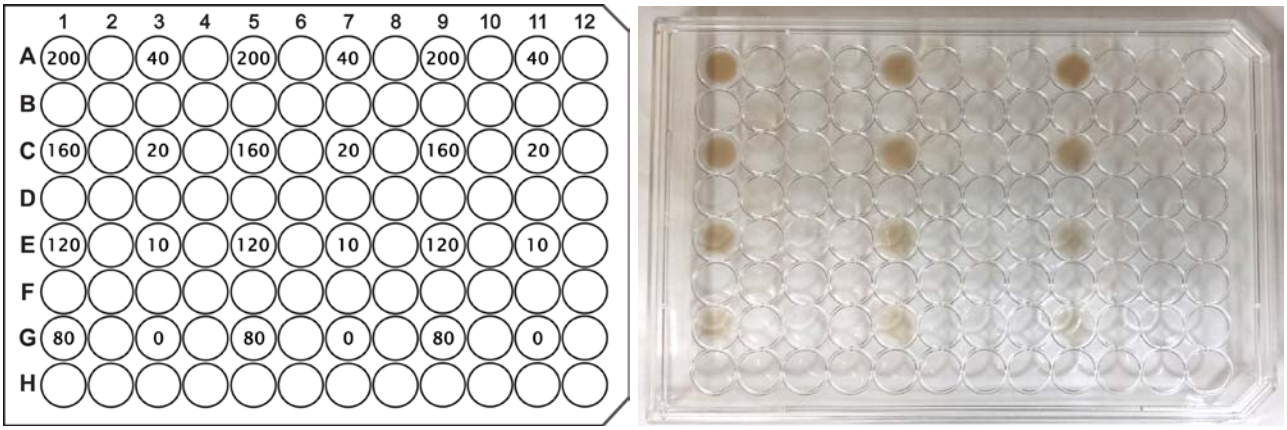
1. Add 300 µL of unknown sample, Sodium Sulfide positive control, or Negative Control to the wells of a 96 well microplate.
2. Cover the 96 well microplate with the Coated Lid (See Preparation of Coated Lid).
3. Incubate plate at room temperature undisturbed for one hour.
4. Transfer the Coated Lid to a new empty 96 well microplate.

5. Read absorbance of each microwell on a spectrophotometer using 405 nm as the primary wavelength.

Example of Results

The following figures demonstrate typical OxiSelect™ Free Hydrogen Sulfide Gas Assay Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

A



B

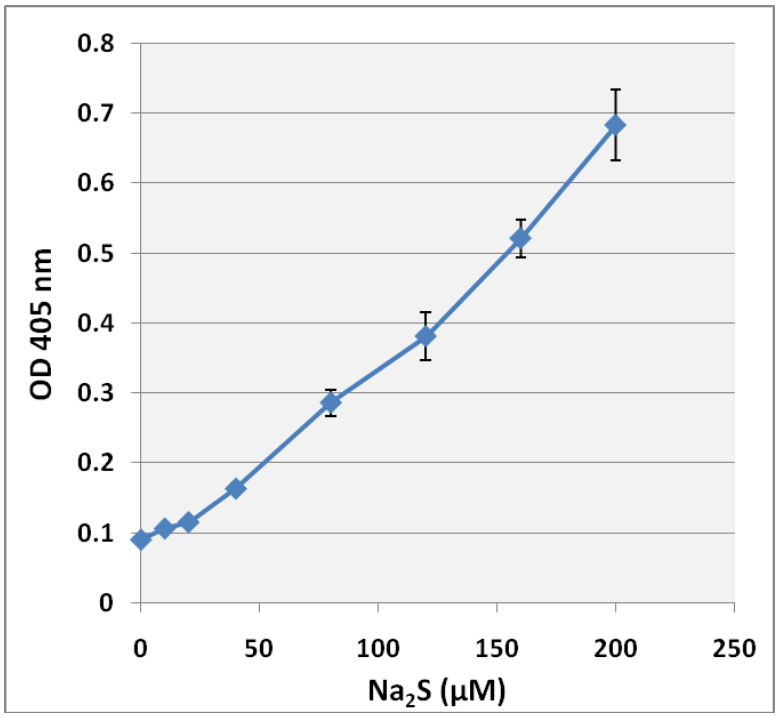


Figure 4. Detection of H_2S from a Hydrogen Sulfide donor. A, Left: Sodium sulfide was prepared at micromolar concentrations indicated and used in the OxiSelect™ Free Hydrogen Sulfide Gas Assay

Kit. **A, Right:** Image of the Coated Lid after one hour of incubation at room temperature according to the Assay Protocol. **B:** Averaged data from (**A**) plotted with standard deviation.

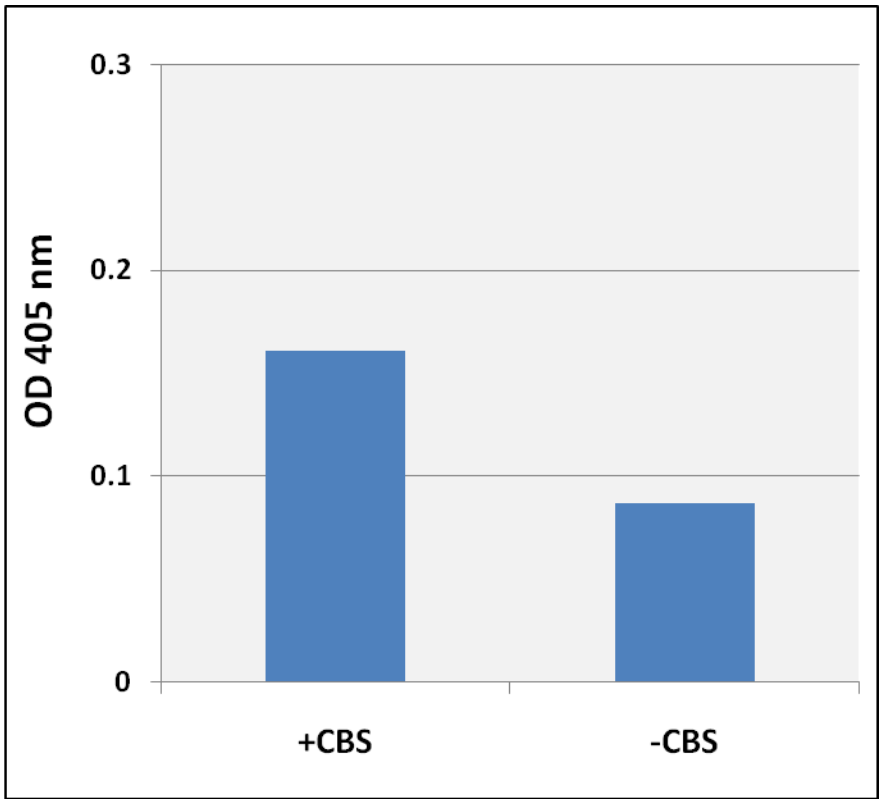


Figure 5. H_2S production by recombinant Cystathionine-β-Synthase (CBS). A Coated Lid was incubated in the absence (-CBS) or presence (+CBS) of 40 µg of recombinant CBS for 1 hour and then measured with a plate spectrophotometer according to the Assay Protocol.

References

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