

Technical Manual (Japanese version) is available at <http://www.dojindo.co.jp/manual/sb01.pdf>

## General Information

It has been recognized that hydrogen sulfide (H<sub>2</sub>S) has an important role as a physiological active substance for vasodilation, cytoprotection, and modulation of insulin secretion. H<sub>2</sub>S is considered as a gaseous molecule such as NO and CO. However, around 80% of the total sulfide exists as hydrogen sulfide anion (HS<sup>-</sup>) under physiological condition, since the pK<sub>a</sub> is about 7 (Fig. 1). In addition, H<sub>2</sub>S easily converts to various biochemical molecules such as persulfides and polysulfides, which react with sulfhydryl moieties in a living body. The functional mechanism of H<sub>2</sub>S has not been well understood. Sodium sulfide (Na<sub>2</sub>S) has been widely used as a H<sub>2</sub>S donor. Na<sub>2</sub>S is readily decomposed and release H<sub>2</sub>S when Na<sub>2</sub>S is dissolved in H<sub>2</sub>O.

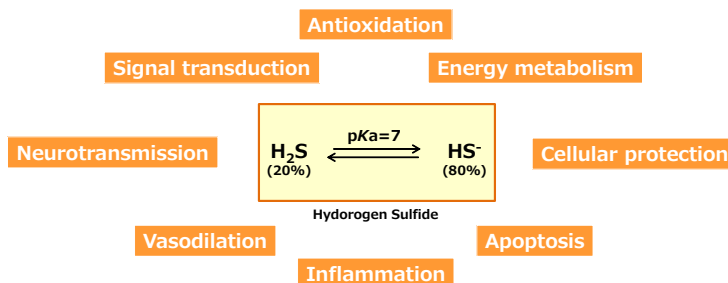


Fig. 1 Physiological functions of hydrogen sulfide

**Contents** -SulfoBiotics- Sodium sulfide (Na<sub>2</sub>S) : 100 mg x 5

**Storage Condition** Store at 0-5 °C

**Precaution** \* Use the reagent after bringing to room temperature, because it is moisture sensitive.  
\* Use up the reagent soon after opening.

**General Protocol** 1) Dissolve 7.8 mg of Na<sub>2</sub>S in 1 ml of deionized H<sub>2</sub>O under nitrogen gas (100 mmol/l Na<sub>2</sub>S solution).  
2) Dilute the 100 mmol/l Na<sub>2</sub>S aqueous solution to an appropriate concentration depending on your experiment.  
\* Purge deionized H<sub>2</sub>O with nitrogen gas for longer than 30 minutes to prevent the oxidation.  
\* Use the aqueous solution as soon as prepared. The solution is not stable enough to store.

## Experimental Example

### - Detection of hydrogen sulfide by methylene blue method -

- 1) 20 µl of 100 mmol/l Na<sub>2</sub>S aqueous solution was added to 980 µl of deionized H<sub>2</sub>O to prepare 2 mmol/l Na<sub>2</sub>S solution.
- 2) 100 µl of 2 mmol/l Na<sub>2</sub>S solution was added to 900 µl of deionized H<sub>2</sub>O to prepare 200 µmol/l Na<sub>2</sub>S solution.
- 3) 200 µmol/l Na<sub>2</sub>S solution was diluted with deionized H<sub>2</sub>O to prepare various concentration of Na<sub>2</sub>S solution by serial dilution (200, 100, 50, 25, 12.5, 6.3, 3.2, 0 µmol/l).
- 4) 300 µl of 1% zinc acetate solution, 50 µl of 20 mmol/l N,N-Dimethyl-p-phenylenediammonium (7.2 mol/l HCl) solution and 50 µl of 30 mmol/l FeCl<sub>3</sub> (1.2 mol/l HCl) solution were added to 250 µl of the Na<sub>2</sub>S solutions and mixed using a vortex.
- 5) The solutions were incubated at room temperature for 30 minutes and transferred 200 µl of the solution to each well (96-well plate).
- 6) Measure the absorbance at 650 nm using a microplate reader (Fig. 2).

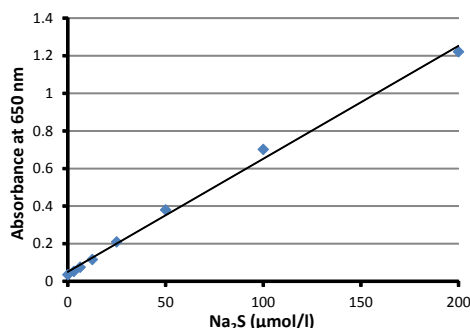


Fig. 2 Absorbance change at 650 nm depending on the concentration of hydrogen sulfide

## References

- 1) R. Greiner, Z. Palinkas, K. Basell, D. Becher, H. Antelmann, P. Nagy and T. P. Dick, "Polysulfides link H<sub>2</sub>S to protein thiol oxidation", *Antioxid. Redox Signal.*, **2013**, *19*, 1749.
- 2) N. S. Lawrence, J. Davis and R. G. Compton, "Analytical strategies for the detection of sulfide: a review", *Talanta*, **2000**, *52*, 771.

If you need more information, please contact Dojindo technical service.

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