-*SulfoBiotics*- Sodium sulfide (Na_2S) Technical Manual

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_2S) has an important role as a physiological active substance for vasodilation, cytoprotection, and modulation of insulin secretion. H_2S is considered as a gaseous molecule such as NO and CO. However, around 80% of the total sulfide exists as hydrogen sulfide anion (HS^-) under physiological condition, since the *pKa* is about 7 (Fig. 1). In addition, H_2S 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_2S has not been well understood.Sodium sulfide (Na_2S) has been widely used as a H_2S donor. Na_2S is readly decomposed and release H_2S when Na_2S is dissolved in H_2O.

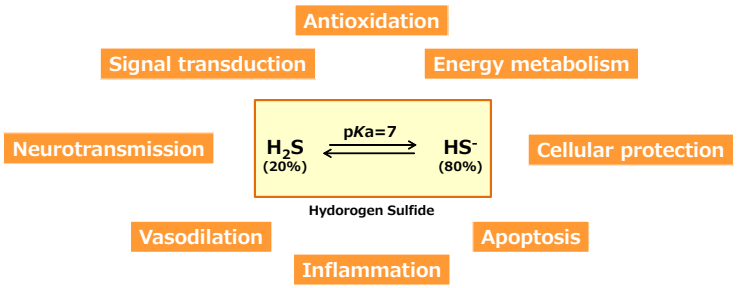

Fig. 1 Physiological functions of hydrogen sulfide

Contents

-*SulfoBiotics*- Sodium sulfide (Na_2S) : 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_2S in 1 ml of deionized H_2O under nitrogen gas (100 mmol/l Na_2S solution).
2) Dilute the 100 mmol/l Na_2S aqueous solution to an appropriate concentration depending on your experiment.

* Purge deionized H_2O 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_2S aqueous solution was added to 980 µl of deionized H_2O to prepare 2 mmol/l Na_2S solution.
2) 100 µl of 2 mmol/l Na_2S solution was added to 900 µl of deionized H_2O to prepare 200 µmol/l Na_2S solution.
3) 200 µmol/l Na_2S solution was diluted with deionized H_2O to prepare various concentration of Na_2S 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 $FeCl_3$ (1.2 mol/l HCl) solution were added to 250 µl of the Na_2S solutions and mixed using a vortex.
5) The solutions were incubated at room temperature for 30 minutes and transfered 200 µl of the solution to each well (96-well plate).
6) Measure the absorbance at 650 nm using a microplate reader (Fig. 2).

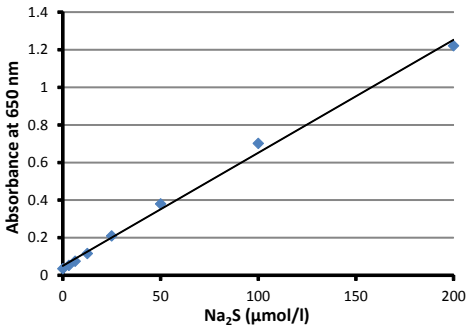

Fig. 2 Absorbance change at 650 nm depending on the concentration of hydrogen sulfide

References

1) R. Greiner, Z. Palinkas, K. Basell, D. Becher, H. Antelmann, P. Nagy and T. P. Dick, "Polysulfides link H_2S 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.

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