-SulfoBiotics- Sodium disulfide (Na_2S_2)
-SulfoBiotics- Sodium trisulfide (Na_2S_3)
-SulfoBiotics- Sodium tetrasulfide (Na_2S_4)
-SulfoBiotics- Sodium Polysulfide Set

Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/sb13.pdf

General Information

It becomes obvious that there are a lot of molecules containing sulfane sulfurs such as persulfides and polysulfides in living body. These molecular species are involved in production, storage and release of hydrogen sulfide, which is recognized as an important physiological mediator. Furthermore, recent studies reveal that persulfides and polysulfides may control intracellular signal transduction through *s*-sulfhydration of proteins, and function *in vivo* as anti-oxidants which have much higher reducing activity than glutathione reduced form and cysteine.

Sodium polysulfides (Na_2S_n) are sulfane sulfur donors which have simple structures, and exist as hydrogen polysulfides in an aqueous solution depending on pH. These reagents are necessary to for research and analysis of sulfane sulfurs *in vivo*.

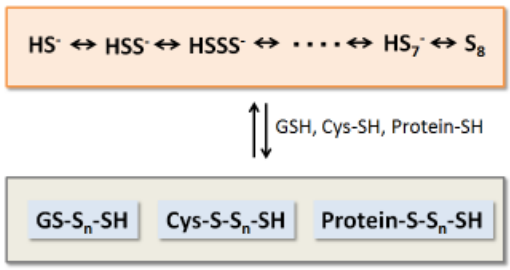


Fig. 1 Chemical species containing sulfane sulfurs

Sodium disulfide (Na_2S_2)
pK_{a1} = 5
pK_{a2} = 9.7

Sodium trisulfide (Na_2S_3)
pK_{a1} = 4.2
pK_{a2} = 7.5

Sodium tetrasulfide (Na_2S_4)
pK_{a1} = 3.8
pK_{a2} = 6.3

※ The pK_a values were refered to the following article.
J. Gun *et al.*, "Electrospray Ionization Mass Spectrometric Analysis of Aqueous Polysulfide Solutions", *Microchim. Acta*, **2004**, *146*, 229

Fig. 2 Structures of Sodium polysulfides (Na_2S_n) and the p*K*a values

Contents

SB02	-SulfoBiotics- Sodium disulfide (Na_2S_2)	: 100 mg x 5
SB03	-SulfoBiotics- Sodium trisulfide (Na_2S_3)	: 100 mg x 5
SB04	-SulfoBiotics- Sodium tetrasulfide (Na_2S_4)	: 100 mg x 5
SB13	-SulfoBiotics- Sodium Polysulfide Set	: Na_2S_2, Na_2S_3, Na_2S_4 100 mg each

Storage Condition

Store at 0-5 °C
*Open the cap after reaching to room temperature because they are moisture sensitive.
Store at 0-5 °C under nitrogen gas, and use up the reagent early after opening.

Preparation of Solutions

1) Prepare 100 mmol/l Na_2S_n aqueous solution with ddH_2O purged with nitrogen gas.
*100 mmol/l Na_2S_n aqueous solution ; Na_2S_2 11 mg/ml, Na_2S_3 14.2 mg/ml, Na_2S_4 17.4 mg/ml
* Purge ddH_2O with nitrogen gas for longer than thirty minutes to prevent oxidation of Na_2S_n.

2) Dilute the 100 mmol/l Na_2S_n aqueous solution to an appropriate concentration depending on your experiment.
* Use 100 mmol/l Na_2S_n aqueous solutions and the diluents as soon as they have been prepared.

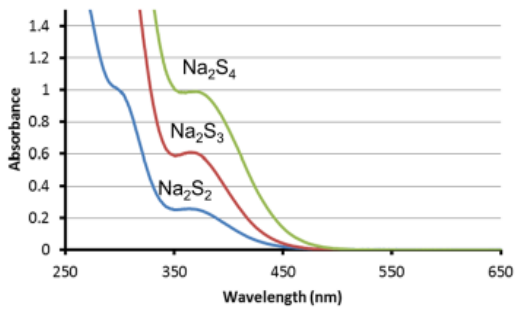


Fig. 3 UV spectra of 1 mmol/l Na_2S_n aqueous solution

Experimental Example 1

- Reducing activity of hydrogen polysulfides -

1) 10 µl of 10 mmol/l Na_2S_n aqueous solution was added to 1 ml of 20 µmol/l WST-8 (PBS) solution.

2) The solution was incubated at room temperature for 30 minutes, transfer 100 µl of the solution to each well, and measure the absorbance at 450 nm using a microplate reader.

* WST-8 is a highly water-soluble tetrazolium salt developed by Dojindo Laboratories, and gives a yellow-color formazan dye by reduction.

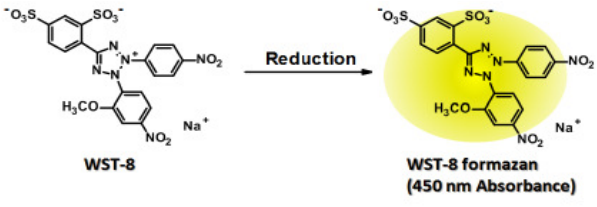


Fig. 4 Reduction of WST-8

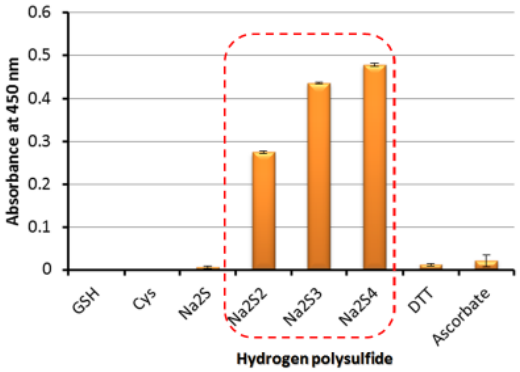


Fig. 5 Absorbances at 450 nm of WST-8 formazans produced with various reducing agents.

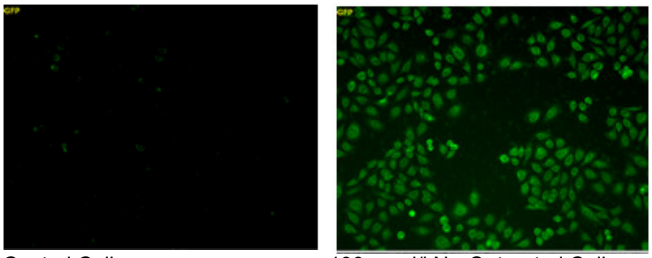
Hydrogen polysulfides gave high absorbances at 450 nm derived from WST-8 formazans. On the other hand, no absorbances at 450 nm were observed when glutathione reduced form (GSH) and cysteine (Cys) were used as reducing agents. These results show that hydrogen polysulfides have much higher reducing activity than the general reducing agents.

Experimental Example 2

- Detection of sulfane sulfurs in cells treated with a sulfane sulfur donor (Na_2S_3) -

1) CHO cell suspensions prepared with serum-containing DMEM were inoculated in a 96-well black clear bottom plate to prepare 10^4 cells/well, and incubated in a humidified incubator (*e.g.*, at 37°C, 5% CO_2) overnight.
2) The culture medium was discarded, and the cells were washed with a serum-free DMEM.
3) 100 µl of a 100 µmol/l Na_2S_3 (serum-free DMEM) was added to the each well, and the cells were incubated for 15 minutes in the incubator.
4) The supernatant was discarded, and the cells were washed with a serum-free DMEM twice.
6) 100 µl of a 20 µmol/l SSP4 (serum-free DMEM) was added to the cells, and the cells were incubated for 15 minutes in the incubator.
5) The supernatant was discarded, and the cells were washed with PBS twice.
6) 100 µl of PBS was added to the each wells, and the cells were analyzed under a fluorescence microscope.

* SSP4 (SB10; *-SulfoBiotics-* SSP4) is a novel fluorescent probe to detect sulfane sulfurs selectively.



Control Cells 100 µmol/l Na_2S_3 treated Cells (Exporsue time: 1000 msec)

Fluorescence signals were observed in cells treated with 100 µmol/l Na_2S_3 (sulfane sulfur donor).

Fig. 6 Fluorescence images of sulfane sulfurs in cells treated with or without Na_2S_3

References

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2) S. Koike, Y. Ogasawara, N. Shibuya, H. Kimura, and K. Ishii, *FEBS Lett.*, **2013**, *587*, 3548.
3) T. Ida, T. Sawa, H. Ihara. Y. Tsuchiya, Y. Watanabe, Y. Kumagai, M. Suematsu, H. Motohashi, S. Fujii, T. Matsunaga, M. Yamamoto, K. Ono, N. O. Devarie-Baez, M. Xian, J. M Fukuto, and T. Akaike, *Proc. Natl. Acad. Sci. USA.*, **2014,** *111*, 7606.

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