

Technical Manual (Japanese version) is available at <http://www.dojindo.co.jp/manual/sb21.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 nitric oxide and carbon monoxide. However, around 80% of the total sulfide exists as hydrogen sulfide anion (HS^-) under physiological condition, since the pKa is about 7. In addition, HS^- easily converts to various biochemical molecules such as persulfides and polysulfides, which react with sulfhydryl moieties in a living body. -SulfoBiotics- HSip-1 is a novel fluorescent probe to detect H_2S selectively and it emits strong green fluorescence when it reacts with H_2S .

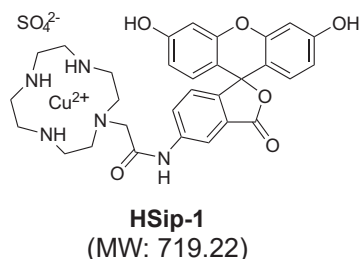
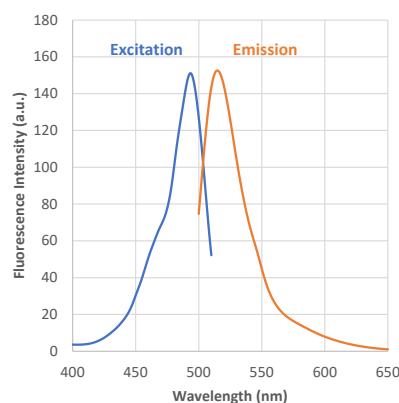


Fig. 1 Chemical structures of HSip-1



λ_{ex} : 491 nm
 λ_{em} : 516 nm

< Recommended filter >
Ex : 470 ~ 500 nm
Em : 500 ~ 550 nm

Fig. 2 Excitation and emission spectra of HSip-1 reacted with H_2S

Contents 1 mg x 1

Storage Conditions Store in a cool dark place.

Required Equipment and Materials
- Purified water
- PBS
- Micropipettes

Preparation of Solutions **Preparation of 10 mmol/l HSip-1 stock solution**
Add 139 μ l of purified water to a tube containing 1 mg of HSip-1 and dissolve it by pipetting.
**Store at -20 °C. The reconstituted solution is stable at -20°C for 1 month.*

Experimental Example

Detection of hydrogen sulfide by HSip-1

- 1) HSip-1 stock solution (10 mmol/l) was diluted with PBS to prepare 200 μ mol/l HSip-1 working solution.
- 2) Sodium Sulfide (-SulfoBiotics- Sodium Sulfide (Na_2S), 7.8 mg) were dissolved in 1 ml of de-oxygenated H_2O prepared by bubbling of nitrogen gas (100 mmol/l Na_2S solution).
- 3) Na_2S solution (100 mmol/l, 20 μ l) was added to 980 μ l of de-oxygenated H_2O to prepare 2 mmol/l Na_2S solution.
- 4) Na_2S solution (2 mmol/l, 100 μ l) was added to 900 μ l of de-oxygenated H_2O to prepare 200 μ mol/l Na_2S solution.
- 5) Na_2S solution (200 μ mol/l) was diluted with de-oxygenated H_2O to prepare various concentrations of Na_2S solution by serial dilution (200, 100, 50, 25, 12.5, 6.3, 3.2, 0 μ mol/l).
- 6) HSip-1 working solution (200 μ mol/l, 350 μ l) was added to 300 μ l of the Na_2S solutions and mixed using a vortex mixer.
- 7) The solutions were incubated at room temperature for 30 minutes and 200 μ l of the solution were transferred to each well (96-well plate).
- 8) The fluorescence intensities were measured at 516 nm (λ_{ex} =491 nm) with a microplate reader.

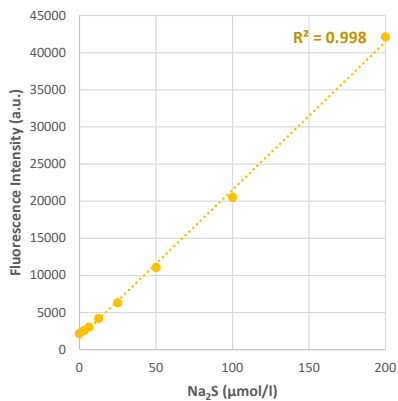


Fig. 3 Fluorescence intensity changes at 516 nm with various concentrations of hydrogen sulfide.
 *Experimental example on HeLa cells is available. It can be found by searching "SB21" on our website.

These products were commercialized under the advisory of Dr. Tetsuo Nagano and Dr. Kenjiro Hanaoka (The University of Tokyo).

Reference

- 1) K. Sasakura, K. Hanaoka, N. Shibuya, Y. Mikami, Y. Kimura, T. Komatsu, T. Ueno, T. Terai, H. Kimura, and T. Nagano, "Development of a Highly Selective Fluorescence Probe for Hydrogen Sulfide", *J. Am. Chem. Soc.*, **2011**, *133*, 18003.

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

Dojindo Laboratories

2025-5 Tabaru, Mashiki-machi, Kamimashiki-gun, Kumamoto
 861-2202, Japan Phone: +81-96-286-1515 Fax: +81-96-286-1525
 E-mail: info@dojindo.co.jp Web: www.dojindo.co.jp

Dojindo Molecular Technologies, Inc.

Tel: +1-301-987-2667 Web: <http://www.dojindo.com/>

Dojindo EU GmbH

Tel: +49-89-3540-4805 Web: <http://www.dojindo.eu.com/>

Dojindo China Co., Ltd

Tel: +86-21-6427-2302 Web: <http://www.dojindo.cn/>