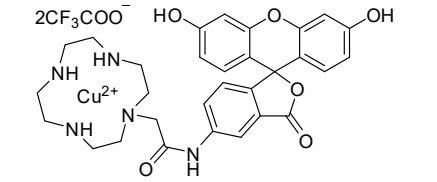
-*SulfoBiotics*- HSip-1
-*SulfoBiotics*- HSip-1 DA

Technical Manual

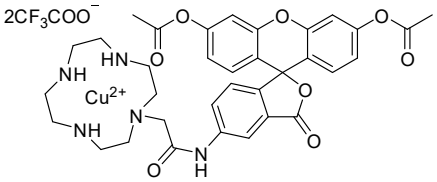
Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/sb21.pdf

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. -*SulfoBiotics*- HSip-1 DA is cell membrane permeable and it enables fluorescent imaging of intracellular H_2S.

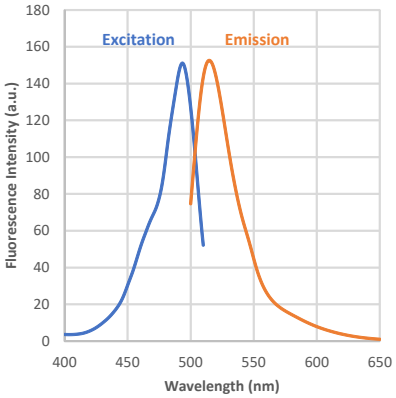


HSip-1
(MW: 849.19)



HSip-1 DA
(MW: 933.26)

Fig. 1 Chemical structures of HSip-1 and HSip-1 DA



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

- HSip-1 1 mg x 1
- HSip-1 DA 50 µg x 1

Storage Conditions

- HSip-1 Store in a cool dark place.
- HSip-1 DA Store at -20°C.

Required Equipment and Materials

- Dimethyl sulfoxide (DMSO)
- Serum-free medium
- HBSS
- PBS
- Micropipettes

Preparation of Solutions

Preparation of 10 mmol/L HSip-1 stock solution

Add 117 µL 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.

Preparation of 1 mmol/L HSip-1 DA stock solution

Add 53 µL of DMSO to a tube containing 50 µg of HSip-1 DA and dissolve it by pipetting.
*Store at -20 °C. The reconstituted solution is stable at -20°C for 1 month.

Experimental Example 1

Detection of hydrogen sulfide by HSip-1

1) HSip-1 stock solution (10 mmol/L) was diluted with PBS to prepare 200 µmol/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 µL) was added to 980 µL of de-oxygenated H_2O to prepare 2 mmol/L Na_2S solution.
4) Na_2S solution (2 mmol/L,100 µL) was added to 900 µL of de-oxygenated H_2O to prepare 200 µmol/L Na_2S solution.
5) Na_2S solution (200 µmol/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 µmol/L).
6) HSip-1 working solution (200 µmol/L, 350 µL) was added to 300 µL of the Na_2S solutions and mixed using a vortex mixer.
7) The solutions were incubated at room temperature for 30 minutes and 200 µL of the solution were transfered to each well (96-well plate).
8) The fluorescence intensities were measured at 516 nm (λ_{ex}=491 nm) with a microplate reader.

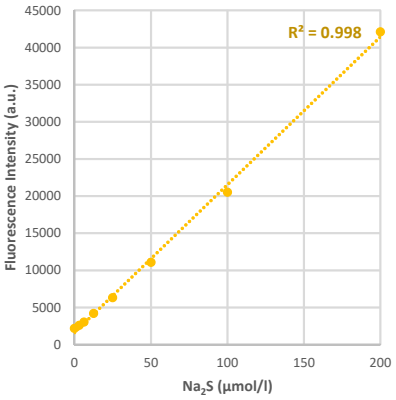


Fig. 3 Fluorescence intensity changes at 516 nm with various concentrations of hydrogen sulfide.

*Experimental example on HeLa cells is available. It can be found by searching “SB21“ on our website.

Experimental Example 2

Fluorescence imaging of hydrogen sulfide with HSip-1 DA

1) HeLa cells were seeded on μ-slide 8 well (Ibidi) and cultured at 37°C overnight in a 5% CO_2 incubator.
2) The culture medium was discarded and the cells were washed with a serum-free medium (MEM) twice.
3) HSip-1 DA stock solution (1 mmol/L) was diluted with a serum-free medium (MEM) to prepare 5 μmol/L HSip-1 DA working solution.

*Please optimize the final concentration of HSip-1 DA depeneding on the cell lines.

4) HSip-1 DA working solution (5 μmol/L, 200 μL) was added to the cells, and the cells were cultured at 37°C for 30 minutes in a 5% CO_2 incubator.
5) The supernatant was discarded, and the cells were washed with HBSS twice.
6) Na_2S solution (200 μmol/L, 200 μL) was added to the each well, and the cells were cultured at 37°C for 30 minutes in a 5% CO_2 incubator.
7) The supernatant was discarded and the cells were washed with HBSS twice.
8) HBSS (200 μL) were added, and the cells were observed by confocal fluorescence microscopy.

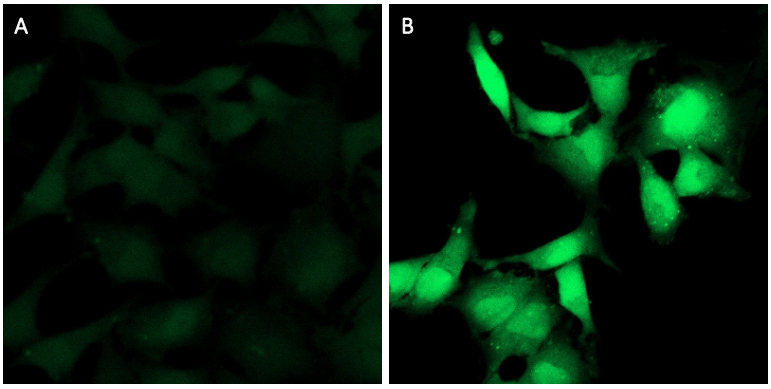


Fig.4 Detection of hydrogen sulfide using HSip-1 DA in HeLa cells treated with Na_2S.
(A: Control, B: 200 μmol/L Na_2S treated)

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.

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