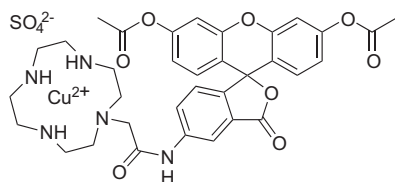


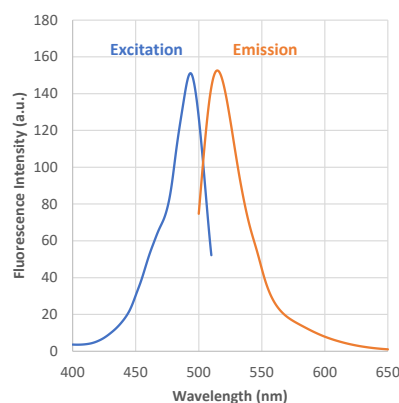
### General Information

It has been recognized that hydrogen sulfide ( $\text{H}_2\text{S}$ ) has an important role as a physiological active substance for vasodilation, cytoprotection, and modulation of insulin secretion.  $\text{H}_2\text{S}$  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 ( $\text{HS}^-$ ) under physiological condition, since the  $\text{pK}_a$  is about 7. In addition,  $\text{HS}^-$  easily converts to various biochemical molecules such as persulfides and polysulfides, which react with sulfhydryl moieties in a living body. -SulfoBiotics- HSip-1 DA is cell membrane permeable and it enables fluorescent imaging of intracellular  $\text{H}_2\text{S}$ .



**HSip-1 DA**  
(MW: 803.29)

Fig. 1 Chemical structures of HSip-1 DA



$\lambda_{\text{ex}}$  : 491 nm  
 $\lambda_{\text{em}}$  : 516 nm

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

Fig. 2 Excitation and emission spectra of HSip-1 reacted with  $\text{H}_2\text{S}$

**Contents** 50  $\mu\text{g} \times 1$

**Storage Conditions** Store at  $-20^\circ\text{C}$ .

**Required Equipment and Materials**

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

**Preparation of Solutions** **Preparation of 1 mmol/l HSip-1 DA stock solution**  
Add 62  $\mu\text{l}$  of DMSO to a tube containing 50  $\mu\text{g}$  of HSip-1 DA and dissolve it by pipetting.  
*\*Store at  $-20^\circ\text{C}$ . The reconstituted solution is stable at  $-20^\circ\text{C}$  for 1 month.*

### Experimental Example

#### Fluorescence imaging of hydrogen sulfide with HSip-1 DA

- 1) HeLa cells were seeded on  $\mu$ -slide 8 well (Ibidi) and cultured at  $37^\circ\text{C}$  overnight in a 5%  $\text{CO}_2$  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  $\mu\text{mol/l}$  HSip-1 DA working solution.  
*\*Please optimize the final concentration of HSip-1 DA depending on the cell lines.*
- 4) HSip-1 DA working solution (5  $\mu\text{mol/l}$ , 200  $\mu\text{l}$ ) was added to the cells, and the cells were cultured at  $37^\circ\text{C}$  for 30 minutes in a 5%  $\text{CO}_2$  incubator.
- 5) The supernatant was discarded, and the cells were washed with HBSS twice.
- 6)  $\text{Na}_2\text{S}$  solution (200  $\mu\text{mol/l}$ , 200  $\mu\text{l}$ ) was added to the each well, and the cells were cultured at  $37^\circ\text{C}$  for 30 minutes in a 5%  $\text{CO}_2$  incubator.
- 7) The supernatant was discarded and the cells were washed with HBSS twice.
- 8) HBSS (200  $\mu\text{l}$ ) were added, and the cells were observed by confocal fluorescence microscopy.

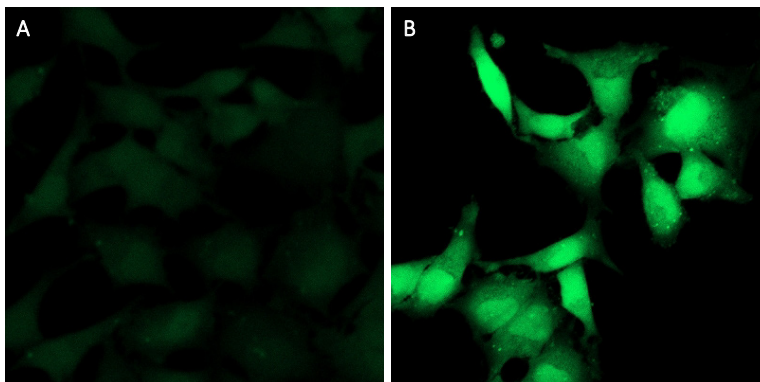


Fig.3 Detection of hydrogen sulfide using HSip-1 DA in HeLa cells treated with Na<sub>2</sub>S.  
(A: Control, B: 200 μmol/l Na<sub>2</sub>S treated)

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.

#### 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](mailto:info@dojindo.co.jp) Web: [www.dojindo.co.jp](http://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/>