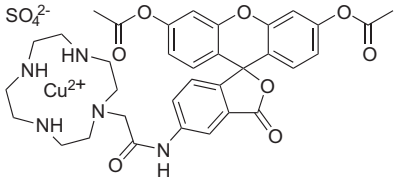
-*SulfoBiotics*- HSip-1 DA

Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/sb22.pdf

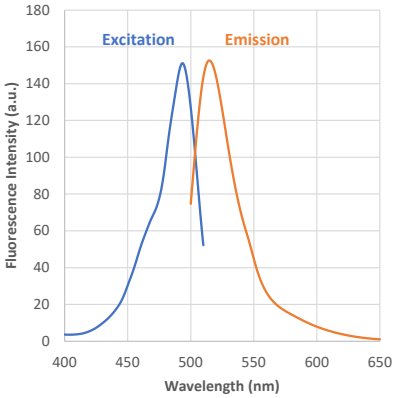
General Information

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 DA is cell membrane permeable and it enables fluorescent imaging of intracellular H_2S.



HSip-1 DA
(MW: 803.29)

Fig. 1 Chemical structures of 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

50 µg x 1

Storage Conditions

Store at -20°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 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

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.

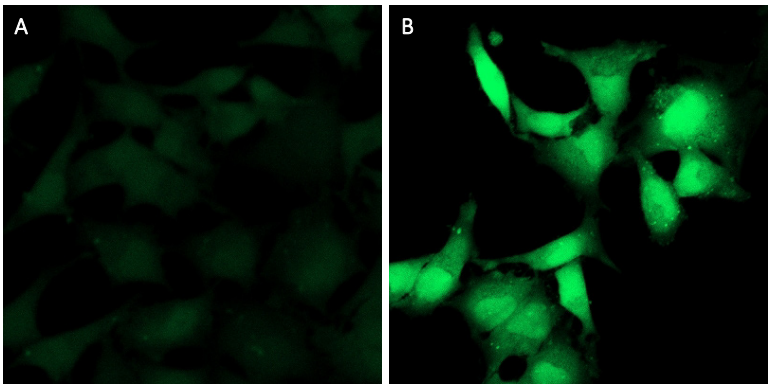


Fig.3 Detection of hydrogen sulfide using HSip-1 DA in HeLa cells treated with Na_2S.
(A: Control, B: 200 µmol/l Na_2S treated)

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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