GORYO CHEMICAL

For research use only

Updated: September 25, 2019

SSip-1 DA

Table 1. Product information

Catalog no.	Product name	Amount	Storage upon receipt	Stability
A402-1	SSip-1 DA	60 nmol × 3	≤−20°C, keep desiccated and protected from light.	1 year (when unopened and stored as described.)

1. About SSip-1 DA

Intracellularly abundant sulfane sulfur species including persulfide (R-S-SH), polysulfide (R-S-S$_n$-S-R) and polysulfide (H$_2$S$_n$) play important roles to maintain intracellular reducing environments. SSip-1 DA is a FRET-based fluorescence probe to specifically detect the intracellular sulfane sulfur species. It shows only week fluorescence without sulfane sulfur, and reversibly fluoresces in response to micromolar concentrations of sulfane sulfur. Its reaction is highly specific and has minimal to no reactivity with H$_2$S, cysteine residues, and sulfur oxides. SSip-1 DA, a cell permeable diacetylated (DA) form of SSip-1 is hydrolyzed by intracellular esterases to generate SSip-1 which retains within the cells. Thus it is suitable to monitor intracellular concentration of sulfane sulfur via live-cell imaging.

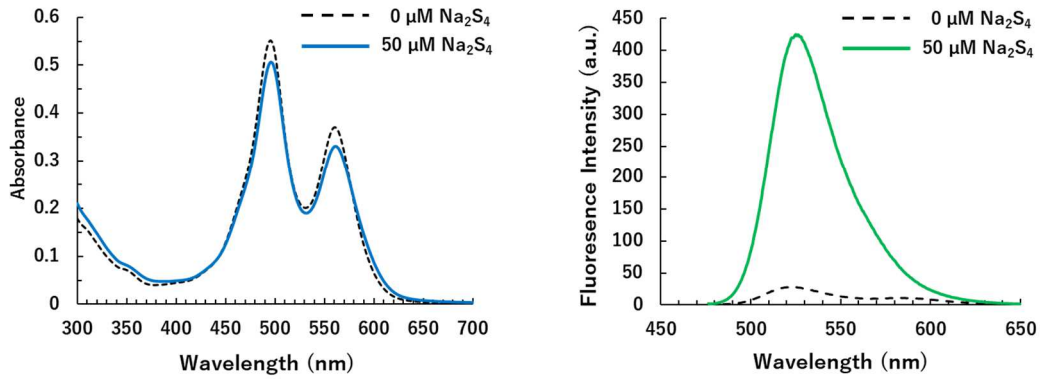
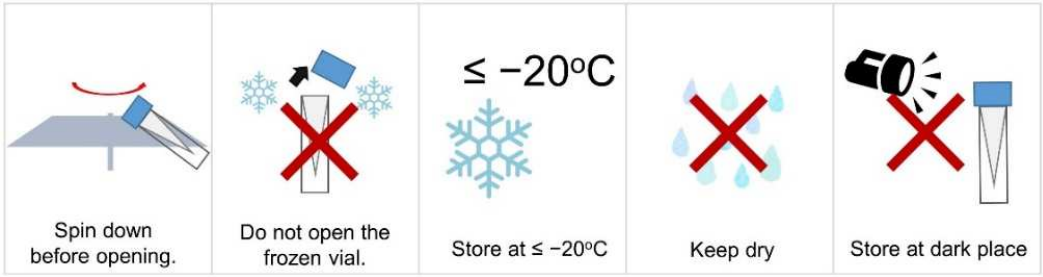


Figure 1. Absorbance (*left*) and fluorescence (*right*) of SSip-1 in 0.1 M phosphate buffer (pH=7.4) in the absence and presence of Na$_2$S$_4$.

■ Storage

Upon receipt, store the product desiccated and protected from light at ≤ −20°C. Storing as a solution is not recommended.



2. Preparation of reagent

■ Materials required but not provided

・Dimethyl sulfoxide (DMSO)
・A protein solution such as bovine serum albumin (BSA)
・Observation buffer (Hank's balanced salt solution (HBSS), etc.). It should be a solution without phenol red.

■ Preparation of reagent

SSip-1 DA is a violet solid. Before opening the cap, warm the vial to the room temperature. Then, use a micro-centrifuge to spin down the solid that might adhere on the cap. Add 60 µL of DMSO to one vial to prepare 1 mM solution. Finally, dissolve the solid entirely by pipetting for more than five times. SSip-1 DA solution will become red-violet.

※SSip-1 DA may aggregates in an aqueous solution. We recommend to dilute SSip-1 DA-DMSO solution with aqueous buffer solution containing proteins such as 1 mg/ml BSA.

3. An example of live cell imaging

■ Detection of intracellular sulfane sulfur in A549 cells

1. Prepare a cell-staining solution by diluting 1 mM SSip-1 DA solution to 10 µM with observation buffer containing 10 mg/mL BSA.
   ※ Fluorescence of SSip-1 DA is diminished by reduced glutathione (GSH). Intracellular GSH concentration is varied among different kinds of cells. Therefore, we recommend optimizing dye concentrations and incubation time in your conditions. In GORYO Chemical, Inc., incubating A549 cells with 1 µM dye solution at 37°C for 60 minutes gave good results.
   ※ We recommend paying attention to intracellular nutrition because the intracellular GSH concentration depends on the nutritional conditions.
2. Remove culture media from the dish and rinse twice with the observation buffer.
3. Add the cell-staining solution to the dish and incubate at 37°C for 60 minutes.
4. After staining, wash twice with the observation buffer.
5. Observe with a fluorescence microscope. You may induce the production of sulfane sulfur by adding Na_2S_4 to the final concentration of 5 µM.

■ Fluorescence observation

Use 488- or 495-nm laser for the laser microscopy. Maximum emission is detected at 525 nm. For fluorescence microscopy, blue-excitation filter sets for GFP or for FITC are appropriate.

■ References

See also the publications below, for other usages and protocols.

D. Ezeriņa, Y. Takano, K. Hanaoka, Y. Urano, T. P. Dick (2018) *Cell Chem. Biol.* **25**: 1–13

R. Miyamoto, S. Koike, Y. Takano, N. Shibuya, Y. Kimura, K. Hanaoka, Y. Urano, Y. Ogasawara, H. Kimura (2017) *Sci. Rep.*, **7**: 45995

Y. Takano, K. Hanaoka, K. Shimamoto, R. Miyamoto, T. Komatsu, T. Ueno, T. Terai, H. Kimura, T. Nagano, Y. Urano (2017) *Chem. Commun.*, **53**: 1064-1067

Table 2. Related Products

Catalog no	Material	Usage
A401-1	QuicGSH3.0	Quantification of intracellular glutathione.
SK1002-01	DAF-2 DA	Green fluorescence probe to detect intracellular nitric oxide (NO).
SK1004-01	DAF-FM DA	Green fluorescence probe to detect intracellular nitric oxide (NO) more than pH 6.
SK1006-01	DAR-4M AM	Orange fluorescence probe to detect intracellular nitric oxide (NO).
SK3001-01 SK3001-02	HPF	Fluorescence probe to detect hydroxyl radical ($^{\cdot}$OH) and peroxynitrite ($ONOO^-$).
SK3002-01 SK3002-02	APF	Fluorescence probe to detect hydroxyl radical ($^{\cdot}$OH), peroxynitrite ($ONOO^-$) and hypochlorous acid (HClO).
SK3003-01	NiSPY-3	Fluorescence probe to detect only peroxynitrite ($ONOO^-$).
GC3004-01	OxiORANGE™	Orange fluorescence probe to detect hydroxyl radical ($^{\cdot}$OH) and hypochlorous acid (HClO).
GC3006-01	HySOx	Fluorescence probe to detect only hypochlorous acid (HClO).
GC3007-01	HYDROP™	Fluorescence probe to detect only intracellular hydrogen peroxide (H_2O_2).