

Technical Manual (Japanese version) is available at <http://www.dojindo.co.jp/manual/sb07.pdf>

## General Information

It has been recognized that hydrogen sulfide (H<sub>2</sub>S) has an important role as a physiological active substance for vasodilation, cytoprotection, modulation of insulin secretion. Although H<sub>2</sub>S is considered as a gaseous molecule such as NO and CO, about 80% of the total sulfide exists as hydrogen sulfide anion (HS<sup>-</sup>) under physiological condition. In addition, H<sub>2</sub>S easily converts to various biochemical molecules such as persulfides and polysulfides, which react with sulfhydryl moieties in a living body. Therefore, the precise action mechanism of H<sub>2</sub>S has not been cleared.

Hydrogen sulfide donor 5a, 8l, and 8o developed by M. Xian *et al.*, release H<sub>2</sub>S upon reaction with reducing agents such as cysteine and glutathione *in vivo*<sup>1-3</sup>). The donors are stable in aqueous solutions, whereas they decompose and release H<sub>2</sub>S in blood, cells and tissues containing sulfhydryl molecules. These hydrogen sulfide donors are expected as useful materials for clarification of H<sub>2</sub>S roles *in vivo*.

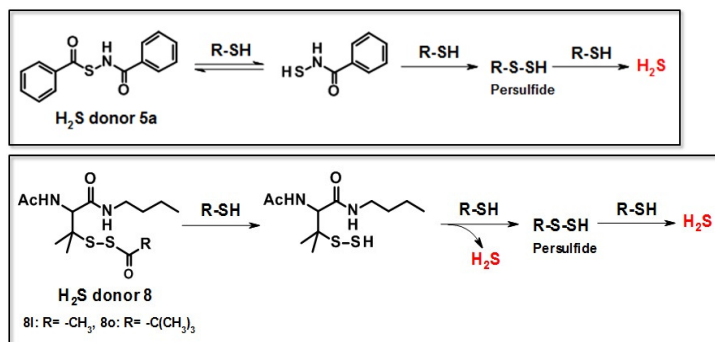


Fig. 1 H<sub>2</sub>S release from H<sub>2</sub>S donor 5a, 8l, 8o by reaction with thiol molecules

Contents	SB07	-SulfoBiotics-	H <sub>2</sub> S donor 5a	10 mg x 1
	SB08	-SulfoBiotics-	H <sub>2</sub> S donor 8l	10 mg x 1
	SB09	-SulfoBiotics-	H <sub>2</sub> S donor 8o	10 mg x 1

Storage Condition Store at 0-5 °C

Precaution These reagents release a toxic hydrogen sulfide gas by reaction with thiol molecules. Refer to the material safety data sheet (MSDS) before using the reagent.

## General Protocol

- 1) Dissolve H<sub>2</sub>S donor 10 mg in DMSO (5a: 1.95 ml, 8l: 1.56 ml, 8o: 1.38 ml) by pipetting to prepare 20 mmol/l H<sub>2</sub>S donor Stock Solution.  
\*Store at -20 °C and use within two months. Aliquot the solution for longer storage.
- 2) Dilute the 20 mmol/l H<sub>2</sub>S donor Stock Solution with a buffer or a medium to an appropriate concentration for your experimental condition.

## Experimental Example

- H<sub>2</sub>S release profile of H<sub>2</sub>S donor 5a, 8l, 8o by reaction with glutathione-

- 1) Ten microliter (10 μl) of 20 mmol/l H<sub>2</sub>S donor Stock Solution was added to 2 ml of PBS to prepare 100 μmol/l H<sub>2</sub>S donor (PBS) solution.
- 2) Glutathione was added to the 100 μmol/l H<sub>2</sub>S donor (PBS) solution to be a final concentration of 5 mmol/l in a screw top vial. Then, the solution was incubated at room temperature.
- 2) The amount of yielded hydrogen sulfide anion in the solution was quantified by methylene blue method.

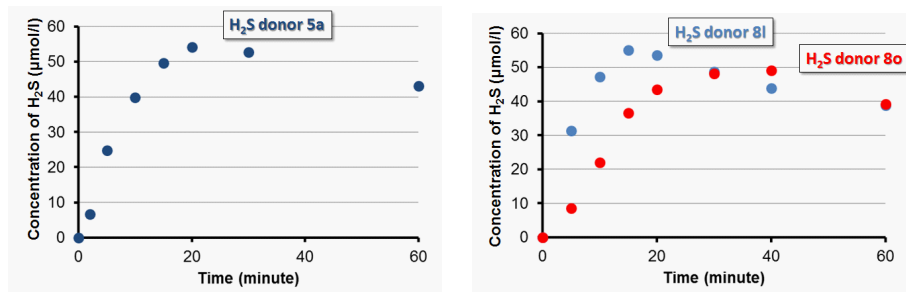


Fig. 2 H<sub>2</sub>S release profile of 100 μmol/l H<sub>2</sub>S donor 5a, 8l, 8o by reaction with 5 mmol/l glutathione

## References

- 1) Y. Zhao, H. Wang and M. Xian, "Cysteine-Activated Hydrogen Sulfide (H<sub>2</sub>S) Donors", *J. Am. Chem. Soc.*, **2011**, 133, 15.
- 2) C-T. Yang, Y. Zhao, M. Xian, J-H. Li, Q. Dong, H-B. Bai, J-D. Xu, M-F. Zhang, "A Novel Controllable Hydrogen Sulfide-Releasing Molecule Protects Human Skin Keratinocytes Against Methylglyoxal-Induced Injury and Dysfunction", *Cell. Physiol. Biochem.*, **2014**, 34, 1304.
- 3) Y. Zhao, S. Bhushan, C. Yang, H. Otsuka, J. D. Stein, A. Pacheco, B. Peng, N. O. Devarie-Baez, H. C. Aguilar, D. J. Lefer and M. Xian, "Controllable Hydrogen Sulfide Donors and Their Activity against Myocardial Ischemia-Reperfusion Injury", *Chem. Biol.*, **2013**, 8, 1283.

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