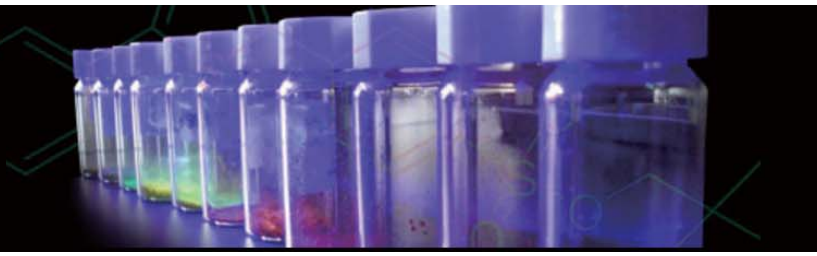
GORYO CHEMICAL

Fluorescent Probe
Application Note
HySOx



HySOx specifically detects HOCl

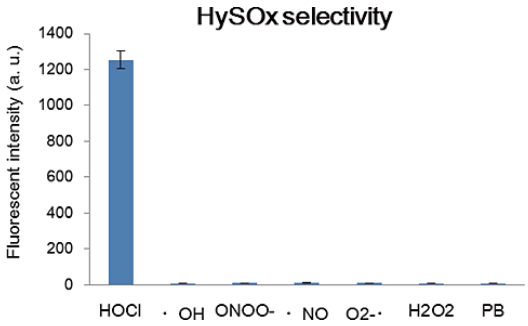


Figure 1. Reactivity of HySOx to various ROS

Fluorescence intensity of HySOx increases up to ~170-fold when it reacts with hypochlorous acid. Other ROS (superoxide, peroxynitrite, nitric oxide, singlet oxygen, and hydrogen peroxide) do not increase the fluorescent intensity of HySOx under identical conditions.

Measurement conditions

Fluorescent intensities of 5 µM HySOx were measured after the addition of various ROS in 0.1 M sodium phosphate buffer (pH 7.4) containing 0.1% DMF as a cosolvent.

Fluorescent intensities were measured at 574 nm, with excitation at 553 nm, slit width 2.5 nm, photon multiplier voltage 700V, using HITACHI F-2700 Fluorescence Spectrophotometer.

ROS generating conditions

HOCl: 5 µM NaOCl
$^{\bullet}$OH: 50 µM $Fe(ClO_4)_2$, 100 µM H_2O_2
$ONOO^-$: 5 µM HOONO
$^{\bullet}$NO: 5 µM NOC18
$O_2^{-\bullet}$: 10 µM KO_2
H_2O_2: 100 µM H_2O_2
PB: 0.1 M Sodium phosphate buffer (pH 7.4) as a control

Dose-dependent changes in the spectrum and intensity of HySOx fluorescence

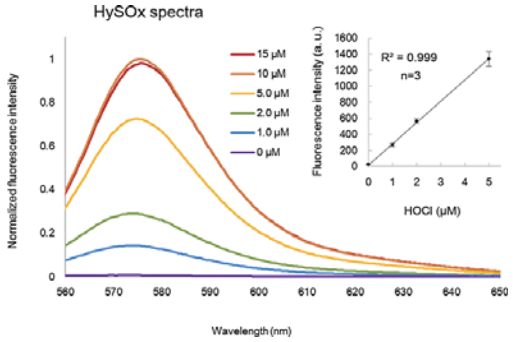


Figure 2. Fluorescent spectrum changes of 5 µM HySOx solution in various concentrations of hypochlorous acid.

The reaction of HySOx with hypochlorous acid saturates at the molar ratio of 1:2.
Inset, fluorescent intensities of HySOx against hypochlorous acid concentrations.

Measurement conditions

Fluorescent spectra of 5 µM HySOx were measured after the addition of hypochlorous acid (0-15 µM) in 0.1 M sodium phosphate buffer at pH 7.4 containing 0.1 % DMF as a cosolvent.

Inset, fluorescent intensities of 5 µM HySOx measured at 574 nm with 0-5 µM of incremental hypochlorous acid concentrations. The solutions were excited at 553 nm with slit width 2.5mm, and measured with photon multiplier voltage of 700V, using HITACHI F-2700 Fluorescence Spectrophotometer.

An application example

Observation of hypochlorous acid production from U937 cell during phagocytosis

1. Induce differentiation of U937 cell, by incubation in the presence of 25 nM PMA for 24 h followed by 24 h incubation without PMA until observation.
2. Dissolve HySOx 1 vial (20 µg) in 51.5 µL DMF to prepare 1 mM solution.
3. Dilute the DMF stock solution with washing buffer or culture media to 5 µM cell staining solution.
4. Add the cell staining solution to the dish and incubate at 37°C for 30 min.
5. Remove the culture medium on the culture dish and wash twice with HBSS.
6. Replace the buffer to new HBSS, and induce the phagocytosis by the addition of ZymosanA, and quickly start the time-lapse imaging.

※ Cells were treated with 50 µg/ml ZymosanA in this test.
※ Microscope: Leica DMI 6000 CS, objective lens: 40×
※ In our test, the fluorescent signal appeared in 10 minutes, and increased after 30 minutes.

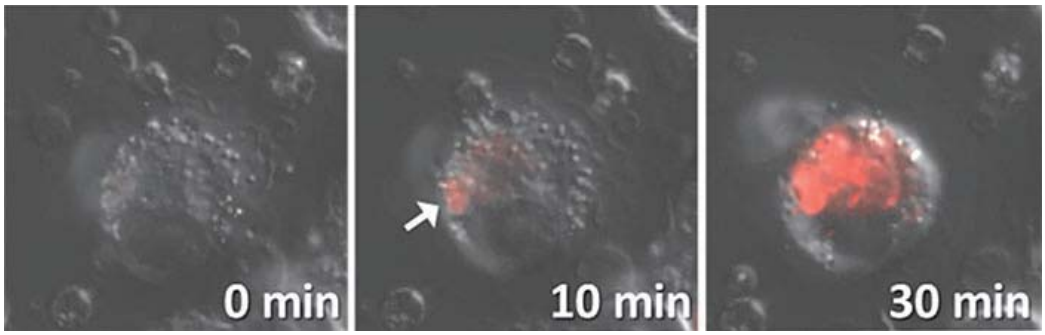


Figure 3. Hypochlorous acid production during phagocyte of ZymosanA by U937 cell

(Left) Immediately after an addition of ZymosanA to U937 cell
(Middle) 10 minutes after the addition of ZymosanA. U937 cell engulfed the ZymosanA and started to show red fluorescent signal (arrow).
(Right) 30 minutes after the addition of ZymosanA. Phagocytosis was completed. Strong red fluorescent signal indicates the production of hypochlorous acid in the cell.

YouTube

U937 cell
https://www.youtube.com/watch?v=PAyBEwesV-Y

RAW264.7 cell
https://www.youtube.com/watch?v=HFGghuqAwCQ

References

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GORYO Chemical, Inc.

Sapporo Head Office EAREE BLDG 5F, Kita 8, Nishi 18-35-100, Chuo-ku, Sapporo, 060-0008 Japan
TEL: +81-11-214-9422 FAX: +81-11-351-1822

Tokyo Office University of Tokyo Entrepreneur Plaza Hongo 7-3-1, Bunkyo-ku, Tokyo, 110-0033, Japan
TEL: +81-3-6240-0781 FAX: +81-3-6745-9204

E-mail info_itnl@goryochemical.com
URL http://goryochemical.com/english/