GORYO CHEMICAL

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For research use only

HySOx

Table 1. Product information

Catalog no.	Product name	Amount	Storage upon receipt	Stability
GC3006-01	HySOx	20 µg × 5	≤−20°C, keep desiccated and protected from light.	1 year (when unopened and stored as described.)

1. About HySOx

HySOx is a fluorescent probe that shows excellent selectivity against hypochlorous acid under physiological environments. Hypochlorous acid is one of highly reactive oxygen species (hROS). ROS are produced by neutrophils and macrophages during phagocytosis or after stimulation with a wide variety of agents. This product can detect hypochlorous acid in living cells, and is suitable for time-lapse imaging because of its fast reactivity and slow photobleaching.

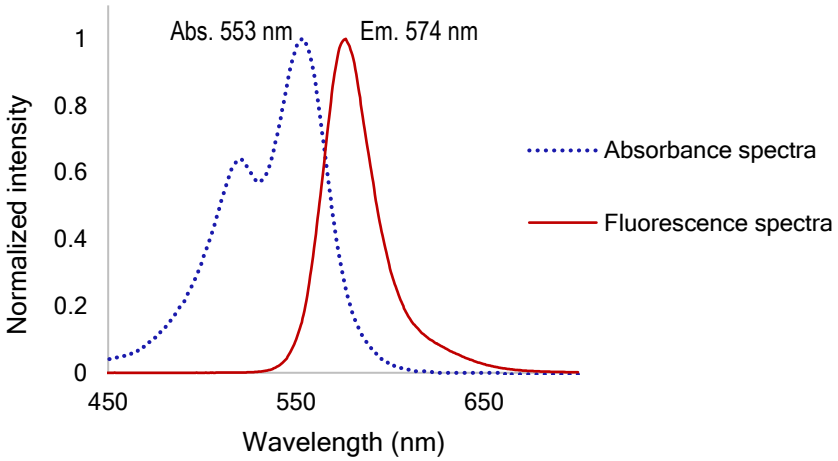

Figure 1. Fluorescence excitation and emission spectra of the oxidized HySOx in phosphate buffer (pH 7.4)

2. An example of live cell imaging

■ **Materials required but not provided**

・N,N-dimethylformamide (DMF)

・Observation buffer (1×PBS pH 7.4, Hank's Balanced Salt Solution (HBSS), Krebs-Ringer phosphate (KRP) buffer, etc. Solutions lacking phenol red may improve imaging.)

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■ Procedure

① Dissolve HySOx 1 vial (20 µg) in 51.5 µL DMF to prepare 1 mM solution.
※ Molecular weight is described on the label and exterior pouch.

② Dilute the 1 mM HySOx solution with the observation buffer or culture media to 5 µM (Cell staining solution).
※ Optimization of the dye concentration and the incubation time are required. In GORYO Chemical, Inc., incubation in 5 µM dye at 37°C for 30 minutes gave good results for human macrophage-like cell line (U937 cells, 10^6 cells/ml), mouse macrophage-like cell line (RAW264.7 cells, 0.5-1 x 10^5 cells/ml or 60-80% of confluent) and pig neutrophils.

③ Remove the culture medium on the dish and wash twice with the observation buffer or culture medium.
※ We recommend using glass bottom dish suitable for fluorescence microscopy.

④ Add the cell staining solution to the dish and incubate at 37°C for 30 min.

⑤ After the staining, wash 2-3 times with the observation buffer.

⑥ Induce the phagocytosis by the addition of ZymosanA (ex. 50 µg/ml), and quickly start the time-lapse imaging.
※ In our test, the fluorescent signal appeared in 10 minutes, and increased after 30 minutes.

■ Fluorescent observation

Use 532 or 543 nm light source for excitation. The wavelength of the maximum emission is 574 nm. Dichroic mirror cubes for Cy3 or equivalent fluorophores (ex. Cy3, G-2A, G-2E/C, and TRITC for Nikon; U-FGWA, U-FRFP, and U-FGNA for Olympus) are recommended.

■ Storage

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

Table 2. Related Products

Catalog no.	Product name	Major applications
SK3001-01	HPF	Detection of hydroxyl radical ($^{\cdot}OH$) and peroxynitrite ($ONOO^-$).
SK3002-01	APF	Detection of hydroxyl radical ($^{\cdot}OH$), peroxynitrite ($ONOO^-$) and hypochlorous acid (HOCl).
SK3003-01	NiSPY-3	Detection of peroxynitrite ($ONOO^-$).
GC301	AcidiFluor™ ORANGE	A pH sensitive probe. Detection of acidic organelles in living cells.
GC901	FeRhoNox™-1	Detection of ferrous ions (Fe^{2+}) in Golgi.

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