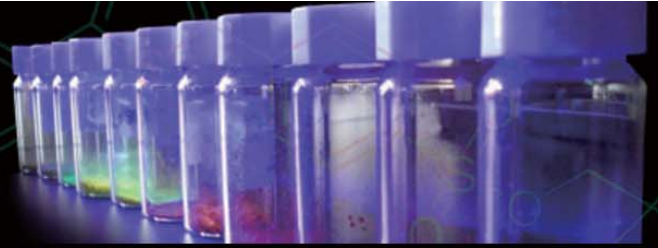


Fluorescent Probe Application Note HYDROP



HYDROP specifically detects H_2O_2

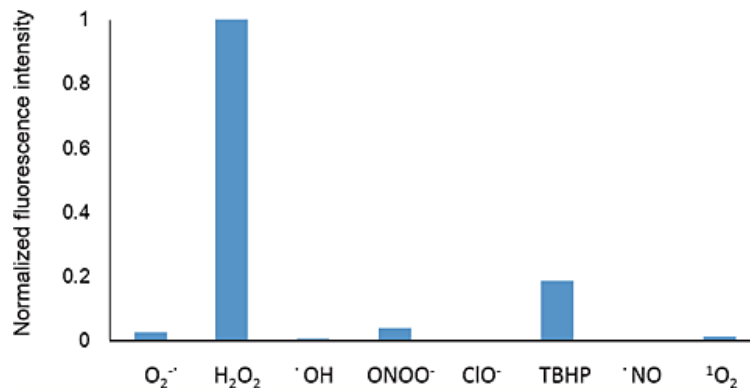


Figure 1. Reaction of HYDROP to various ROS

Fluorescence increase of hydrolyzed HYDROP is observed by the addition of hydrogen peroxide at physiological pH of around 7.4 or higher.

Fluorescence intensity of 10 μM hydrolyzed HYDROP after addition of various ROS (final conc. 50 μM) in 0.1 M sodium phosphate buffer at pH 7.4 containing 0.1 % DMF as a cosolvent.

Fluorescence intensities were measured at 490 nm, with excitation at 520 nm.

ROS generating system

$O_2^{\bullet-}$: KO_2

H_2O_2 : H_2O_2 , 37°C, 60 min

$\bullet OH$: $Fe(ClO_4)_2 : H_2O_2 = 10:1$, 37°C, 60 min

$ONOO^-$: $ONOO^-$, 25°C, 5 min

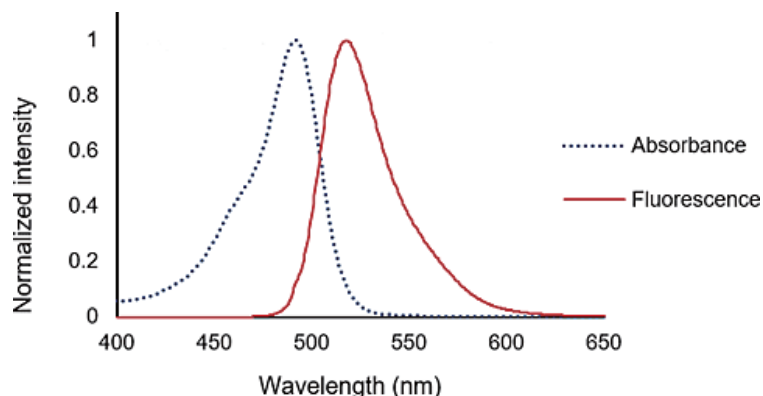
ClO^- : NaOCl, 25°C, 5 min

TBHP: tert-Butyl hydroperoxide

$\bullet NO$: NOC13, 37°C, 30 min

1O_2 : EP-1, 37°C, 30 min

Spectra

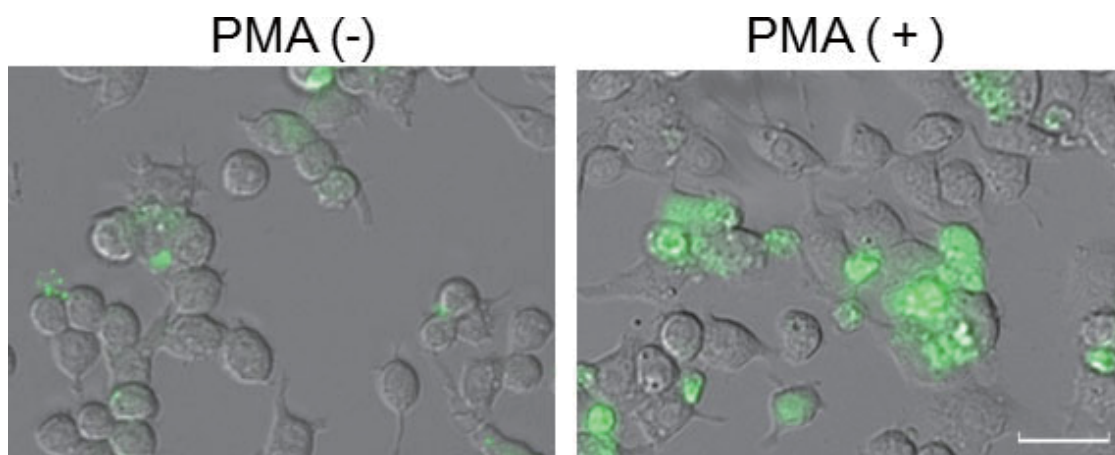


Fluorescence intensity of 5 μM HYDROP after addition of 50 μM hydrogen peroxide in 0.1 M sodium phosphate buffer at pH 7.4 containing 0.1 % DMF as a cosolvent.

Absorbance intensities were measured with slit width 1.5 nm using HITACHI U-2910 Spectrophotometer.

Fluorescence intensities were measured 518 nm with excitation at 492 nm, slit width 2.5 mm, photon multiplier voltage 700 V, using HITACHI F-2700 Fluorescence Spectrophotometer.

Images



Visualization of hydrogen peroxide production by RAW 264.7 cells (1×10^5 cell/ml) using HYDROP. Green fluorescent signal is overlaid to DIC image. Hydrogen peroxide production was induced by the addition of $0.001 \mu\text{g/ml}$ phorbol myristate acetate (PMA). Cells were stained with $1 \mu\text{M}$ HYDROP for 20 min. Bar, $25 \mu\text{m}$.

Reference

Masahiro Abo, Yasuteru Urano, Kenjiro Hanaoka, Takuya Terai, Toru Komatsu, and Tetsuo Nagano
J. Am. Chem. Soc., 2011, 133 (27), pp 10629–10637 doi: 10.1021/ja203521e



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/>

