GORYO CHEMICAL

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For research use only

HYDROP™, HYDROP-EX™

Table 1. Product information

Catalog no.	Material	Amount	Storage upon receipt	Stability
GC3007-01	HYDROP	30 nmol × 3	≤−20°C, keep desiccated and protected from light.	1 year (when unopened and stored as described.)
GC3008-01	HYDROP-EX	30 nmol × 3		

1. About HYDROP and HYDROP-EX

HYDROP and HYDROP-EX are fluorescent probes to specifically detect hydrogen peroxide (H_2O_2), one of the reactive oxygen species (ROS). These are initially non-fluorescent but fluoresce upon reaction with hydrogen peroxide in physiological conditions.

HYDROP is a probe to specifically detect intracellular H_2O_2. It is diacetylated and highly cell-permeable, but not reactive to H_2O_2 before entering into cells. After hydrolysis of diacetyl groups by intracellular esterases, it becomes highly reactive to H_2O_2.

Cell impermeable HYDROP-EX is suitable to detect or quantify extracellular H_2O_2 or that in solutions

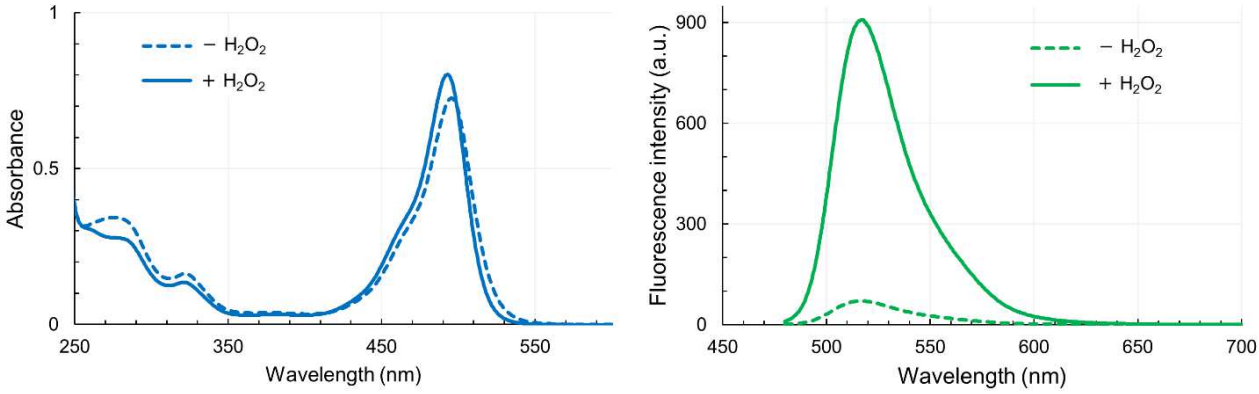
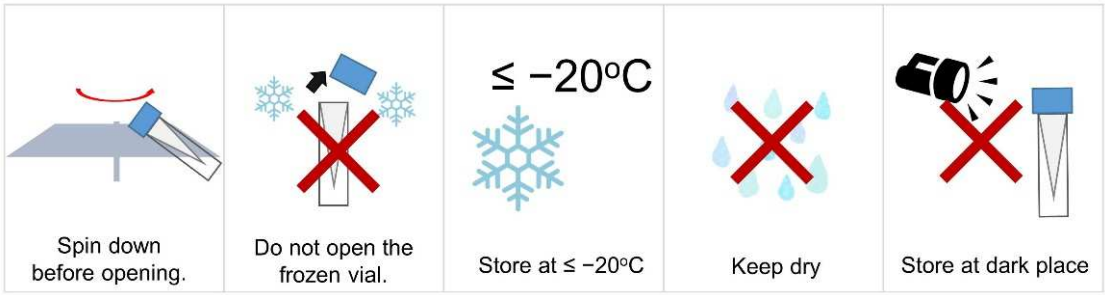


Figure 1. Absorbance of 10 μM HYDROP-EX (*left*) and fluorescence of 0.5 μM HYDROP-EX (*right*) in 0.1 M phosphate buffer (pH=7.4).

■ Storage

Probes are shipped at room temperature in a nitrogen gas-filled vial. Upon receipt, store the product desiccated and protected from light at ≤ −20°C. Storing the reagent as a solution is not recommended.



2. Preparation of reagents

Prepare N,N-dimethylformamide (DMF) as a solvent.

HYDROP is a colorless solid, HYDROP-EX is an orange-colored solid. Before opening the cap, warm the vial to the room temperature and use micro-centrifuge to spin down the solid that might be adhered on the cap. Add 30 µL of DMF to one vial to prepare 1 mM solution. Dissolve the solid completely by pipetting for more than five times. HYDROP solution will be colorless, whereas HYDROP-EX solution will be orange.

3. Quantification of H_2O_2 in solutions using HYDROP-EX

1. Prepare hydrogen peroxide (H_2O_2) solutions to generate a standard curve. Commercially available 30−35% H_2O_2 solution is about 10 M. Dilute the solution 1000 times with pure water and measure absorbance at 240 nm (*A240*).
2. Concentration of the H_2O_2 can be obtained by the equation: $C = A240 / \varepsilon \times \text{dilution factor}$ (M), whereas ε = 43.6 ($M^{-1}cm^{-1}$) is the molar extinction coefficient of H_2O_2.
3. Dilute the H_2O_2 to the final concentration of 0−100 µM using a solution. The diluting solution condition should be equivalent to that in which you measure H_2O_2 concentrations. Add HYDROP-EX to final concentration of 1−10 µM, react at 37ºC for 1 hour. These concentrations and reaction conditions should be adjusted to the expected conditions to be measured.
4. Measure fluorescence intensities using a fluorescence spectrometer or a fluorescence microplate reader. Before the measurement, dilute the solution to make the final HYDROP-EX concentration to be <1 µM, or absorbance of the solution at 490 nm should be less than 0.1. We recommend to set the excitation wavelength within 470−490 nm and emission wavelength to be 520−530 nm.
5. Plot the measured fluorescence intensity (I) against H_2O_2 concentration (C) and fit using the following equation,
$$I = \frac{aC}{k - C} + b$$
to obtain the parameters, a, b, and k.
6. Add the same concentration of HYDROP-EX to the solution you would like to measure, mix well and incubate in the same conditions (temperature and time). After the incubation, measure fluorescence.
7. Obtain H_2O_2 concentrations using the following equation,
$$C = \frac{I - b}{I + a - b}k$$

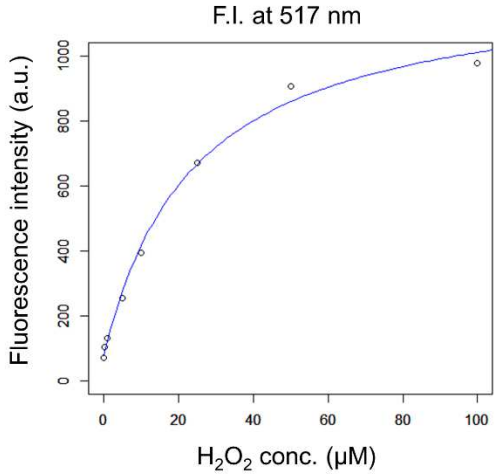


Fig. 2. An example standard curve of HYDROP-EX.

4. Detection of intracellular H_2O_2 with HYDROP

1. Dilute the 1 mM HYDROP solution with observation buffer or culture media to 1−5 µM (Cell staining solution).

※ We recommend to optimize the dye concentration and the incubation time. In GORYO Chemical, incubation in 5 µM dye at 37°C for 20 min gave good results for HeLa cells (human cervical cancer cell line), A431 cells (human epidermis carcinoma cell line), and 1 µM dye at 37°C for 20 min for RAW276.4 cells (mouse macrophage-like cell lines).

2. Remove the culture medium on the dish and wash twice with the observation buffer or the culture medium.
3. Add the cell staining solution to the dish and incubate at 37°C for 20 min.
4. After the staining, wash 2 times with the observation buffer.
5. Induce the production of hydrogen peroxide by the addition of PMA and start microscope observation.
   A dose response curve with PMA and vehicle (blank/control solution without PMA) is recommended.

※ We detected the fluorescence signal by microscope observation, 30 min after stimulation.

■ Fluorescence observation

Use 488 nm blue light for excitation. Maximum emission is observed at 516 nm. For fluorescence microscopy, blue-excitation filter sets for GFP or FITC is appropriate.

Table 2. Related Products

Catalog no.	Material	Usage
SK3001-1 SK3001-2	HPF	Specific fluorescence probe to detect hydroxyl radical ($^{\cdot}$OH) and peroxynitrite ($ONOO^-$).
SK3002-01 SK3002-02	APF	Specific fluorescence probe to detect hydroxyl radical ($^{\cdot}$OH), peroxynitrite ($ONOO^-$) and hypochlorous acid (HClO).
SK3003-01	NiSPY-3	Specific fluorescence probe to detect only peroxynitrite ($ONOO^-$).
GC3006-01	HySOx	Specific fluorescence probe to detect only hypochlorous acid (HClO).
GC301	AcidiFluor™ ORANGE	A fluorescence probe to detect acidic organelles in living cells
GC901	FeRhoNox™-1	A fluorescence probe to detect ferrous ion (Fe^{2+}) in Golgi.
A401-1	QuicGSH3.0	Quantification of intracellular glutathione.

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