

HPF

Fluorescent probe for hROS detection

Cell permeable fluorescent reagent (Ex(max): 490nm; Em(max): 515nm) for the detection of highly reactive oxygen species (hROS). Immediately reacts with hROS such as hydroxyl radical and peroxynitrite, and the fluorescence intensity greatly increases. In addition, peroxynitrite can be detected in distinction from nitric oxide and superoxide since HPF does not react with nitric oxide, superoxide and hydrogen peroxide. Moreover, HPF is resistant to light-induced autooxidation. HPF does not react with hypochlorite (OCl^-) either and thus can be used in combination with APF (Prod. No. ALX-620-075) which detects OCl^- to elucidate reliably the roles of OCl^- in biological systems such as neutrophils. Moreover, HPF is resistant to light-induced autooxidation. *Not for sale in Japan.*

Citations: 3

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Ordering Information

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ALX-620-074-M001	1mg
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Manuals, SDS & CofA

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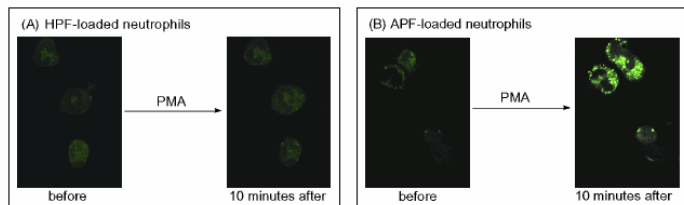


Figure 4: Fluorescence images of HPF- or APF-loaded neutrophils. The fluorescence intensity of HPF-loaded neutrophils was not changed upon stimulation with PMA whereas that of APF-loaded neutrophils greatly increased. This suggests that $^{\cdot}\text{OCI}$ produced by MPO (myeloperoxidase) upon stimulation with PMA could be selectively visualized in distinction from other ROS.

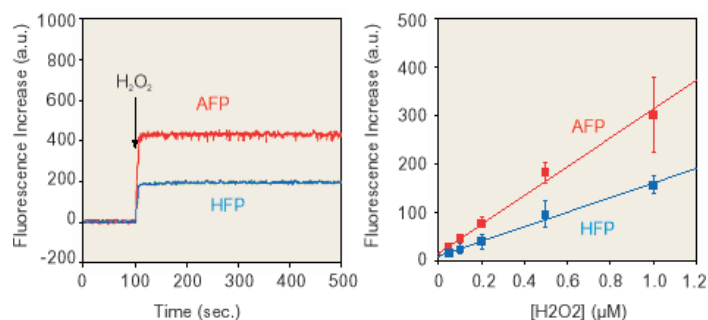


Figure 5: Detection of hROS in the HRP/H₂O₂ system using HPF and APF. The fluorescence intensity increased immediately upon the addition of H₂O₂. Further, it was found that HPF and APF could detect hROS generated in the HRP/H₂O₂ system in a dose-dependent manner.

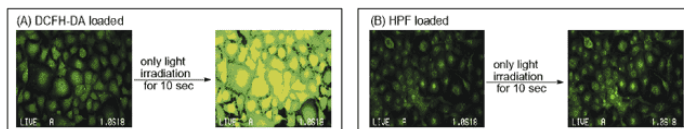


Figure 3: Light-induced autoxidation. Compared with DCFH, HPF and APF were hardly autoxidized by light irradiation.

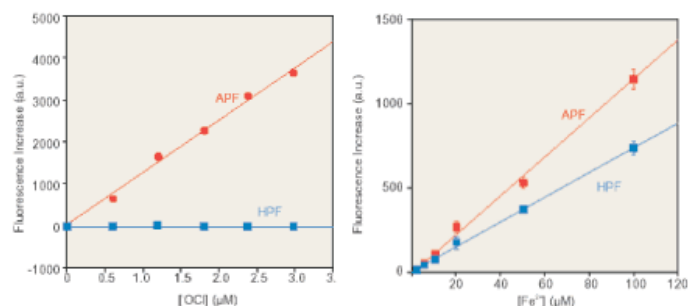


Figure 2: HPF and APF enable the selective detection of highly reactive oxygen species and they are hardly autoxidized by light irradiation. Left panel: detection of $^{\cdot}\text{OCI}$. Right panel: detection of $^{\cdot}\text{OH}$ generated by the Fenton reaction.

ROS	HPF	APF	DCFH
$\cdot\text{OH}^a$	730	1200	7400
ONOO^{-b}	120	560	6600
OCI^c	6	3600	86
$^1\text{O}_2^d$	5	9	26
$\text{O}_2^{\cdot e}$	8	6	67
H_2O_2^f	2	<1	190
NO^g	6	<1	150
$\text{ROO}^{\cdot h}$	17	2	710
Autoxidation ⁱ	<1	<1	2000

Figure 6: Fluorescence probe reagents were added to sodium phosphate buffer (0.1M, pH 7.4) (final 10 μM ; 0.1% DMF as a cosolvent). The fluorescence intensities of HPF, APF and DCFH were measured at excitation wavelength of 490, 490 and 500nm and fluorescence emission wavelength of 515, 515 and 520nm, respectively.

^a 100 μM of Ferrous perchlorate (II) and 1mM of H_2O_2 were added.

^b 3 μM (final) of ONOO^- was added.

^c 3 μM (final) of NaOCl was added.

^d 100 μM of 3-(1,4-Dihydro-1,4-epidioxo-1-naphthyl)propionic acid was added.

^e 100 μM of KO_2 was added.

^f 100 μM of H_2O_2 was added.

^g 100 μM of 1-Hydroxy-2-oxo-3-(3-aminopropyl)-3-methyl-1-triazene was added.

^h 100 μM of 2,2

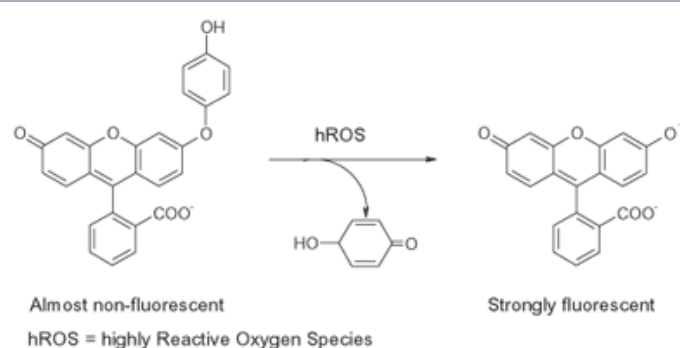
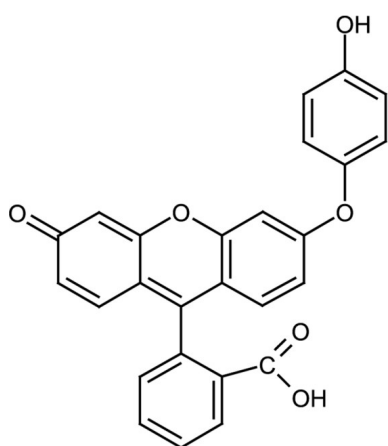


Figure 1: Reaction of Hydroxyphenyl Fluorescein (HPF) with hROS.



Handling & Storage

Use/Stability	As indicated on product label or CoA when stored as recommended. Prepare 500-5'000-fold dilution (~10-1µM) in phosphate buffer (0.1M phosphate, pH 7.4) immediately before use. BSA, phenol red and amines may affect the fluorescence and must be used with caution. Do not store the dilutions.
Handling	After opening, prepare aliquots and store at +4°C. Protect from light. Keep under inert gas.
Long Term Storage	+4°C
Shipping	Blue Ice

Regulatory Status

RUO - Research Use Only

Product Details

Alternative Name	Hydroxyphenyl fluorescein, 2-[6-(4'-Hydroxy)phenoxy-3H-xanthen-3-on-9-yl]benzoic acid
Appearance	Pale yellow.
CAS	359010-69-8
Couple Target	ROS
Couple Type	Ligand
Formula	$C_{26}H_{16}O_6$
Formulation	Dissolved in 0.47ml dimethylformamide.
MW	424.4
Purity	≥98% (HPLC)

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