# 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 View Online »

## Ordering Information Order Online »

| | |
|---|---|
| **ALX-620-074-M001** | 1mg |

## Manuals, SDS & CofA View Online »

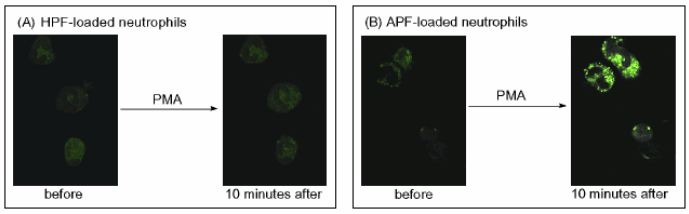



**Figure 4:** Fluorescence images of HPF- or APF-loaded neutrophils. The fluorescence intensity of HPF-loaded neutrophils was not changed upon stimulation with PMA whereas that of APF-loaded neutrophils greatly increased. This suggests that $^{-}$OCl produced by MPO (myeloperoxidase) upon stimulation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

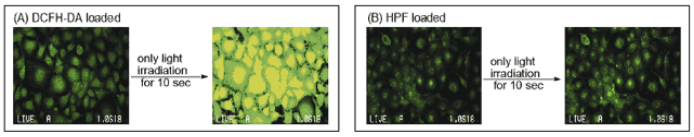



**Figure 3:** Light-induced autoxidation. Compared with DCFH, HPF and APF were hardly autoxidized by light irradiation.

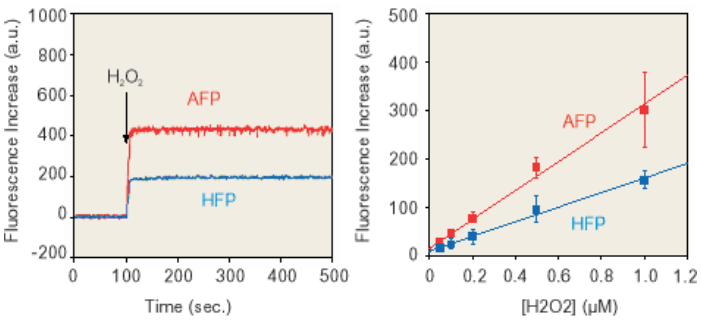



**Figure 5:** Detection of hROS in the HRP/$H_2O_2$ system using HPF and APF. The fluorescence intensity increased immediately upon the addition of $H_2O_2$. Further, it was found that HPF and APF could detect hROS generated in the HRP/$H_2O_2$ system in a dose-dependent manner.

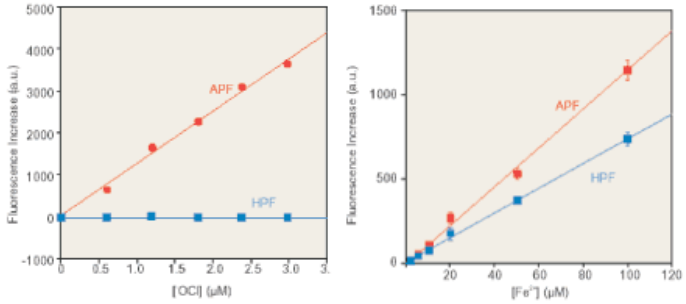



**Figure 2:** HPF and APF enable the selective detection of highly reactive oxygen species and they are hardly autoxidized by light irradiation. Left panel: detection of $^{-}$OCl.Right panel: detection of $^{\bullet}$OH generated by the Fenton reaction.

| ROS | HPF | APF | DCFH |
|---|---|---|---|
| $^{\cdot}OH^{a}$ | 730 | 1200 | 7400 |
| $ONOO^{-b}$ | 120 | 560 | 6600 |
| $^{-}OCl^{c}$ | 6 | 3600 | 86 |
| $^{1}O_2{}^{d}$ | 5 | 9 | 26 |
| $O_2^{\cdot-e}$ | 8 | 6 | 67 |
| $H_2O_2{}^{f}$ | 2 | <1 | 190 |
| $NO^{g}$ | 6 | <1 | 150 |
| $ROO^{\cdot h}$ | 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 10μM; 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] 100μM of Ferrous perchlorate (II) and 1mM of $H_2O_2$ were added.
[b] 3μM (final) of $ONOO^{-}$ was added.
[c] 3μM (final) of NaOCl was added.
[d] 100μM of 3-(1,4-Dihydro-1,4-epidioxy-1-naphthyl)propionic acid was added.
[e] 100μM of $KO_2$ was added.
[f] 100μM of $H_2O_2$ was added.
[g] 100μM of 1-Hydroxy-2-oxo-3-(3-aminopropyl)-3-methyl-1-triazene was added.
[h] 100μM of 2,2

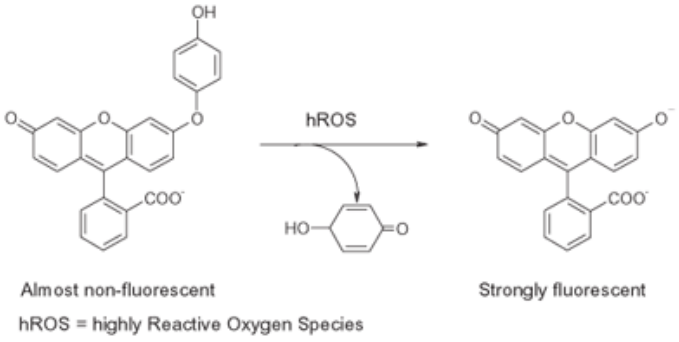
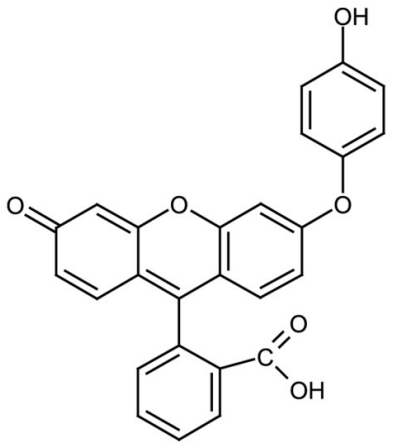



**Figure 1**: Reaction of Hydroxyphenyl Fluorescein (HPF) with hROS.



## Handling & Storage

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|---|---|
| **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) |

**Last modified: May 29, 2024**

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