

# Hydroxyphenyl Fluorescein (HPF) Aminophenyl Fluorescein (APF)

Table 1. Product information

Code.	Product name	Property	Amount	Storage upon receipt	Stability
SK3001-01	Hydroxyphenyl Fluorescein (HPF)	5 mM DMF solution	0.47 mL (contains 1 mg HPF)	≤ 4°C, protect from light and keep away from fire.	1 year (when unopened and stored as described.)
SK3001-02	Hydroxyphenyl Fluorescein (HPF)	solid	1 mg	≤ -20°C, keep desiccated and protected from light.	
SK3002-01	Aminophenyl Fluorescein (APF)	5 mM DMF solution	0.47 mL (contains 1 mg APF)	≤ 4°C, protect from light and keep away from fire.	
SK3002-02	Aminophenyl Fluorescein (APF)	solid	1 mg	≤ -20°C, keep desiccated and protected from light.	

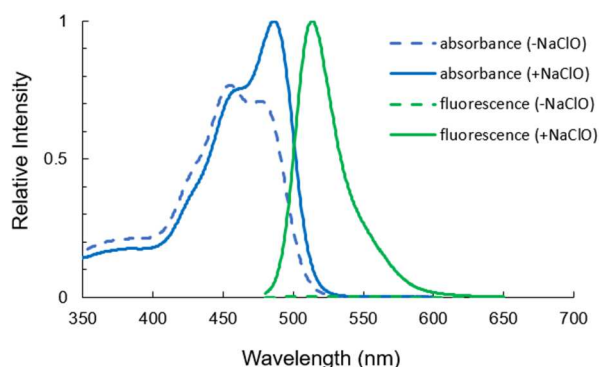
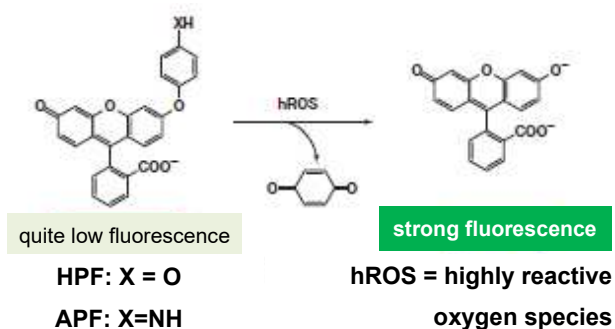
Reagents of DMF solution will be shipped by either in dry-ice or in blue-ice.

## 1. Features

- The reagents can detect highly reactive oxygen species (hROSs) including hydroxy radicals ( $\cdot\text{OH}$ ) and peroxynitrite ( $\text{ONOO}^-$ ). They do not react with  $\text{O}_2$ ,  $\text{H}_2\text{O}_2$ ,  $^1\text{O}_2$ , and  $\text{NO}$ .
- APF can also detect hypochlorous acid ( $\text{HOCl}$ ). In contrast, HPF has low reactivity with  $\text{HOCl}$ .
- Reliable detection of hROSs because of the lower level of autooxidation.
- Suitable for live cell imaging.

## 2. Principle of the hROS detection

APF and HPF have almost no fluorescence in neutral physiological aqueous solutions and fluoresce after reaction with hROSs. The reaction generates fluorescein (excitation maximum at 490 nm, emission maximum at 515 nm) which can be observed with fluorescence microscope, etc.



### 3. Usage

For solid reagents (SK3001-02, SK3002-02), prepare N,N-dimethylformamide (DMF) as a solvent. Dimethyl sulfoxide (DMSO) is not appropriate because it is known as a quencher of hydroxyl radicals which perturbs the detection and compromises sensitivity. Warm the vial to the room temperature and add 470  $\mu$ L of DMF to 1 mg solid and mix well to prepare 5 mM solution. Liquid reagents (SK3001-01, SK3002-01) are shipped as a solution in DMF. Dilute the DMF solution with physiological buffer solution (ex. 0.1 M phosphate buffer pH 7.4) to prepare 1 – 10  $\mu$ M solution just before use. Appropriate concentration and reaction condition varies depending on your purpose.

The reagents become yellowish color when diluted in neutral aqueous buffer. This solution has almost no fluorescence. After the reaction with hROSs, fluorescence can be detected by fluorescence microscopy. Blue excitation filters for FITC, GFP, etc. can be used. 488 nm laser excitation is also appropriate

### 4. References

K. Setsukinai, Y. Urano, K. Kakinuma, H. J. Majima and T. Nagano (2003) *J. Biol. Chem.* **278**, 3170-3175

Many research reports using these reagents have been published. Please refer to our website:

<http://www.goryochemical.com/english/products/rosfluor/hpf-apf.html>

### 5. Precautions

- Dilute solutions in an aqueous solution just before use. The reagents can be oxidized after the opening the vial and we provide no warranty for the reagents stored after the opening the cap.
- Use buffer solutions of pH 7.0-7.5. Bovine serum albumin and phenol red may interfere with the measurements.
- DMF in SK3001-01, SK3002-01 is flammable. Keep fire away and store in accordance with legal regulations.

Table 2. Related Products

Catalog no.	Product name	Major applications
GC3004-01	OxiORANGE™	Detection of hROSs ( $\cdot$ OH, HOCl) with orange fluorescence.
GC3006-01	HySOx	Specific detection and live cell imaging of hypochlorous acid (HOCl).
GC3007-01	HYDROP™	Specific detection of intracellular hydrogen peroxide ( $H_2O_2$ ).
SK3003-01	NiSPY-3	Specific detection of peroxynitrite ( $ONOO^-$ ).
GC901	FeRhoNox™-1	Detection of ferrous ions ( $Fe^{2+}$ ) in Golgi.
SK2001-01	ZnAF-2	Detection of $Zn^{2+}$ ions.
SK2002-01	ZnAF-2 DA	For live cell imaging of $Zn^{2+}$ ions.