Indicators for Highly Reactive Oxygen Species

A36003 3′-(p-aminophenyl) fluorescein (APF) *5 mM solution in DMF*
H36004 3′-(p-hydroxyphenyl) fluorescein (HPF) *5 mM solution in DMF*

Quick Facts

Storage upon receipt:

- 2–6°C

Ex/Em of reaction product: 490/515 nm

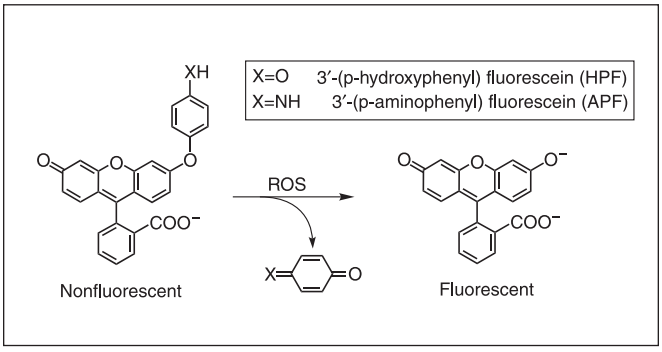

Figure 1. Nonfluorescent hydroxyphenyl fluorescein (HPF) and aminophenyl fluorescein (APF) become fluorescent in the presence of a reactive oxygen species (ROS).

Introduction

Aminophenyl fluorescein (APF, A36003) and hydroxyphenyl fluorescein (HPF, H36004), two new ROS indicators developed by Nagano, offer greater specificity and stability than dichlorodihydrofluorescein diacetate (H_2DCFDA).[1] Also known as dichlorofluores*cin* diacetate (sometimes abbreviated DCF), H_2DCFDA is probably the most commonly used reagent for detecting intracellular ROS species, despite its nonspecificity and auto-oxidation in the presence of light. The nonfluorescent H_2DCFDA becomes fluorescent in the presence of a wide variety of ROS including, but not limited to, peroxyl (ROO•) and hydroxyl (•OH) radicals and the peroxynitrite anion ($ONOO^-$). In contrast, APF and HPF show much more limited reactivity and higher resistance to light-induced oxidation (Table 1). Both of these new fluorescein derivatives are nonfluorescent until they react with the hydroxyl radical or peroxynitrite anion (Figure 1); APF will also react with the hypochlorite anion (^-OCl). Together, these two ROS indicators can selectively detect hypochlorite anion. Upon oxidation, both APF and HPF exhibit bright green fluorescence (excitation/emission maxima ~490/515 nm), making them compatible with fluorescence instrumentation capable of visualizing fluorescein. Using APF, researchers have been able to detect the hypochlorite anion generated by activated neutrophils, a feat that has not been possible with traditional ROS indicators.

Materials

Contents

APF and HPF are provided in a unit size of 470 µL, as a 5 mM solution in dimethylformamide (DMF).

Storage and Handling

Upon receipt, store APF and HPF at 2–6°C protected from light until required for use. The solutions are stable for at least six months when properly stored. Allow the solutions to warm to room temperature before opening. For long-term storage, divide the DMF stock solution into aliquots. Prepare working solutions of these reagents immediately before use, and discard any excess diluted reagent at the end of the work session.

Application

DMF stock solutions of APF and HPF may be diluted into aqueous buffers. Bovine serum albumin (BSA) and phenol red can affect the fluorescence and should be used with caution. APF and HPF are sold for research use only and should not be used for *in vitro* diagnostics. Both APF and HPF can be used in solution assays or for intracellular measurements.

Solution Assays

The optimal dilution buffer and working concentration must be determined empirically. A suggested starting concentration range is 1–10 µM.

Cell-Based Assays

The following protocol is provided as an introductory guide; optimal loading concentration, time, and temperature must be determined empirically. In general it is desirable to use the minimum dye concentration required to yield fluorescence signals

Table 1. Fluorescence response of HPF, APF, and H_2DCFDA to various reactive oxygen species (ROS).[1]

ROS	ROS Generation Method	APF *	HPF *	H_2DCFDA *
•OH	100 µM of ferrous perchlorate (II) and 1 mM of H_2O_2	1200	730	7400
$ONOO^-$	3 µM (final) of $ONOO^-$	560	120	6600
^-OCl	3 µM (final) of ^-OCl	3600	6	86
$^1O_2$	100 µM of 3-(1,4-dihydro-1,4-epidioxy-1-naphthyl)propionic acid	9	5	26
$•O_2^-$	100 µM of KO_2	6	8	67
H_2O_2	100 µM of H_2O_2	<1	2	190
NO	100 µM of 1-hydroxy-2-oxo-3-(3-aminopropyl)-3-methyl-1-triazene	<1	6	150
ROO•	100 µM of 2,2′-azobis(2-amidinopropane), dihydrochloride (AAPH)	2	17	710
Auto-oxidation	2.5 hours exposure to fluorescent light source	<1	<1	2000

* 10 µM of APF, HPF, or H_2DCFDA were added to sodium phosphate buffer (0.1 M, pH 7.4), ROS were generated as indicated, and fluorescence was measured using excitation/emission wavelengths of 490/515 nm (for APF and HPF) or 500/520 nm (for H_2DCFDA).

with adequate signal-to-noise ratios. Subcellular compartmentalization of the dye is usually lessened by lowering the incubation temperature.

1.1 Prepare viable cells in suspension or on a slide.

1.2 Dilute the DMF stock solution into a suitable buffer. A suggested starting concentration range is 1–10 µM.

1.3 Incubate the cells with the diluted APF or HPF for 20–60 minutes at 4–37°C. Adherent cultures do not need to be trypsinized for loading.

1.4 Wash the cells to remove excess probe. Replace with fresh buffer or medium.

1.5 Fluorescence excitation and emission maxima are 490 and 515 nm, respectively. Because these wavelengths are very similar to fluorescein, detection systems designed for fluorescein, or FITC can be used.

Reference

1. J Biol Chem 278, 3170 (2003).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
A36003	3′-(p-aminophenyl) fluorescein (APF) *5 mM solution in DMF*	470 µL
H36004	3′-(p-hydroxyphenyl) fluorescein (HPF) *5 mM solution in DMF*	470 µL

Contact Information

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