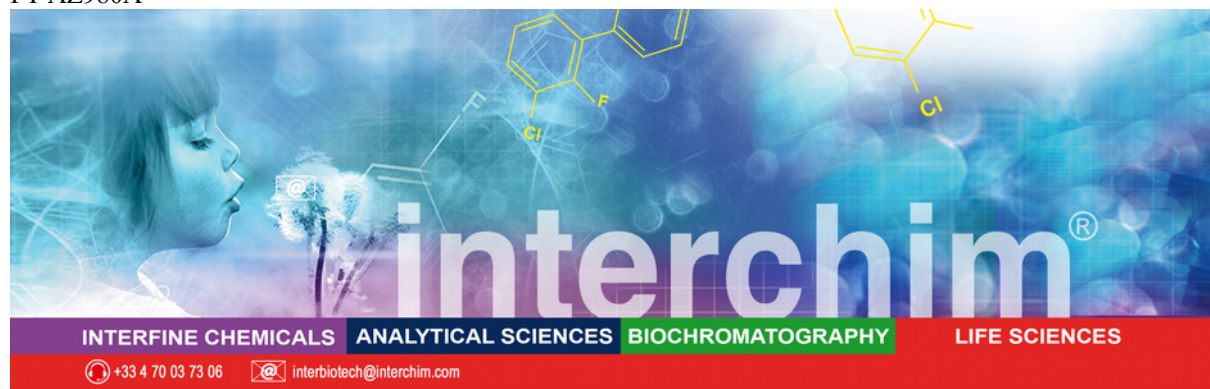


FT-AZ980A

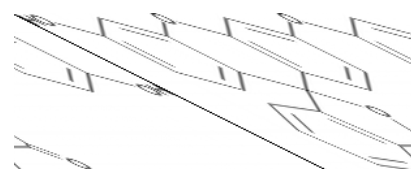


HPF, ROS Probe

For detection of hydroxyl radical and peroxynitrite

Product Description

Name :	Hydroxyphenyl Fluorescein (HPF) (2-[6-(4'-hydroxy)phenoxy-3H-xanthene-3-on-9-yl]benzoic acid)
Catalog Number :	FP-AZ980A, 1mg CAS [359010-69-8]
Molecular Weight :	MW= 424.4
Soluble in :	DMSO, DMF, ethanol
Absorption / Emission :	$\lambda_{exc} \backslash \lambda_{em} = 490/515 \text{ nm}$



Storage: -20°C Protect from light and moisture

Introduction

The biology of highly reactive oxygen radical species is of great interest in many biomedical research disciplines, including neurodegeneration, aging, cancer, and infectious diseases. There are a number of fluorescent reagents, such as 2,7-dichlorodihydrofluorescein (DCDHF), that can be used to detect free radicals, but they have significant limitations due to their facile oxidation by light and numerous non-radical oxidants such as hydrogen peroxide (H₂O₂). HPF is a cell-permeable aromatic amino-fluorescein derivative that has little intrinsic fluorescence. It undergoes oxidation only by highly reactive oxygen species (hROS) such as the hydroxyl radical, peroxynitrite, and hROS generated from a peroxidase/H₂O₂ system. It is inert to hypochlorite ion, nitric oxide, hydrogen peroxide (H₂O₂), superoxide, and other oxidants. Upon oxidation, HPF is converted to the highly fluorescent molecule fluorescein, allowing the simple direct detection of highly reactive biological radicals.

Directions for use

Guidelines for use

Solvent of choice can be added to the provided powder. Solvents such as ethanol, DMSO and dimethyl formamide purged with an inert gas can be used. The solubility of HPF in these solvents is approximately 20mg/ml.

HPF is sparingly soluble in aqueous buffers. For maximum solubility in aqueous buffers, the methyl acetate solution of HPF should be diluted with the aqueous buffer of choice. HPF has a solubility of approximately

FT-AZ980A

0.15 mg/ml in a 1:8 solution of ethanol:PBS (pH 7.2) using this method. We do not recommend storing the aqueous solution for more than one day.

This following protocol only provides a guideline, and should be modified according to your specific needs. Treat cells as desired before making the working solution.

- 1) Prepare a 10 to 20 mM HPF stock solution in DMSO. Make 1 to 10 μ M working solution by diluting the DMSO stock solution into Hanks solution with 20 mM Hepes buffer (HHBS).
- 2) Treat cells as desired (e.g., RASM cells are treated with 50-100 nM angiotensin II for 3-5 hours)
- 3) Incubate the cells with HPF (1-10 μ M, from Step #1) for 20 -60 minutes at 37 °C.
- 4) Replace the dye-loading solution with HHBS buffer.
- 5) Analyze the cells with a proper fluorescence instrument at Ex/Em = 490/525 nm (cut off = 515 nm) with bottom read mode (e.g., FITC filter set for a fluorescence microscope, FL1 filter for a flow cytometer).

Note: BSA and phenol red can affect the fluorescence and should be used with caution. Both APF and HPF can be used in solution assays or for intracellular measurements.

References

- 1- Matés, J.M. *et al.* Antioxidant enzymes and human diseases. Clin Biochem 32(8) 595-603 (1999).
- 2- Hempel, S.L., *et al.* Dihydrofluorescein diacetate is superior for detecting intracellular oxidants: Comparison with 2',7'-dichlorodihydrofluorescein diacetate, 5-(and 6)-carboxy-2',7'-dichlorodihydrofluorescein diacetate, and dihydrorhodamine 123. Free Radic Biol Med 27(1) 146-159 (1999).
- 3- Setsukinai, K., *et al.* Development of novel fluorescence probes that can reliably detect reactive oxygen species and distinguish specific species. J Biol Chem 278(5) 3170-3175 (2003).

Technical and scientific information

Related products

- APF, FP-AZ9811
- H2DCFDA, FP-467312
- Dihydrorhodamine 123, FP-83775A

Ordering information

Catalog size quantities and prices may be found at <http://www.interchim.com>.

Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06

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