



Cell Technology, Inc



Fluoro Hypochlorite™

Hypochlorite (OCl^-) Detection Kit

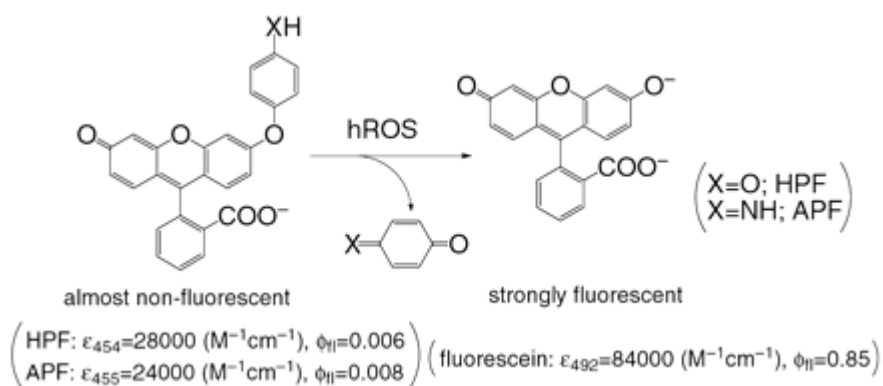
Key Benefits

- Can monitor multiple time points to follow real time kinetics.
- Quenched cell permeable dye.
- One-step, no wash assay.
- Adaptable for High Throughput format
- Non-destructive cell based assay allows monitoring of additional parameters.

Introduction

The two new novel probes, Aminophenyl fluorescein (APF) and Hydroxyphenyl fluorescein (HPF) developed by Tetsuo Nagano et. al. (1), are selective for the detection of highly reactive oxygen species (hROS). Both probes have little reactivity towards other ROS such as: singlet oxygen (O_2^1), superoxide ($\text{O}_2^{\cdot -}$), hydrogen peroxide (H_2O_2), nitric oxide (NO^\bullet), and alkyl peroxide (RO_2^\bullet) (see table below)¹. HPF/APF are cell permeable and can be used in combination to detect hypochlorite (OCl^-) production in cells (see fig 1). Hypochlorite can be detected by loading two samples, one with APF and the other with HPF. Hypochlorite production is visualized by increase in fluorescence of APF loaded cells and no increase in fluorescence in HPF loaded cells.

Assay Principle



Reactivity Profile of APF/HPF:

ROS (RFU)	HPF (RFU) Ex:499 Em:515	APF (RFU) Ex:499 Em:515	DCFH-DA (RFU) Ex:500 Em:520
Hydroxyl Radical: $\cdot\text{OH}$	730	1200	7400
Peroxynitrite: ONOO^-	120	560	6600
Hypochlorite: OCl^-	6	3600	86
Oxygen Radical: $\cdot\text{O}_2$	5	9	26
Superoxide: $\text{O}_2^{\cdot-}$	8	6	67
Hydrogen Peroxide : H_2O_2	2	<1	190
Nitric Oxide: NO	6	<1	150
Alkylperoxyl Radical: $\text{ROO}\cdot$	17	2	710
Autoxidation	<1	<1	2000

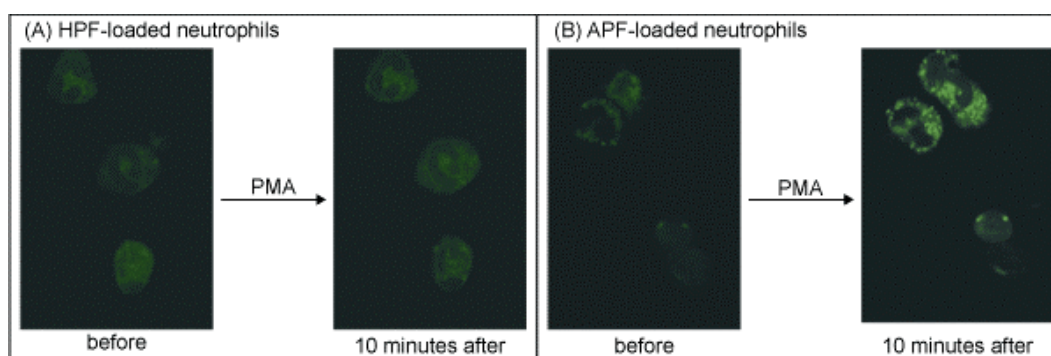


Figure 1: Detection of Hypochlorite (OCl^-) in neutrophils. Neutrophils were isolated from porcine blood, washed in Krebs-Ringers phosphate buffer (as described in step V 1. above) and seeded in glass bottom dishes. The cells were then loaded with APF or HPF ($10\mu\text{M}$ final) by incubation for 30 minutes at room temperature. The Dye-loaded neutrophils were stimulated with PMA (2ng/mL). Fluorescence images were acquired before and 10 minutes after stimulation. Excitation: 488nm emission: 505-550 nm barrier filters¹. Hypochlorite production can be detected with increase APF fluorescence and no increase in HPF fluorescence.

Catalog #	Contents	Size	
FLOCL100-2	APF and HPF One vial each	150 Tests*	

*Test size will depend on dilution and volume used per test. For example:

Dilution of APF/HPF	Volume of test	Number of tests
1:500 ($10\mu\text{M}$ final)	0.2 mL	147
1: 1000 ($5\mu\text{M}$ final)	0.2mL	294

Manufactured: by Daiichi Pure Chemicals Co. Ltd. Japan

References:

¹ Ken-ichi Setsukinai, Yasuteru Urano, Katsuko Kakinuma, Hideyuki J. Majima, and Tetsuo Nagano. Development of Novel Fluorescence Probes That Can Reliably Detect Reactive Oxygen Species and Distinguish Specific Species. THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 278, No. 5, Issue of January 31, pp. 3170–3175, 2003



211 bis Avenue Kennedy - BP 1140
03103 Montluçon - France
33 (0) 4 70 03 88 55
Fax 33 (0) 4 70 03 82 60
e-mail interchim@interchim.com

Agence Paris - Normandie
33 (0) 1 41 32 34 40
Fax 33 (0) 1 47 91 23 90
e-mail interchim.paris@interchim.com