Amplite™ Fluorimetric Hypochlorite (Hypochlorous Acid) Assay Kit

Catalog number: 13846
Unit size: 200 Tests

Component	Storage	Amount
Component A: Oxirite™ Hypochlorite Sensor (light sensitive)	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (20 mL)
Component C: Hypochlorite Standard	Freeze (<-15 °C), Minimize light exposure	1 vial (1M, 300 µL)
Component D: DMSO	Freeze (<-15 °C)	1 vail (100 µL)

OVERVIEW

Hypochlorite anion (ClO-) and its protonated form, hypochlorous acid (HClO) are critical reactive oxygen species (ROS) in biological systems. Uncontrolled production of hypochlorite (hypochlorous acid) can lead to tissue damage and diseases including arthritis, renal failure and cancers. In addition, sodium hypochlorite (NaClO) has been widely used as a bleaching agent for surface cleaning, odor removal and water disinfection in our daily lives. Exposure to large amount of sodium hypochlorite can lead to poisoning with the symptoms of serious breathing problems, stomach irritation, redness and pain on skin and eye. Therefore, highly selective and sensitive detection of hypochlorite (hypochlorous acid) is of toxicological and environmental importance. Amplite™ Fluorimetric Hypochlorite (Hypochlorous Acid) Assay Kit offers a sensitive fluorescence-based assay for measuring hypochlorite (hypochlorous acid) with high specificity. Upon selective reaction with hypochlorite (hypochlorous) the weakly fluorescent Oxirite™ Hypochlorite Sensor generates a strongly fluorescent product that gives more than 100-fold fluorescence enhancement. The fluorescence signal can be measured by a fluorescence microplate reader. With this Fluorimetric Hypochlorite (Hypochlorous Acid) Assay Kit, as low as 3 µM hypochlorite was detected in a 100 µL reaction volume.

AT A GLANCE

Protocol summary

1. Prepare Hypochlorite working solution (50 µL)
2. Add Hypochlorite standards or test samples (50 µL)
3. Incubate at room temperature for 10 - 30 min
4. Monitor fluorescence intensity at Ex/Em = 540/590 nm (Cutoff = 570 nm)

Important To achieve the best results, it's strongly recommended to use the black plates. Thaw one vial of each kit component at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 nm
Recommended plate:	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. Oxirite™ Hypochlorite Sensor stock solution (200X):
Add 50 µL of DMSO (Component D) into the vial of Oxirite™ Hypochlorite Sensor (Component A) to make 200X stock solution. Protect from light.

PREPARATION OF STANDARD SOLUTION

Hypochlorite standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/13846

Add 50 µL of Hypochlorite Standard (Component C) into 450 µL of Assay Buffer (Component B) to get 100 mM Hypochlorite standard solution (H7). Take 100 mM (H7) Hypochlorite standard solution and perform 1:10 serial dilutions in Assay Buffer (Component B) to get 10, 1, 0.1, and 0.01 mM serially diluted Hypochlorite standards (H6 - H3). Then, 0.01 mM Hypochlorite standard (H3) and perform 1:3 in Assay Buffer (Component B) to get 0.003 and 0.001 mM diluted Hypoclorite standards (H2 - H1).

PREPARATION OF WORKING SOLUTION

Add 25 µL of 200X Oxirite™ Hypochlorite Sensor stock solution into 5 mL of Assay Buffer (Component B) and mix well to make Hypochlorite working solution.

Note This Hypochlorite working solution is enough for one 96-well plate. It is not stable, use it promptly.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Hypochlorite standards and test samples in a 96-well solid black microplate. H= Hypochlorite Standards (H1 - H7, 0.001 to 100 mM), BL=Blank Control, TS=Test Samples.

BL	BL	TS	TS
H1	H1	...	...
H2	H2	...	...
H3	H3		
H4	H4		
H5	H5		
H6	H6		
H7	H7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
H1 - H7	50 µL	Serial Dilutions (0.001 to 100 mM)
BL	50 µL	Assay Buffer
TS	50 µL	test sample

1. Prepare Hypochlorite standards (H), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

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2. Add 50 µL of Hypochlorite working solution to each well of Hypochlorite standard, blank control, and test samples to make the total Hypochlorite assay volume of 100 µL/well. For a 384-well plate, add 25 µL of Hypochlorite working solution into each well instead, for a total volume of 50 µL/well.

3. Incubate the reaction at room temperature for 10 - 30 minutes, protected from light.

4. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 540/590 nm (Cutoff = 570 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Hypochlorite samples. We recommend using the Online Four Parameter Logistics Calculator which can be found at:

https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-online-calculator

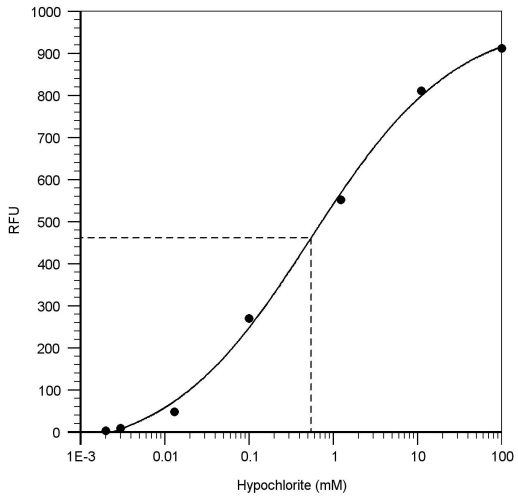

Figure 1. Hypochlorite was measured with Amplite™ Fluorimetric Hypochlorite/Hypochlorous Acid Assay Kit in a 96-well solid black plate.

DISCLAIMER