Amplite™ Colorimetric Hypochlorite (Hypochlorous Acid) Assay Kit

Catalog number: 13845
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 (300 µL)
Component D: DMSO	Freeze (<-15 °C)	1 vial (600 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™ Colorimetric Hypochlorite (Hypochlorous Acid) Assay Kit offers a sensitive absorption-based assay for measuring hypochlorite (hypochlorous acid) with high specificity. Upon selective reaction with hypochlorite (hypochlorous) the colorless Oxirite™ Hypochlorite Sensor generates a strong color product. The color signal can be measured by a absorption microplate reader at ~580 nm. With this Colorimetric Hypochlorite (Hypochlorous Acid) Assay Kit, trace amount of hypochlorite can be detected.

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 3 - 5 min
4. Monitor absorbance increase at OD of 555 ± 5 nm

Important Thaw one vial of each kit component at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Absorbance microplate reader
Absorbance:	555 ± 5 nm
Recommended plate:	Clear bottom

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 (20X):
Add 500 µL of DMSO (Component D) into the vial of Oxirite™ Hypochlorite Sensor (Component A) to make 20X Oxirite™ Hypochlorite Sensor stock solution.

PREPARATION OF STANDARD SOLUTION

Hypochlorite standard

For convenience, use the Serial Dilution Planner:

https://www.aatbio.com/tools/serial-dilution/13845

Add 100 µL of Hypochlorite Standard (Component C) into 400 µL of Assay Buffer (Component B) to get 1% Hypochlorite standard solution (H7). Take 1% Hypochlorite standard solution (H7) and perform 1:3 serial dilutions in Assay Buffer (Component B) get serially diluted Hypochlorite standards (H6 - H1).

PREPARATION OF WORKING SOLUTION

Add 250 µL of 20X 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. Protect from light.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Hypochlorite standards and test samples in a 96-well clear bottom microplate. H = hypochlorite standard (H1 - H7, 0.001% to 1%), BL = blank control, TS = test sample.

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 Dilution (0.001% to 1%)
BL	50 µL	Assay Buffer (Component B)
TS	50 µL	Test Sample

1. Prepare Hypochlorite standards (H), blank controls (BL), and test samples (TS) in a 96-well clear bottom microplate as provided in Table 1 and Table 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.
2. Add 50 µL of Hypochlorite working solution to each well of Hypochlorite standards, 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 working solution into each well instead, for a total volume of 50 µL/well.
3. Incubate the reaction at room temperature for 3 - 5 minutes, protected from light.

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4. Monitor the absorbance increase with an absorbance plate reader at OD = 555 ± 5 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Absorbance) 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

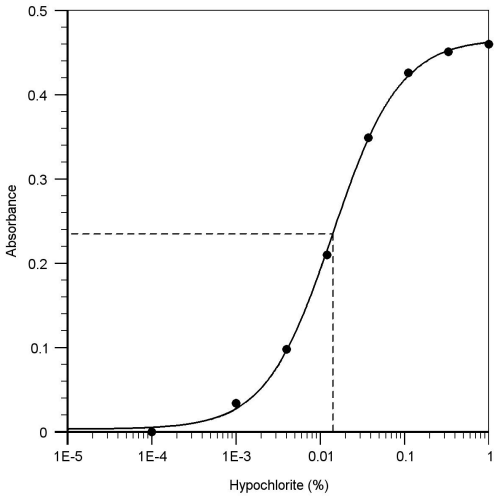

Figure 1. Hypochlorite was measured with Amplite™ Colorimetric Hypochlorite/Hypochlorous Acid Assay Kit in a 96-well clear bottom plate. As low as 0.001% (10 ppm) sodium hypochlorite (NaClO) was detected with 3-5 minutes incubation.

DISCLAIMER