Amplite™ Colorimetric Hypochlorite (Hypochlorous Acid) Assay Kit

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 13845 (200 tests)	Frozen	Absorbance microplate reader

Introduction

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 life. 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 assay for measuring hypochlorite (hypochlorous acid) with high specificity. The Oxirite™ Hypochlorite Sensor selectively reacts with hypochlorite (hypochlorous) to generate a red color product. The assay can be measured with an absorbance microplate reader around 550 nm.

Kit Components

Components	Amount
Component A: Oxirite™ Hypochlorite Sensor (light sensitive)	1 vial
Component B: Assay Buffer	1 bottle (20 mL)
Component C: Hypochlorite Standard	1 vial (300 µL)
Component D: DMSO	1 vail (600 µL)

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare hypochlorite assay mixture (50 µL) → Add hypochlorite or test samples (50 µL) → Incubate at room temperature for 3-5 min → Monitor absorbance increase at OD of 555±5 nm

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

1. Prepare Oxirite™ hypochlorite sensor stock solution (20X):

Add 500 µL of DMSO into the vial of Oxirite™ hypochlorite sensor (**Component A**) to make 20X stock solution.
Note: Make single use aliquots, and store unused 20X Oxirite™ hypochlorite sensor stock solution at -20ºC, avoid light and repeat freeze-thaw cycles.

2. Prepare hypochlorite assay mixture:

Add 250 µL of Oxirite™ hypochlorite sensor stock solution (from Step 1) into 5 mL of assay buffer (**Component B**), and mix well to make hypochlorite assay mixture (**Component A+B**).
Note: This hypochlorite assay mixture is enough for one 96-well plate. It is not stable, use it promptly.

3. Prepare serial dilutions of hypochlorite standards (0 to 1%):

3.1 Prepare hypochlorite standard dilutions: Add 100 µL of hypochlorite standard (**Component C**) into 400 µL of assay buffer (**Component B**) to get 1% hypochlorite standard solution. Perform 1:3 serial dilutions in assay buffer (**Component B**) get approximately 1%, 0.3%, 0.1%, 0.03%, 0.01%, 0.003%, 0.001% and 0% serially diluted hypochlorite standards.
Note: The unused hypochlorite standard (Component C) should be divided into single use aliquots and stored at -20 °C.

3.2 Add serial dilutions of hypochlorite standard and hypochlorite/hypochlorous acid containing test samples into a 96-well clear bottom microplate microplate as described in Tables 1 and 2.

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Table 1 Layout of hypochlorite standards and test samples in a 96-well clear bottom microplate

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

Note: H= Hypochlorite Standards, BL=Blank Control, TS=Test Samples.

Table 2 Reagent composition for each well

Hypochlorous Acid Standard	**Blank Control**	**Test Sample**
Serial Dilutions*: 50 µL	Assay Buffer : 50 µL	50 µL

**Note: Add the serially diluted hypochlorite standards from 0.001% to 1% into wells from H1 to H7 in duplicate.*

4. Run hypochlorite/hypochlorous acid assay:

4.1 Add 50 µL of hypochlorite assay mixture (from Step 2) to each well of hypochlorite standards, blank control, and test samples (see Step 3.2) to make the total volume of 100 µL/well.
Note: For a 384-well plate, add 25 µL of sample and 25 µL of hypochlorite assay mixture into each well.

4.2 Incubate the reaction at room temperature for 3-5 minutes, protected from light.

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

Data Analysis

The absorbance increase in blank wells (with assay buffer and hypochlorite assay mixture only) is used as a control, and is subtracted from the values of those wells with the hypochlorite standards and test samples. A hypochlorite standard curve is shown in Figure 1.

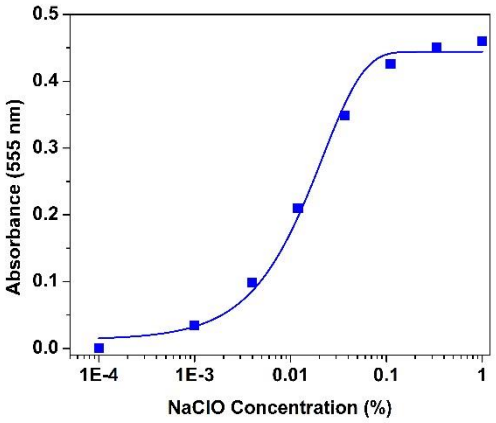

Figure 1. Hypochlorite was measured with Amplite™ Colorimetric Hypochlorite/Hypochlorous Acid Assay Kit (Cat#13845) in a 96-well clear bottom plate. As low as 0.001% (10 ppm) sodium hypochlorite (NaClO) was detected with 3-5 minutes incubation.

Reference

1. C.C. Winterbourn. (2002) Biological reactivity and biomarkers of the neutrophil oxidant, hypochlorous acid. Toxicology, 181, 223-227.
2. F. Racioppi, P.A. Daskaleros, N. Besbelli, A. Borges, C. Deraemaeker, S.I. Magalini, R. Martinez Arrifta, C. Pulce, M.L. Ruggerone, P. Vlachos. (1994) Household bleaches based on sodium hypochlorite: Review of acute toxicology and poison control center experience. Food and Chemical Toxicology, 32, 845-864.