Technical Bulletin

Hypochlorite (Hypochlorous Acid) Assay Kit

Catalogue number MAK556

Product Description

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.

The 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 Hypochlorite Sensor generates a strong color product. The color signal can be measured by an absorption microplate reader at ~555 nm. With this Assay Kit, trace amounts of hypochlorite can be detected.

Components

The kit is sufficient for 200 colorimetric assays in 96-well plates.

- Hypochlorite Sensor 1 Vial
  Catalogue Number MAK556A
- Assay Buffer 20 mL
  Catalogue Number MAK556B
- Hypochlorite Standard 300 µL
  Catalogue Number MAK556C
- DMSO 600 µL
  Catalogue Number MAK556D

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories.
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped at room temperature. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate to room temperature prior to use.

Procedure

All Samples and Standards should run in duplicate.

Preparation of Stock Solution

Hypochlorite Sensor Stock Solution (20X): Add 500 µL of DMSO into the vial of Hypochlorite Sensor.

Note: Unused stock solution should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

Preparation of Hypochlorite Standard Solution

1. 1% Hypochlorite Standard solution (H1): Add 100 µL of Hypochlorite Standard into 400 µL of

Assay Buffer to get 1% Hypochlorite Standard solution (H1).

2. Take the 1% Hypochlorite Standard solution (H1) and perform 1:3 serial dilutions in Assay Buffer to get serially diluted Hypochlorite Standards (H2 – H7) as shown in Table 1.

Table 1.

Serial Dilution of Hypochlorite Standards

Dilution	$HClO^-$ Standard Volume (µL)	Serial Dilution Source	Assay Buffer Volume (µL)	Conc (%)
H1	225	From 1% Stock	0	1
H2	75	From H1	150	0.33
H3	75	From H2	150	0.11
H4	75	From H3	150	0.037
H5	75	From H4	150	0.012
H6	75	From H5	150	0.004
H7	75	From h6	150	0.001

Preparation of Working Solution

Note: Prepare immediately before use in assay reaction. The working solution is enough for one 96-well plate. Protect from light.

Hypochlorite Working Solution: Add 250 µL of 20X Hypochlorite Sensor stock solution into 5 mL of Assay Buffer and mix well.

Assay Reaction

1. Add 50 µL of Samples, Standards, and blanks to separate wells of a 96-well plate. For 384-well plates, use 25 µL.
2. Add 50 µL of the Working Solution to each well containing a Sample, Standard, and blank for a total 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.

Measurement

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

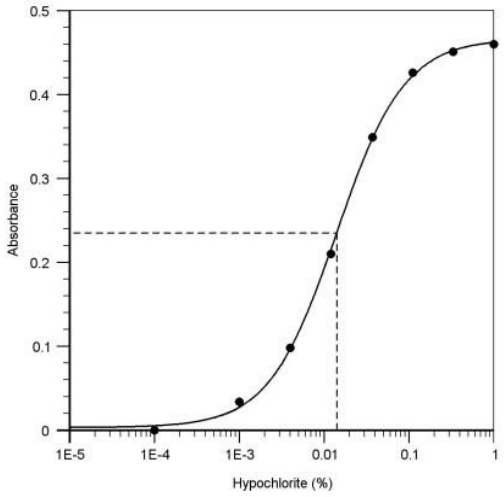
1. The reading (Absorbance) obtained from the blank Standard well is used as a negative control.
2. Subtract the blank value from the Standards' readings to obtain the base-line corrected values.
3. Plot the Standards' readings to obtain a

Standard curve and equation.

4. The concentration of Hypochlorite in the Samples may be determined from the Standard curve.

Figure 1.

Typical Hypochlorite Standard Curve



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