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ab219930 Hypochlorite Detection Kit (Fluorometric)

For the rapid, sensitive and accurate measurement of Hypochlorite in various samples.

This product is for research use only and is not intended for diagnostic use.

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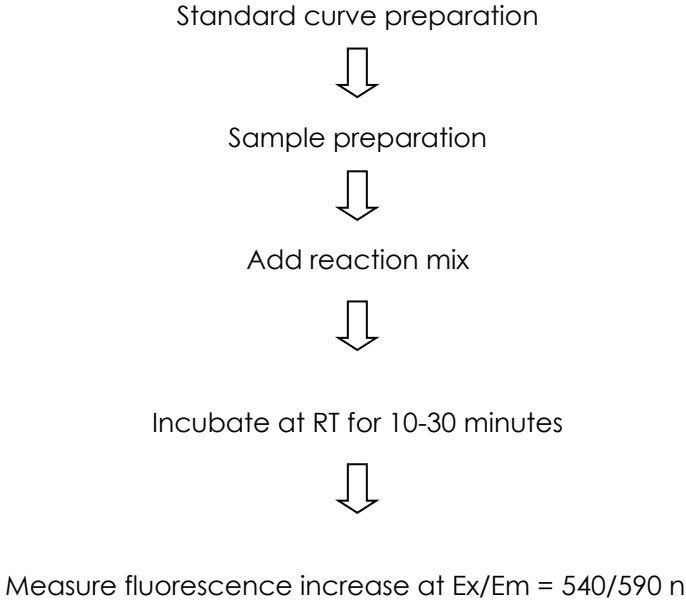
1. Overview

Hypochlorite Detection Kit (Fluorimetric) (ab219930) provides a sensitive fluorescence-based assay for measuring hypochlorite (HClO, hypochlorous acid) with high specificity in cell and tissue lysates and biological fluids. Upon selective reaction with hypochlorite (hypochlorous), the weakly fluorescent Hypochlorite Sensor used in the assay selectively reacts with hypochlorite (hypochlorous) generates a strongly fluorescent product with more than 100-fold fluorescence enhancement. The fluorescence signal can be measured by a fluorescence microplate reader at Ex/Em = 540/590 nm. The assay can detect as low as 3 µM hypochlorite was detected in a 100 µL reaction volume.

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.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer	20 mL	-20°C	-20°C
Hypochlorite Standard	300 µL	-20°C	-20°C
Hypochlorite Sensor	1 vial	-20°C	-20°C
DMSO	100 µL	-20°C	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring fluorescence at Ex/Em = 540/590 nm
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom, preferably black
- Dounce homogenizer (if using tissue)
- (Optional) Mammalian Cell Lysis Buffer 5X (ab179835)

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Assay Buffer (20 mL):

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 Hypochlorite Standard (300 µL):

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot so that you have enough volume to perform the desired number of assays. Store at -20°C.

9.3 DMSO (100 µL):

Ready to use as supplied. Warm by placing in a 37°C bath for 1-5 min to thaw the DMSO solution before use.

Δ Note: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for a few minutes at 37°C. Repeat this step every time probe is needed.

Store at -20°C. Avoid repeated freeze-thaw cycles.

9.4 Hypochlorite Sensor (light sensitive):

Prepare a **200X Hypochlorite Sensor** stock solution by adding 50 µL of DMSO to the vial of Hypochlorite Sensor. Mix well by pipetting up and down. Aliquot so that you have enough to perform the desired number of assays. Store at -20°C protected from light. Avoid repeated freeze-thaw cycles.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

10.1 Prepare a 100 mM hypochlorite standard by diluting 50 µL of the provided standard (Step 9.2) into 450 µL of Assay Buffer. Mix well by pipetting up and down.

10.2 Using the Hypochlorite standard provided, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Sample to dilute	Volume standard in well (µL)	Assay Buffer (µL)	End conc Hypochlorite in well
1	100 mM	250	0	100 mM
2	Std #1	50	450	10 mM
3	Std #2	50	450	1 mM
4	Std #3	50	450	0.1 mM
5	Std #4	50	450	0.01 mM
6	Std #5	100	200	0.003 mM
7	Std #6	100	200	0.001 mM
8 (blank)	0	0	200	0 mM

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

11. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice and proceed with the Sample Preparation step. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Cell lysates:

Δ Note: For ease of use, mammalian adherent or suspension cells lysates can be easily prepared using Mammalian Cell Lysis Buffer 5X (ab179835). Follow product protocol and proceed to Section 12.

11.1.1 Harvest the number of cells necessary for each assay (initial recommendation: 2-5 x 10^5 cells).
11.1.2 Wash cells with cold PBS.
11.1.3 Resuspend or scrape cells in 100 µL of cold PBS.
11.1.4 Homogenize cells quickly by pipetting up and down a few times.
11.1.5 Centrifuge 5 minutes at 4°C at 13,000 x*g* in a cold microcentrifuge to remove any insoluble material.
11.1.6 Collect supernatant and transfer to a new tube.
11.1.7 Keep on ice.

11.2 Tissue lysates:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation: 20 mg).
11.2.2 Wash tissue with cold PBS.
11.2.3 Homogenize tissue in 400 µL Assay Buffer using a Dounce homogenizer.
11.2.4 Centrifuge homogenate at 2,500 rpm for 5 – 10 minutes at 4°C.
11.2.5 Transfer supernatant to a new tube. Discard pellet.
11.2.6 Keep sample on ice.

11.3 Plasma, Serum and Urine (and other biological fluids):

Samples can be used directly or diluted in Assay Buffer for testing.

Δ Note: We suggest using different volumes of sample to ensure readings are within the standard curve range.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.
- The protocol describe in this section is for 1 x 96-well plate. To perform the assay in a 384-wp, scale down volumes by half.

12.1 Reaction wells set up:

- Blank control = 50 µL Assay Buffer.
- Standard wells = 50 µL standard dilutions.
- Sample wells = 1-50 µL samples (adjust volume to 50 µL/well with Assay Buffer).

12.2 Run Hypochlorite Assay:

12.2.1 Prepare Hypochlorite Reaction Mix as described in the table below. The volume given in the table is enough for 1 x 96-well plate.

Component	Hypochlorite Reaction Mix
Assay Buffer	5 mL
200X Hypochlorite Sensor solution	25 µL

Δ Note: The hypochlorite assay mixture is not stable, use it promptly.

12.2.2 Add 50 µL of Hypochlorite Reaction Mix into each reaction well (total volume = 100 µL/well). Mix well.

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

12.3 Measurement:

12.3.1 Monitor fluorescence increase at Ex/Em = 540/590 nm (cut off 570 nm) on a microplate reader in kinetic mode, every 2-3 minutes, at RT for at least 10-30 minutes, protected from light.

13. Calculations

– Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

13.1 Average the duplicate reading for each standard and sample.

13.2 Subtract the mean fluorescence value of the blank (Standard #7) from all standard and sample readings. This is the corrected fluorescence.

13.3 Plot the corrected fluorescence values for each standard as a function of the final concentration of Hypochlorite.

13.4 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.5 Apply the corrected sample OD reading to the standard curve to get Hypochlorite (B) amount in the sample wells.

13.6 Concentration of Hypochlorite (mM) in the test samples is calculated as:

$$\text{Hypochlorite concentration} = \frac{B}{V} * D$$

Where:

B = amount of Hypochlorite in the sample well calculated from standard curve (mM).

V = sample volume added in the sample wells (µL).

D = sample dilution factor if sample is diluted to fit within the standard curve range.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

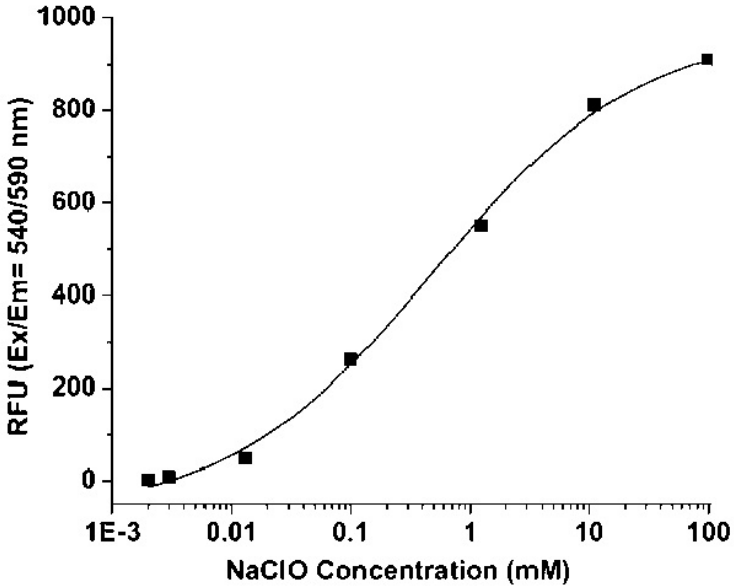


Figure 1. Typical Hypochlorite standard calibration curve. Hypochlorite was measured with in a 96 well solid black plate. As low as 0.003 mM (~3 µM) sodium hypochlorite (NaClO) was detected with 10-30 min incubation.

15. Quick Assay Procedure

Δ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare reagents and aliquot; get equipment ready.
- Prepare Hypochlorite standard dilution [100-0.001 mM].
- Prepare samples in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate for standard (50 µL) and samples (50 µL).
- Prepare Reaction Mix for one 96-well plate.

Component	Hypochlorite Reaction Mix
Assay Buffer	5 mL
200X Hypochlorite Sensor solution	25 µL

- Add 50 µL of Reaction Mixture into each well.
- Incubate plate at RT for 10 - 30 minutes.
- Measure Fluorescence increase with a fluorescence plate reader at Ex/Em = 540/590 nm (cut off: 570 nm).

16. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Sample with erratic readings	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Reason	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions described in the protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. Notes

Technical Support

Austria
wissenschaftlicherdienst@abcam.com | 019-288-259
France
supportscientifique@abcam.com | 01.46.94.62.96
Germany
wissenschaftlicherdienst@abcam.com | 030-896-779-154
Spain
soportecientifico@abcam.com | 91-114-65-60

Switzerland
technical@abcam.com
Deutsch: 043-501-64-24 | Français: 061-500-05-30
UK, EU and ROW
technical@abcam.com | +44(0)1223-696000

Canada
ca.technical@abcam.com | 877-749-8807
US and Latin America
us.technical@abcam.com | 888-772-2226

Asia Pacific
hk.technical@abcam.com | (852) 2603-6823
China
cn.technical@abcam.com | 400 921 0189 / +86 21 2070 0500
Japan
technical@abcam.co.jp | +81-(0)3-6231-0940
Singapore
sg.technical@abcam.com | 800 188-5244

Australia
au.technical@abcam.com | +61-(0)3-8652-1450
New Zealand
nz.technical@abcam.com | +64-(0)9-909-7829