ab219929
Hypochlorite Detection Kit (Colorimetric)

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.
Table of Contents

1. Overview 1
2. Protocol Summary 2
3. Precautions 3
4. Storage and Stability 3
5. Limitations 4
6. Materials Supplied 4
7. Materials Required, Not Supplied 5
8. Technical Hints 6
9. Reagent Preparation 7
10. Standard Preparation 8
11. Sample Preparation 9
12. Assay Procedure 11
13. Calculations 12
14. Typical Data 13
15. Quick Assay Procedure 14
16. Troubleshooting 15
17. Notes 17
1. Overview

Hypochlorite Detection Kit (Colorimetric) (ab219929) provides a sensitive assay for measuring hypochlorite (HClO, hypochlorous acid) with high specificity in cell and tissue lysates and biological fluids. The hypochlorite sensor used in the assay selectively reacts with hypochlorite (hypochlorous) to generate a red color product which can be measured with an absorbance microplate reader at OD 555 nm.

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

Standard curve preparation
↓
Sample preparation
↓
Add reaction mix
↓
Incubate at RT for 3-5 minutes
↓
Measure absorbance increase at OD555 (± 5 nm)
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 in the dark 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

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Storage temperature (before prep)</th>
<th>Storage temperature (after prep)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Buffer</td>
<td>20 mL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Hypochlorite Standard</td>
<td>300 µL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Hypochlorite Sensor</td>
<td>1 vial</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>DMSO</td>
<td>600 µL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
</tbody>
</table>
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 absorbance at OD = 555 nm
– PBS
– 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
– 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 (600 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.

△ 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 protected from light. Avoid repeated freeze-thaw cycles.

9.4 Hypochlorite Sensor (light sensitive):
Prepare a 20X Hypochlorite Sensor stock solution by adding 500 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 Using the Hypochlorite standard provided, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

<table>
<thead>
<tr>
<th>Standard #</th>
<th>Sample to dilute</th>
<th>Volume standard in well (µL)</th>
<th>Assay Buffer (µL)</th>
<th>End Hypochlorite amount in well</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Stock</td>
<td>100</td>
<td>400</td>
<td>1 %</td>
</tr>
<tr>
<td>2</td>
<td>Std #1</td>
<td>100</td>
<td>200</td>
<td>0.3 %</td>
</tr>
<tr>
<td>3</td>
<td>Std #2</td>
<td>100</td>
<td>200</td>
<td>0.1 %</td>
</tr>
<tr>
<td>4</td>
<td>Std #3</td>
<td>100</td>
<td>200</td>
<td>0.03 %</td>
</tr>
<tr>
<td>5</td>
<td>Std #4</td>
<td>100</td>
<td>200</td>
<td>0.01 %</td>
</tr>
<tr>
<td>6</td>
<td>Std #5</td>
<td>100</td>
<td>200</td>
<td>0.003 %</td>
</tr>
<tr>
<td>7</td>
<td>Std #6</td>
<td>100</td>
<td>200</td>
<td>0.001 %</td>
</tr>
<tr>
<td>8 (blank)</td>
<td>0</td>
<td>0</td>
<td>200</td>
<td>0 %</td>
</tr>
</tbody>
</table>

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:

⚠️ 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 Collect supernatant and transfer to a new tube.
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.

△ 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.

<table>
<thead>
<tr>
<th>Component</th>
<th>Hypochlorite Reaction Mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Buffer</td>
<td>5 mL</td>
</tr>
<tr>
<td>20X Hypochlorite Sensor solution</td>
<td>250 µL</td>
</tr>
</tbody>
</table>

⚠️ 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.

12.3 Measurement:

12.3.1 Incubate reaction at room temperature for 3-5 minutes.
12.3.2 Monitor the absorbance increase with an absorbance plate reader at OD = 555 ± 5 nm.
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 absorbance value of the blank (Standard #8) from all standard and sample readings. This is the corrected absorbance.
13.3 Plot the corrected absorbance 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 Amount of Hypochlorite in the test samples is calculated as:

\[
\text{Hypochlorite amount} = \frac{B}{V} \times D
\]

Where:
- \(B\) = amount of Hypochlorite (%) in the sample well calculated from standard curve (\(\mu\text{mol}\)).
- \(V\) = sample volume added in the sample wells (\(\mu\text{L}\)).
- \(D\) = 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.

![Typical Hypochlorite standard calibration curve](image)

**Figure 1.** Typical Hypochlorite standard calibration curve. Hypochlorite was measured in a 96 well clear bottom plate. As low as 0.001% (10 ppm) sodium hypochlorite (NaClO) was detected with 3-5 minutes incubation.
15. Quick Assay Procedure

△ 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 [1-0.001%].
- 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.

<table>
<thead>
<tr>
<th>Component</th>
<th>Hypochlorite Reaction Mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Buffer</td>
<td>5 mL</td>
</tr>
<tr>
<td>20X Hypochlorite Sensor Solution</td>
<td>250 µL</td>
</tr>
</tbody>
</table>

- Add 50 µL of Reaction Mix into each well.
- Monitor the absorbance increase with an absorbance plate reader at OD = 555 ± 5 nm in kinetic mode for 3-5 minutes.
## 16. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Reason</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Assay not working</strong></td>
<td>Use of ice-cold buffer</td>
<td>Buffers must be at assay temperature</td>
</tr>
<tr>
<td></td>
<td>Plate read at incorrect wavelength</td>
<td>Check the wavelength and filter settings of instrument</td>
</tr>
<tr>
<td></td>
<td>Use of a different microplate</td>
<td>Colorimetric: clear plates, Fluorometric: black wells/clear bottom plates, Luminometric: white wells/clear bottom plates</td>
</tr>
<tr>
<td><strong>Sample with erratic readings</strong></td>
<td>Cells/tissue samples not homogenized completely</td>
<td>Use Dounce homogenizer, increase number of strokes</td>
</tr>
<tr>
<td></td>
<td>Samples used after multiple free/thaw cycles</td>
<td>Aliquot and freeze samples if needed to use multiple times</td>
</tr>
<tr>
<td></td>
<td>Use of old or inappropriately stored samples</td>
<td>Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use</td>
</tr>
<tr>
<td></td>
<td>Presence of interfering substance in the sample</td>
<td>Check protocol for interfering substances; deproteinize samples</td>
</tr>
<tr>
<td><strong>Lower/higher readings in samples and standards</strong></td>
<td>Improperly thawed components</td>
<td>Thaw all components completely and mix gently before use</td>
</tr>
<tr>
<td></td>
<td>Allowing reagents to sit for extended times on ice</td>
<td>Always thaw and prepare fresh reaction mix before use</td>
</tr>
<tr>
<td></td>
<td>Incorrect incubation times or temperatures</td>
<td>Verify correct incubation times and temperatures in protocol</td>
</tr>
<tr>
<td>Problem</td>
<td>Reason</td>
<td>Solution</td>
</tr>
<tr>
<td>---------</td>
<td>--------</td>
<td>----------</td>
</tr>
<tr>
<td><strong>Standard readings do not follow a linear pattern</strong></td>
<td>Pipetting errors in standard or reaction mix</td>
<td>Avoid pipetting small volumes (&lt; 5 µL) and prepare a master mix whenever possible</td>
</tr>
<tr>
<td></td>
<td>Air bubbles formed in well</td>
<td>Pipette gently against the wall of the tubes</td>
</tr>
<tr>
<td></td>
<td>Standard stock is at incorrect concentration</td>
<td>Always refer to dilutions described in the protocol</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Unanticipated results</th>
<th>Measured at incorrect wavelength</th>
<th>Check equipment and filter setting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Samples contain interfering substances</td>
<td>Troubleshoot if it interferes with the kit</td>
</tr>
<tr>
<td></td>
<td>Sample readings above/below the linear range</td>
<td>Concentrate/Dilute sample so it is within the linear range</td>
</tr>
</tbody>
</table>
17. Notes
Technical Support

Copyright © 2017 Abcam, All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.

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
UK, EU and ROW
technical@abcam.com | +44(0)1223-696000

Canada
can.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 | +86-21-5110-5938 | 400-628-6880
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