ab83372  
Chloride Assay Kit  
(Colorimetric)  

Instructions for Use  

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

This product is for research use only and is not intended for diagnostic use.
# Table of Contents

1. Overview  
   - page 3
2. Protocol Summary  
   - page 4
3. Components and Storage  
   - page 5
4. Assay Protocol  
   - page 7
5. Data Analysis  
   - page 8
6. Troubleshooting  
   - page 10
1. Overview

Chloride is the anionic form of chlorine. It is the most common of the anions found in living organisms. Chloride ions play a variety of important physiological roles. Chloride channels are found in a variety of cells and are responsible for setting resting cell membrane potential and regulating cell volume.

In the nervous system, the action of glycine and GABA are related to chloride levels in specific neurons. Chloride is also instrumental in maintaining the acid-base balance in blood. The kidneys are instrumental in closely regulating serum chloride levels. There are a number of pathologies associated with defective chloride transport; the most well-known being Cystic Fibrosis, caused by a mutation in CFTR a membrane chloride transporter.

Abcam’s Chloride Assay Kit provides a quick, simple method for quantification of Chloride in a variety of biological samples. Blood and urine can be used directly after dilution with water. The assay is based upon the competition of Hg\(^{2+}\) and Fe\(^{2+}\) for TPTZ. The preferred Hg-TPTZ adduct exhibits no color. In the presence of Chloride, Hg\(^{2+}\) forms HgCl\(_2\) freeing up TPTZ which then binds the available Fe\(^{2+}\) giving a very intense absorbance with a \(\lambda_{\text{max}}\)~ 620nm. The assay is linear in the range 20 to 120 nmol Chloride/well with detection sensitivity ~0.4 mM chloride.
2. Protocol Summary

Sample Preparation

↓

Standard Curve Preparation

↓

Add Chloride Reagent

↓

Measure Optical Density
3. Components and Storage

A. Kit Components

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloride Reagent</td>
<td>15 mL</td>
</tr>
<tr>
<td>Chloride Standard (10 μmol; Lyophilized)</td>
<td>1 vial</td>
</tr>
</tbody>
</table>

* Store kit at room temperature, keep tightly capped.

**CAUTION:** This kit contains small amounts of mercury. Waste generated from using this kit should be disposed properly.

CHLORIDE REAGENT: Ready to use as supplied. Store at room temperature. Stable for at least 6 months.

CHLORIDE STANDARD: Dissolve in 1 ml dH₂O to generate a 10 mM solution. Store at room temperature.
B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader
- 96 well plate
- Orbital shaker
4. Assay Protocol

1. Sample Preparation:
Tissue or cell samples: 20-50 mg of tissue or 1 million cells should be rapidly homogenized with 100 µL lysis buffer (pH 6.5-8). Centrifuge at 13000 rpm for 10 min to remove insoluble materials. Liquid or solution samples: can be directly diluted in the Assay Buffer.

Sample Chloride concentrations can vary over a rather wide range. Liquid or solution samples should be diluted 10-100X. Cell samples should be diluted 30-100 fold. Tissue samples should be diluted 50 – 100 fold.

Take 10-50 µl samples and adjust the well volume to 50 µl with dH2O.

For unknown samples, we suggest testing several different amounts of sample to ensure the readings are within the standard curve

2. Standard Curve Preparation:
Add 0, 2, 4, 6, 8, 10 µl of the 10 mM Chloride standard to a series of wells. Adjust volume to 50 µl/well with water to generate 0, 20, 40, 60, 80 and 100 nmol per well of the Chloride Standard.
3. Development:
Add 150 μL of the Chloride Reagent to each well containing Chloride Standard or test samples. Incubate at room temperature for 15 minutes.

4. Measure OD at 620 nm in a microplate reader.
5. Data Analysis

Subtract the zero Chloride OD reading from all standard and sample readings. This corrects for absorbance due to buffer or plate.
Plot the Chloride standard curve for the zero corrected Chloride standards (nmol/well vs. standard readings).
Apply corrected sample readings (E) to the standard curve to get the amount of Chloride in the sample wells.

**Note:**
There is a slight non-linearity below 20 nmol Chloride. Any samples below 20 nmol Chloride should be repeated with 3-5X higher sample.

The Chloride concentration in the test samples

\[
\text{Concentration} = \frac{Ay}{Sv} \text{ (nmol/μl, or μmol/ml, or mM)}
\]

Where:

*Ay* is the amount of Chloride (nmol) in sample well from the standard curve.

*Sv* is the sample volume (μl) added to the sample well.

**Chloride molecular weight:** 35.5 g/mol.

Assuming a sample dilution of 10X and a sample volume of 10 μL was added into the reaction well, 80 nmol/well corresponds to 80 mmol/L (80 mM) chloride in the original sample.
Chloride Standard Curve: Assays were performed following the kit protocol.
## 6. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Reason</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay not working</td>
<td>Assay buffer at wrong temperature</td>
<td>Assay buffer must not be chilled - needs to be at RT</td>
</tr>
<tr>
<td></td>
<td>Protocol step missed</td>
<td>Re-read and follow the protocol exactly</td>
</tr>
<tr>
<td></td>
<td>Plate read at incorrect wavelength</td>
<td>Ensure you are using appropriate reader and filter settings (refer to datasheet)</td>
</tr>
<tr>
<td></td>
<td>Unsuitable microtiter plate for assay</td>
<td>Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells</td>
</tr>
<tr>
<td>Unexpected results</td>
<td>Measured at wrong wavelength</td>
<td>Use appropriate reader and filter settings described in datasheet</td>
</tr>
<tr>
<td></td>
<td>Samples contain impeding substances</td>
<td>Troubleshoot and also consider deproteinizing samples</td>
</tr>
<tr>
<td></td>
<td>Unsuitable sample type</td>
<td>Use recommended samples types as listed on the datasheet</td>
</tr>
<tr>
<td></td>
<td>Sample readings are outside linear range</td>
<td>Concentrate/ dilute samples to be in linear range</td>
</tr>
<tr>
<td>Samples with inconsistent readings</td>
<td>Unsuitable sample type</td>
<td>Refer to datasheet for details about incompatible samples</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>-----------------------</td>
<td>----------------------------------------------------------</td>
</tr>
<tr>
<td>Samples prepared in the wrong buffer</td>
<td>Use the assay buffer provided (or refer to datasheet for instructions)</td>
<td></td>
</tr>
<tr>
<td>Samples not deproteinized (if indicated on datasheet)</td>
<td>Use the 10kDa spin column (ab93349)</td>
<td></td>
</tr>
<tr>
<td>Cell/tissue samples not sufficiently homogenized</td>
<td>Increase sonication time/number of strokes with the Dounce homogenizer</td>
<td></td>
</tr>
<tr>
<td>Too many freeze-thaw cycles</td>
<td>Aliquot samples to reduce the number of freeze-thaw cycles</td>
<td></td>
</tr>
<tr>
<td>Samples contain impeding substances</td>
<td>Troubleshoot and also consider deproteinizing samples</td>
<td></td>
</tr>
<tr>
<td>Samples are too old or incorrectly stored</td>
<td>Use freshly made samples and store at recommended temperature until use</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lower/Higher readings in samples and standards</th>
<th>Not fully thawed kit components</th>
<th>Wait for components to thaw completely and gently mix prior use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Out-of-date kit or incorrectly stored reagents</td>
<td>Always check expiry date and store kit components as recommended on the datasheet</td>
<td></td>
</tr>
<tr>
<td>Reagents sitting for extended periods on ice</td>
<td>Try to prepare a fresh reaction mix prior to each use</td>
<td></td>
</tr>
<tr>
<td>Incorrect incubation time/temperature</td>
<td>Refer to datasheet for recommended incubation time and/or temperature</td>
<td></td>
</tr>
<tr>
<td>Incorrect amounts used</td>
<td>Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)</td>
<td></td>
</tr>
<tr>
<td>Problem</td>
<td>Reason</td>
<td>Solution</td>
</tr>
<tr>
<td>------------------------------</td>
<td>---------------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Standard curve is not linear</td>
<td>Not fully thawed kit components</td>
<td>Wait for components to thaw completely and gently mix prior use</td>
</tr>
<tr>
<td></td>
<td>Pipetting errors when setting up the standard curve</td>
<td>Try not to pipette too small volumes</td>
</tr>
<tr>
<td></td>
<td>Incorrect pipetting when preparing the reaction mix</td>
<td>Always prepare a master mix</td>
</tr>
<tr>
<td></td>
<td>Air bubbles in wells</td>
<td>Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates</td>
</tr>
<tr>
<td></td>
<td>Concentration of standard stock incorrect</td>
<td>Recheck datasheet for recommended concentrations of standard stocks</td>
</tr>
<tr>
<td></td>
<td>Errors in standard curve calculations</td>
<td>Refer to datasheet and re-check the calculations</td>
</tr>
<tr>
<td></td>
<td>Use of other reagents than those provided with the kit</td>
<td>Use fresh components from the same kit</td>
</tr>
</tbody>
</table>
UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp

Copyright © 2016 Abcam, All Rights Reserved. The Abcam logo is a registered trademark.
All information / detail is correct at time of going to print.