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.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 5b, Last Updated: 23 May 2024

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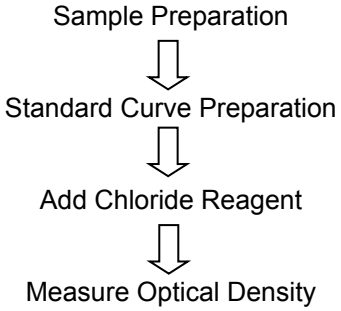
1. Overview

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 λ_{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



3. Components and Storage

A. Kit Components

Item	Quantity
Chloride Reagent	15 mL
Chloride Standard/Chloride Standard (10 µmol; Lyophilized)	1 vial

* 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 dH_2O 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:

Sample Chloride concentrations can vary over a rather wide range.

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. Tissue samples should be diluted 50 – 100-fold. Cell samples should be diluted 30-100-fold.

Liquid or solution samples: should be diluted 10-100X.

Take 10-50 µl samples and adjust the well volume to 50 µl with dH_2O.

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

Concentration = Ay / Sv (nmol/µl, or µmol/ml, or mM)

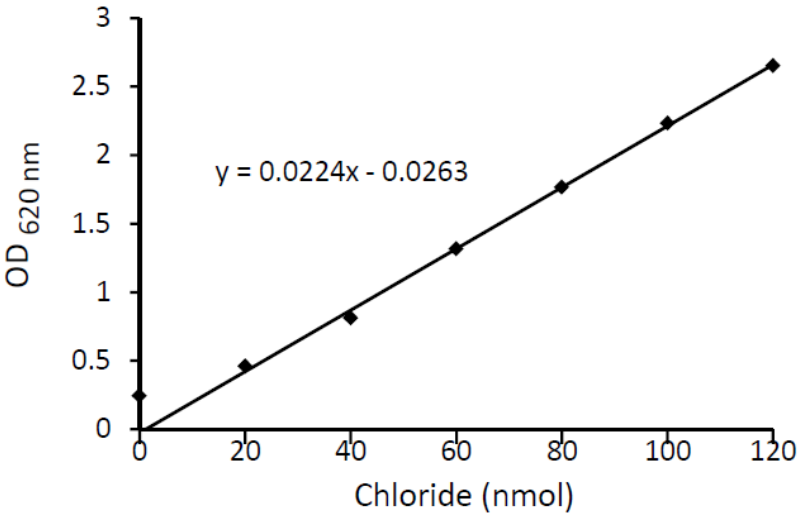
Where:

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

Problem	Reason	Solution
Assay not working	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Refer to datasheet for instructions)

	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	**Reason**	**Solution**
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use

	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)