

Chloride Assay Kit (Colorimetric)

LS-K548-100 (100 Tests) • Store at RT



Introduction

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 is 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. LSBio'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 OD 620nm. The assay is linear in the range 20 to 120 nmol Chloride/well with detection sensitivity ~ 0.4 mM chloride.

Components

Component	K548-100	Cap Code
	100 Tests	
Chloride Reagent	15 ml	WM
Chloride Standard (10 μmol)	Lyophilized	Yellow

Storage Conditions and Reagents Preparation

Store kit at room temperature, keep tightly capped. 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.

Assay Procedure

1. Standard Curve Preparations: 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.
2. Sample Preparation: Sample Chloride concentrations can vary over a rather wide range. Urine and serum 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, it may be necessary to test several different amounts of sample to ensure the readings are within the standard curve.
3. Development: Add 150 μl of the Chloride Reagent to each well containing Chloride Standard or test samples.
4. Incubate at room temperature for 15 min.
5. Reading: Read OD at 620 nm. The signals are stable for many hours.

FOR RESEARCH USE ONLY! Not for use in humans.

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121
www.LSBio.com • (206) 464-1554 • TechnicalSupport@LSBio.com

Chloride Assay Kit (Colorimetric)

LS-K548-100 (100 Tests) • Store at RT



6. Calculation: Subtract the 0 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 0 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 nonlinearity 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:

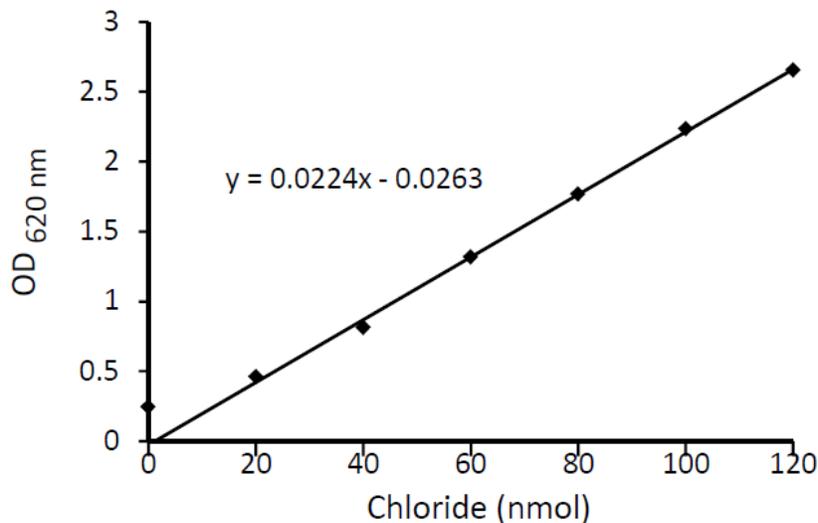
$$C = Ay/Sv \text{ (nmol/}\mu\text{l, or }\mu\text{mol/ml, or mM)}$$

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.

Sample Data



Chloride Standard Curve: Assays were performed following the kit protocol.

Version: V.08.09.2018

FOR RESEARCH USE ONLY! Not for use in humans.

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121

www.LSBio.com • (206) 464-1554 • TechnicalSupport@LSBio.com