Chloride Colorimetric Assay Kit

(Catalog #K530-100; 100 assays; Store at room temperature)

I. 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 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. BioVision'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.

II. Kit Contents:

Components	K530 -100	Cap Code	Part Number
Chloride Reagent Chloride Standard (10 µmol)	15 ml Lyophilized	WM Yellow	K530 -100-1 K530 -100-2

III. Storage and Handling:

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.**

IV. Reagent Preparation and Storage Conditions:

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.

V. Chloride Assay Protocol:

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 hr.
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.

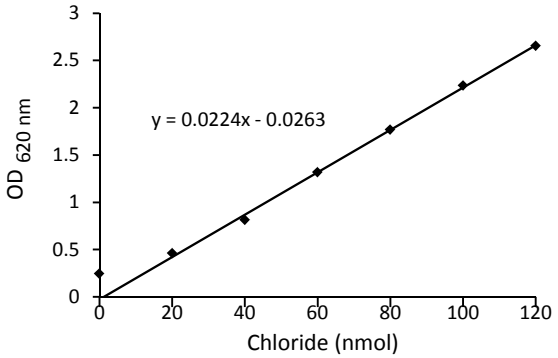
   NOTE: There is a slight nonlinearity below 20 nmol Chloride. Any samples below 20 nmol Chloride should be repeated with 3-5X higher sample.

   The Chloride concentration in the test samples:

   C = Ay/Sv (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.

RELATED PRODUCTS:

Cobalt Assay Kit	Ammonia Assay Kit
Iron Assay Kit	Heme Assay Kit
Phosphate Assay Kit	Sarcosine Assay Kit
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Ethanol assay Kit	Starch Assay Kit
Urea Assay Kit	Uric Acid Assay Kit
ATP & ADP Assays	Sucrose Assay Kit
Glucose Assay Kit	Cholesterol Assay Kit
Lactate Assay Kit	HDL/LDL Assay Kit
Glutathione Assay Kit	Phenylalanine Assay Kit
Nitric Oxide Assay Kit	Oxaloacetate Assay Kit
Pyruvate Assay Kit	Malate Assay Kit
FAD Assay Kit	Maltose Assay Kit
Glycerol Assay Kit	Isocitrate Assay Kit
Lactose Assay Kit	GST Assay Kit
Ascorbic Acid Assay Kit	Acetyl CoA Assay Kit

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