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 HgCl₂ and Fe³⁺ for TPTZ. The preferred Hg-TPTZ adduct exhibits no color. In the presence of Chloride, HgCl₂ forms HgCl₃ which then binds the available Fe³⁺ giving a very intense absorbance with a OD 620 nm. The assay is linear in the range 20 to 120 nmol Chloride/well with detection sensitivity ~0.4 mM chloride.

II. Kit Contents:

<table>
<thead>
<tr>
<th>Components</th>
<th>K530 -100</th>
<th>Cap Code</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloride Reagent</td>
<td>15 ml</td>
<td>WM</td>
<td>K530 -100-1</td>
</tr>
<tr>
<td>Chloride Standard (10 µmol)</td>
<td>Lyophilized</td>
<td>Yellow</td>
<td>K530 -100-2</td>
</tr>
</tbody>
</table>

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 dH₂O 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 dH₂O. 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.

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

VI. Calculation:
6. 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 3F5X higher sample.

The Chloride concentration in the test samples:

\[ C = Ay/Sv \text{ (nmol/µl, or } \mu\mu \mu\mu\text{mol/ml, or mM)} \]

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 mM chloride in the original sample.

RELATED PRODUCTS:
- Cobalt Assay Kit
- Iron Assay Kit
- Phosphate Assay Kit
- Calcium Assay Kit
- Ethanol assay Kit
- Ethanol Kit
- ATP & ADP Assays
- Urea Assay Kit
- Glucose Assay Kit
- Lactate Assay Kit
- Glutathione Assay Kit
- Nitric Oxide Assay Kit
- Pyruvate assay Kit
- FAD assay Kit
- Glycerol assay Kit
- Lactose assay Kit
- Ascorbic Acid assay Kit
- Ammonia Assay Kit
- Heme Assay Kit
- Sarcosine Assay Kit
- Sialic Acid Assay Kit
- Phenylalanine Assay Kit
- Oxalacetic Acid Kit
- Malate assay Kit
- Sucrose assay Kit
- Cholesterol Assay Kit
- HDL/LDL Assay Kit
- Phenyalanine Assay Kit
- Acetyl CoA assay Kit

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