

Technical Bulletin

Chloride Assay Kit

Catalog number MAK510

Product Description

Chloride is the major extracellular anion in human body fluids. Chloride plays a key role in maintaining proper water distribution, osmotic pressure, and electrolyte balance. Low chloride concentrations may be found with prolonged vomiting, extensive burns, metabolic acidosis, Addisonian crisis and renal diseases. Elevated chloride concentrations are associated with dehydration, congestive heart failure, hyperventilation, and urinary obstructions. Determination of chloride in sweat is useful in diagnosing cystic fibrosis.

Simple, direct, and automation-ready procedures for measuring chloride concentration in biological samples are becoming popular in Research and Drug Discovery. The chloride assay kit is designed to measure chloride directly in biological samples without any pretreatment. The improved Fried method utilizes mercuric 2,4,6-tripyridyl-s-triazine, which forms a colored complex specifically with chloride. The intensity of the color, measured at 610 nm, is directly proportional to the chloride concentration in the sample. The optimized formulation substantially reduces interference by substances in the raw samples.

The linear detection range of the kit is 0.7 mg/dL (0.2 mM) to 35 mg/dL (10 mM) Cl⁻. This kit is suitable for Chloride activity determination in food, beverages, and environmental samples such as water, and soil. It can also be used on samples such as serum and plasma, urine, saliva, sweat, as well as in determining the effects of drugs on chloride metabolism.

Components

The kit is sufficient for 250 colorimetric assays in 96-well plates.

- Reagent 50 mL
Catalog Number MAK510A
- Chloride Standard (35 mg/dL Cl⁻) 1 mL
Catalog Number MAK510B

Equipment Required but Not Provided

- Pipetting devices and accessories (for example multichannel pipettor)
- Multiwell plate reader, Clear flat-bottom 96-well plates for colorimetric assay. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL centrifuge tubes

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped at room temperature. Store components at 2-8 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

Important: Equilibrate to room temperature and shake well prior use.

Procedure

All Samples and Standards should be run in duplicate.

Sample preparation

1. Serum, plasma, urine, and milk Samples should be diluted 20-fold in water.
2. Water Samples can be assayed undiluted.

Colorimetric Standard Curve Preparation

1. Dilute Chloride Standard (35 mg/dL Cl⁻) in purified water as shown in Table 1.
2. Store diluted Standards at 4 °C for future use.

Table 1.

Preparation of Chlorine standards

Well	Chloride Standard	Purified Water	Cl ⁻ (mg/d L)
1	100 µL	0 µL	35.0
2	80 µL	20 µL	28.0
3	60 µL	40 µL	21.0
4	40 µL	60 µL	14.0
5	30 µL	70 µL	10.5
6	20 µL	80 µL	7.0
7	10 µL	90 µL	3.5
8	0 µL	100 µL	0

Measurement

Using 96-well plate:

1. Transfer 5 µL diluted Standards and Samples to wells of a clear bottom 96-well plate.
2. Add 200 µL reagent and tap lightly to mix.
3. Incubate 5 min at room temperature and read optical density (OD) at 610 nm (550 nm - 650 nm).

Using cuvette:

1. Set up test tubes labeled Standards and Samples.
2. Transfer 10 µL diluted standards and samples to appropriately labeled tubes.
3. Add 1000 µL Reagent and vortex to mix.
4. Incubate 5 min at room temperature, transfer to cuvette and read OD at 610 nm.

Results

1. Calculate ΔOD by subtracting the reading of Standard #8 (Blank) from the remaining Standard reading values.
2. Plot the ΔOD against the standard concentrations and determine the slope of the standard curve.
3. Calculate Chloride concentration of the sample using the below given equation:

$$[\text{Chloride}] = \frac{R_{\text{Sample}} - R_{\text{Blank}}}{\text{Slope}} \times DF$$

where:

R_{Sample} = OD reading of Sample

R_{Blank} = OD reading of Blank

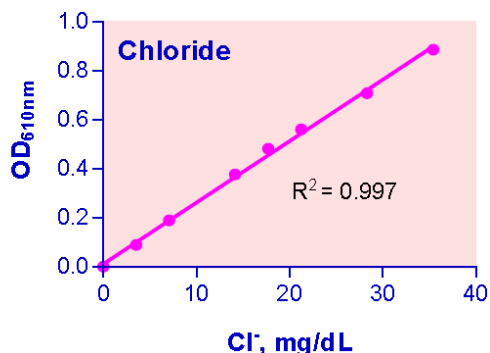
DF = Sample dilution factor (DF = 1 for undiluted Samples)

(DF = 20 for serum, plasma, milk, urine that are diluted 20-fold)

Conversions: 1 mg/dL Cl⁻ equals 282 µM, 0.001% or 10 ppm.

Figure 1.

Typical Colorimetric Chloride Standard Curve in purified water.



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