QuantiChrom™ Chloride Assay Kit (DICL-250)

Quantitative Colorimetric Chloride Determination at 610nm

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, Addisonia 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. BioAssay Systems' 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 610nm, is directly proportional to the chloride concentration in the sample. The optimized formulation substantially reduces interference by substances in the raw samples.

KEY FEATURES

Sensitive and accurate. Use as little as 5 µL samples. Linear detection range 0.7 mg/dL (0.2mM) to 35 mg/dL (10mM) Cl^- in 96-well plate assay.
Simple and high-throughput. The procedure involves addition of a single working reagent and incubation for 5 min. Can be readily automated as a high-throughput assay for thousands of samples per day.
Improved reagent stability and versatility. The optimized formulation has greatly enhanced reagent and signal stability. Cuvet or 96-well plate assay.
Low interference in biological samples. No pretreatments are needed. Assays can be directly performed on raw biological samples i.e., in the presence of lipid, protein and minerals such as magnesium, iron and zinc.

APPLICATIONS

Direct Assays: Cl^- in serum, plasma, urine, saliva, sweat etc.
Drug Discovery/Pharmacology: effects of drugs on chloride metabolism.
Food and Beverages: chloride determination.
Environment: chloride determination in water and soil.

KIT CONTENTS (250 tests in 96-well plates)

Reagent: 50 mL Chloride standard: 1 mL 35 mg/dL Cl^-

Storage conditions. The kit is shipped at room temperature. Store Reagent and Standard at 4°C. Shelf life: 12 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Important: bring reagents to room temperature and shake well before use.

Procedure using 96-well plate:

1. Dilute standards in distilled water as shown in the table. Serum, plasma, urine and milk samples should be diluted 20-fold in water. Transfer 5 µL diluted standards and samples to wells of a clear bottom 96-well plate. Store diluted standards at 4°C for future use.

No	STD + H_2O	Vol (µL)	Cl^- (mg/dL)
1	100µL + 0µL	100	35.0
2	80µL + 20µL	100	28.0
3	60µL + 40µL	100	21.0
4	40µL + 60µL	100	14.0
5	30µL + 70µL	100	10.5
6	20µL + 80µL	100	7.0
7	10µL + 90µL	100	3.5
8	0µL + 100µL	100	0

2. Add 200 µL working reagent and tap lightly to mix.
3. Incubate 5 min at room temperature and read optical density at 610 nm (550-650nm nm).

Procedure using cuvette:

1. Set up test tubes labeled Standards and Samples. Transfer 10 µL diluted standards and samples to appropriately labeled tubes.
2. Add 1000 µL working reagent and vortex to mix. Incubate 5 min, transfer to cuvet and read OD at 610nm.

CALCULATION

Subtract blank OD (water, #8) from the standard OD values and plot the OD against Cl^- standard concentrations. Determine the slope using linear regression fitting. Chloride concentration of the sample is calculated as

$$= \frac{OD_{SAMPLE} - OD_{BLANK}}{Slope} \times n \quad (mg/dL)$$

OD_{SAMPLE} and OD_{BLANK} are OD_{610nm} values of sample and sample blank (water or buffer in which the sample was diluted). *n* is the dilution factor (*n* = 20 for serum, plasma, milk, urine).
Conversions: 1 mg/dL Cl^- equals 282 µM, 0.001% or 10 ppm.

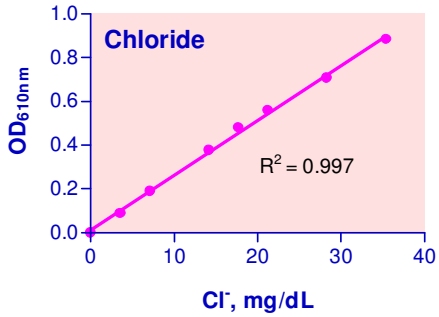
MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices and accessories (e.g. 5 µL), clear flat-bottom 96-well plates and plate reader, or spectrophotometer and cuvets.

EXAMPLES (96-well plate assay):

	Cl^- (mg/dL)
1	324 ± 5
2	341 ± 3
3	127 ± 1
4	5.71 ± 0.09
5	0.27 ± 0.17
6	< 0.17
7	< 0.17
8	< 0.17
9	0.25 ± 0.11

Biological Samples: 1. Human serum. 2. Fresh human urine. 3. Commercial 2% reduced fat milk (Kirkland). **Water samples:** 4. Tap water (Hayward, CA). 5. Tap water (San Bruno, CA). **Food and Beverages:** 6. Crystal Geyser natural alpine spring water. 7. Coca-cola® classic coke. 8. Lipton Lemon iced tea. **Environmental:** 9. Soil extract. 5.6 g of soil (Hayward, CA) was extracted with 10 mL MilliQ water. The supernatant was centrifuged to remove any insoluble particles. Clear supernatant was assayed.



Standard Curve in 96-well plate assay

PUBLICATIONS

1. Ahmad, T. et al (2009). Influence of varying dietary electrolyte balance on broiler performance under tropical summer conditions. J Anim Physiol Anim Nutr (Berl). 93(5):613-621.

2. Sink, T.D. and Neal, J.W. (2009). Stress response and posttransport survival of hybrid striped bass transported with or without clove oil. North American Journal of Aquaculture 2009; 71: 267-275.

3. Borenshtein, D. et al. (2009). Decreased expression of colonic Slc26a3 and carbonic anhydrase iv as a cause of fatal infectious diarrhea in mice. Infect Immun. 77(9):3639-50.