Chloride Assay Kit (Colorimetric/Fluorimetric)

LS-K213-250 (250 Tests) • **Store at 4°C**

Introduction

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. LSBio's 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. Linear detection range in 96-well plate: 0.1 to 50 U/L for colorimetric assays and 0.01 to 2 U/L for fluorimetric assays run at 25°C for 30 min.

Applications

- 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.

Components

Component	K213-250
	250 Tests
Reagent	50 mL
Chloride Standard (35 mg/dL Cl^-)	1 mL

Materials Not Supplied

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

Storage

The kit is shipped at room temperature. Store Reagent and Standard at 4°C.

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

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 610nm (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.

Calculations

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

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

ODSAMPLE and ODBLANK are OD610nm 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.

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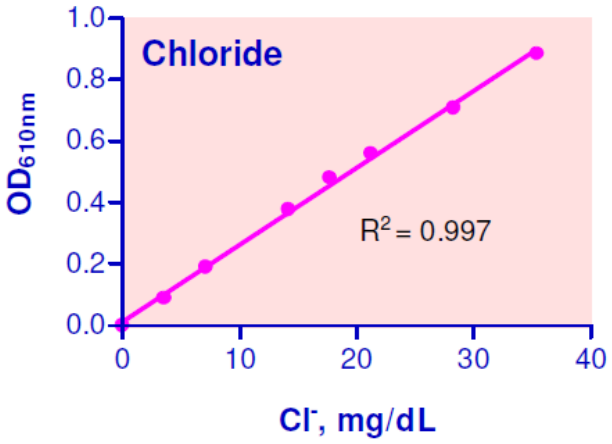
Sample Data

	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

Version: V.08.09.2018

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