CATACHEM

CHLORIDE REAGENT KIT
C195-0A

Contents	Product No.	Package
CHLORIDE REAGENT KIT	**C195-0A**	
Chloride Sample Diluent (R1)	C195-01	3 x 60 mL
Chloride Color (R2)	C195-02	3 x 20 mL

REAGENT PREPARATION

The Catachem reagents are packaged ready for use.
No preparation of either reagent is required.

REAGENT STORAGE AND STABILITY

Store unopened reagents at 15-30°C.
When stored as directed, both unopened reagents are stable until the expiration date stated on the label.

NOT FOR USE IN UNPROFESSIONAL SETTINGS

FOR TECHNICAL ASSISTANCE:
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CHLORIDE REAGENT KIT
C195-0A
MANUAL/AUTOMATED APPLICATION

Intended Use
For **IN VITRO quantitative** determination of Chloride in serum using manual or automated applications.

Clinical Significance
Determinations of Chloride are primarily used for diagnosing chronic pyelonephritis, metabolic acidosis, dehydration, congestive heart failure, and hypercalcemia, as well as for monitoring the causes and treatments. (1)

Method History
In 1956 D.M. Zall, et al (2), published a Chloride method for small amounts of Chloride in water. The method of Zall, et al, suffers from two major disadvantages. The method is non-linear and mercury is discharged to waste as a pollutant. A non-mercurometric linear Chloride method was published by B. Fingerhut (3) in 1972. Catachem's Chloride method is based upon the work reported by Fingerhut.

Method Principle
Chloride ions present in the serum sample interact with Ferric ions in dilute acid to form a yellow chloro-complex with maximum absorption at 340nm. The intensity of the color thus produced is directly proportional to the concentration of the Chloride ion in the serum sample. The reaction scheme below illustrates the reaction that occurs in this method.

$$Fe + 6\,Cl \longrightarrow Fe(Cl)_6$$

Reagent Content
The concentrations of the active ingredients in the reagents are approximately as follows.

Chloride Acid Reagent (R1)
Each liter contains:

Methane Sulfonic Acid	1.56 Mol/L
Ferric nitrate	25 mM/L

Surfactant and non-reactive ingredients

Chloride Color Reagent (R2)

Methane Sulfonic Acid	1.56 Mol/L
Ferric Nitrate	150 mM/L

Surfactant and non-reactive ingredients

Precautions
Avoid contact of reagent with skin and eyes. Should contact with skin and eyes occur, wash affected area with plenty of cold water. **DO NOT PIPETTE REAGENTS BY MOUTH.**

Preparation Of Working Reagents
Catachem Chloride Reagents are packaged in ready-to-use form. No preparation is required.

Reagent Storage And Stability
Store Catachem Chloride Reagents at 15-30°C. When stored as directed, the reagents are stable until the expiration date stated on the label. The Catachem Chloride Reagents have been tested to reflect shipping conditions and are stable for the lifespan of the product if frozen up to 5 times or upon reaching temperatures up to 40°C for up to one week.

Specimen Collection And Preparation
Test sera should be fresh, clear, and unhemolyzed. When blood is drawn, it should be processed as soon as possible and the serum should be isolated from the clot without delay.

Quality Control
To monitor the performance of the Reagent and the procedure used, we recommend the regular use of a normal and abnormal control serum.

Interfering Substances
Several substances have been reported to interfere with the Chloride determination in serum. (4) The following substances, if present in the sample, reagents, or system can produce erroneous results. a) the bromide ion interferes in any quantity. Hemolysis normally will decrease the Chloride values; b) anticoagulants should be avoided. A summary of the influence of drugs on clinical laboratory procedures may be found by consulting D.S. Young, et al. (4)

Expected Values
The range of expected values determined for this method in humans is 96-106 meq/L. These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located and for the species under test.

Directions For Use
Catachem Chloride method requires two reagents. The reagents are packaged ready for use. No preparation is necessary.

Materials Required (Not Provided)

Spectrophotometer	
Cuvettes	1 cm light path
Timer	to time incubation time
Pipette	1 ml adjustable for reagent
Pipette	0.1 ml adjustable for sample

Materials Provided
Chloride Reagent

Analytical Parameters

Wavelength	340nm
Pathlength	1 cm
Reaction Mode	endpoint
Reaction Time	2 minutes
Reagent Volume R1	0.5 ml
Reagent Volume R2	0.17 ml
Sample Volume	0.03 ml
Total Volume	0.7 ml
Sample-to-Reagent Ratio	1:23

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Assay Procedure

1. Pipette 0.5 ml of Chloride R1 Reagent into each of three cuvettes marked “calibrator”, “sample” and “blank”.
2. Pipette 0.17 ml of Chloride R2 Reagent into the same cuvettes marked “calibrator”, “sample” and “blank”.
3. Incubate for approx.1 minute
4. Set spectrophotometer wavelength at 340 nm and read all cuvettes.
3. Pipette 0.03 ml of calibrator or sample into their respective cuvettes. Use 0.03 ml of distilled water for the blank. Mix all cuvettes well.
4. Incubate all cuvettes for 1 minute at room temperature.
4.At spectrophotometer wavelength of 340 nm re-read the absorbencies of all the cuvettes.
6. Calculate the Chloride concentration (meq/L) in the sample(s), as shown in calculations and results.

Calculations And Results

$$\text{Chloride (meq/L)} = \frac{\text{Sample Absorbance} - \text{Blank Absorbance}}{\text{Calibrator Absorbance- Blank Absorbance}} \times \text{Calibrator(meq/L)}$$

Example:

	Assay OD
Sample-Blank	0.240
Calibrator-Blank	0.250
Calibrator	= 100 meq/L

$$\text{Chloride (meq/L)} = \frac{0.240}{0.250} \times 100 \text{ meq/L} = 96 \text{ meq/L}$$

Method Performance Characteristics

Sensitivity: The sensitivity of this method is 0.004 - 0.0045 absorbance units per meq/L.
Linear Range: In this method there is no significant nonlinearity over the range of 80-130 meq/L.
Precision: Within-run and day-to-day precision is summarized below.

Precision Study

Chloride meq/L	Total Precision SD	Total CV%
83	**1.22**	**1.46**
109	**1.68**	**1.54**
133	**1.97**	**1.97**

Chloride meq/L	Within-run Precision SD	Within-run CV%
83	**0.42**	**0.51**
109	**0.81**	**0.77**
133	**0.79**	**0.59**

Correlation

A comparison of this method using an automated analyzer running a similar chloride chemistry resulted in the following regression statistics.

Range	=	82-126 meq/L
N	=	121
Y	=	0.993x + 1.9
r	=	0.972
Sy.x	=	1.7

References

1. Fundamentals of Clinical Chemistry. Edited by Norbert Teitz. W.V Saunders, Philadelphia (1976).
2. Zall D.M, Fisher D, Garner MQ. Photometric determination of chlorides in water. Anal Chem 28:166 5-1668 (1956).
3. Fingerhut, B. A known mercurometric automated method for serum chloride. Clin Chim Acta 41 :247-253 (1972).
4. Young D.S, Pestaner LC, Gibberman V. Effect of drugs on clinical laboratory tests. Clin Chem 21 (5): ID-432D (1975).

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