3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Chloride Assay Kit

Catalog Number **MAK023**
Store at Room Temperature

TECHNICAL BULLETIN

Product Description
Chloride is an essential anion needed for the proper functioning of many critical aspects of metabolism, including aiding in maintenance of the body's acid-base balance. In Cystic Fibrosis, mutations to the CF transmembrane conductance regulator (*CFTCR*) gene result in altered sodium and chloride ion transport channels.

The Chloride Assay kit provides a simple and direct procedure for measuring chloride in a variety of samples, including blood and urine. Chloride concentration is determined by a competition reaction between Hg^{2+} and Fe^{2+} for 2,4,6-Tris(2-pyridyl)-*s*-triazine(TPTZ). The preferred Hg-TPTZ complex exhibits no color. In the presence of chloride, Hg^{2+} forms $HgCl_2$, which precipitates, allowing TPTZ to complex with Fe^{2+}. The Fe-TPTZ complex results in a colorimetric (620 nm) product proportional to the chloride present.

Components
The kit is sufficient for 100 assays in 96 well plates.

Chloride Reagent Catalog Number MAK023A	15 mL
Chloride Standard Catalog Number MAK023B	1 vl

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Note: This kit contains small amounts of mercury. Waste generated from using this kit should be disposed of properly.

Preparation Instructions
Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents.

Chloride Reagent – Ready to use as supplied. Stable for 6 months when stored at room temperature.

Chloride Standard – Reconstitute with 1 mL of water to generate a 10 mM standard solution. Mix well by pipetting, then aliquot and store, protected from light, at room temperature.

Storage/Stability
The kit is shipped and storage at room temperature, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Chloride Standards for Colorimetric Detection
Add 0, 2, 4, 6, 8, and 10 µL of the 10 mM standard solution into a 96 well plate, generating 0 (blank), 20, 40, 60, 80, and 100 nmole/well standards. Add water to each well to bring the volume to 50 µL.

Sample Preparation
Urine and serum samples should be diluted 10 to 100-fold. Add 10–50 µL of sample to wells. Bring samples to a final volume of 50 µL with water.

Note: Sample chloride concentrations can vary over a wide range. For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Assay Reaction
1. Add 150 µL of the Chloride Reagent to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 15 minutes at room temperature. Cover the plate and protect from light during the incubation.

2. Measure the absorbance at 620 nm (A_{620}).

Results
Calculations
The background for the assay is the value obtained for the 0 (blank) Chloride standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings.

Use the values obtained from the appropriate Chloride standards to plot a standard curve.
Note: A new standard curve must be set up each time the assay is run.

Using the corrected measurement, the amount of chloride present in the samples may be determined from the standard curve.
Note: There is slight nonlinearity below 20 nmoles of chloride. Samples falling below 20 nmoles should be repeated with 3 to 5-fold higher sample.

Concentration of Chloride

$$S_a/S_v \times D = C \text{ (nmole/µL, µmole/mL, or mM)}$$

S_a = Amount of chloride in unknown sample (nmole) from standard curve
S_v = Sample volume (µL) added to reaction well
D = Dilution of original sample
C = Concentration of chloride in sample

Chloride atomic weight: 35.5 g/mole

Sample Calculation
Amount of chloride (S_a) = 45.8 nmole (from standard curve)
Sample volume (S_v) = 50 µL

Concentration of chloride in sample
45.8 nmole/50 µL = 0.916 nmole/µL

$$0.916 \text{ nmole/µL} \times 35.5 \text{ ng/nmole} = 32.52 \text{ ng/µL}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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