TECHNICAL MANUAL

Chlorine (Cl) Colorimetric Assay Kit

- **SKU CODE:** MAES0139
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Measure OD values

2. Intended use

This kit can be used to measure chlorine ion (Cl-) content in serum, plasma and animal tissue samples.

3. Detection principle

Chloride ions in biological fluids are replaced by mercury ions in mercury thiocyanate through ion replacement, which results in the formation of difficult-to-dissociate mercury chloride. The substituted thiocyanate ions combine with ferric nitrate to form a red complex. The content of chlorine ion can be calculated indirectly by measuring the OD value at 460 nm.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	100 mmol/L Standard Solution	1 mL x 1 vial	2-8 °C, 12 months
Reagent 2	Chromogenic Agent A	10 mL x 1 vial	2-8 °C, 12 months
Reagent 3	Chromogenic Agent B	20 mL x 1 vial	2-8 °C, 12 months, shading light
Reagent 4	Chromogenic Agent C	1 mL x 1 vial	2-8 °C, 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (440-480 nm, optimum wavelength: 460 nm), Micropipettor, Incubator, Vortex mixer, Centrifuge

Reagents:

Double distilled water

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. Preheat Chromogenic Agent B for 2-3 min in 90-95 °C water bath before use.
3. **Preparation of working solution:** Before testing, prepare sufficient working solution according to the test wells. For example, prepare 306 µL of working solution (mix well 100 µL of Chromogenic Agent A, 200 µL of Chromogenic Agent B and 6 µL of Chromogenic Agent C). The working solution should be prepared fresh.
4. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 100 mmol/L standard solution with double distilled water to serial concentrations. The recommended dilution gradient is: 0, 5, 10, 20, 30, 40, 50, 60 mmol/L.

7. Sample preparation

Sample preparation:

Serum (plasma): Detect directly. If not detected on the same day, the serum or plasma can be stored at -80 °C for a month.

Tissue samples:

1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

2. Wash tissue in cold PBS (0.01 M, pH 7.4).

3. Homogenize 20 mg tissue in 180 µL double distilled water with a dounce homogenizer at 4 °C.

4. Centrifuge at 10,000 xg for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

The recommended dilution factor for different samples is as follows (for reference only):

- 10% Mouse liver tissue homogenate: 2-3
- 10% Mouse kidney tissue homogenate: 2-3
- 10% Rat spleen tissue homogenate: 2-3
- Human serum: 5-10
- Mouse serum: 5-10
- Cynomolgus monkey serum: 5-10

Note: The diluent is double distilled water. For the dilution of other sample types, please perform a pre-test to confirm the dilution factor.

8. The key points of the assay

1. Prevent the formation of bubbles when adding liquid to the microplate.
2. It is recommended to use double distilled water instead of normal saline or phosphate buffered solution to prepare tissue homogenate and avoid chlorine ion contamination.

9. Operating steps

1. Standard well: Add 10 µL of standard solution with different concentrations to the corresponding wells. Sample well: Add 10 µL of sample to the corresponding wells.
2. Add 250 µL of working solution to each well.
3. Stand at room temperature for 5 min, and measure the OD value of each well at 460 nm with microplate reader.

10. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #1) from all standard readings. This is the absolute OD value.

3. Plot the standard curve using absolute OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

The sample:

1. Liquid sample:

Chlorine ion content (mmol/L) = (deltaA$_{460}$ - b) ÷ a x f

2. Tissue sample:

Chlorine ion content (mmol/gprot) = (deltaA$_{450}$ - b) ÷ a x f ÷ C_{pr}

[Note]

f: Dilution factor of sample before test.

deltaA$_{460}$: OD_{Sample} - OD_{Blank}.

C_{pr}: Concentration of protein in sample (gprot/L).

11. Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Recovery

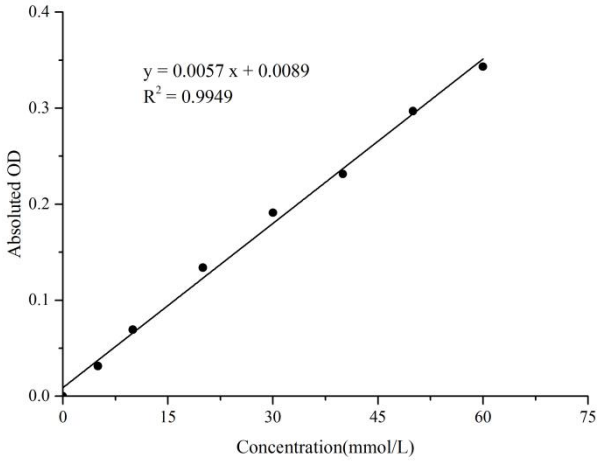
Three samples of high concentration, middle concentration and low concentration were tested with each concentration analyzed 6 times in parallel to obtain an average recovery rate of 105%.

Sensitivity

The analytical sensitivity of the assay is 1 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), the standard curve and data are provided below for reference only.



12. Appendix II Example Analysis

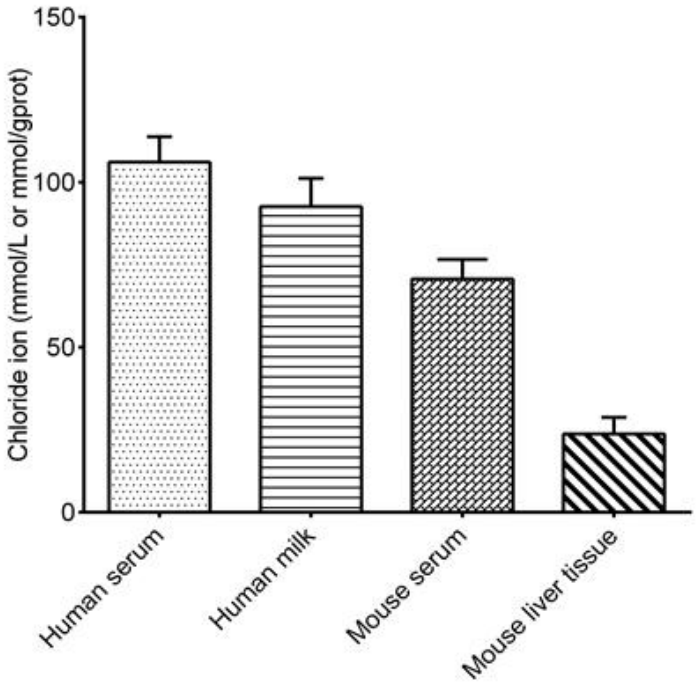
Example analysis:

For human serum, take 10 µL of sample diluted with double distilled water 5 times, and perform the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0057 x + 0.0089, the average OD value of the sample is 0.194, the average OD value of the blank is 0.064, and the calculation result is:

Cl- content (mmol/L) = (0.194 - 0.064 - 0.0089) ÷ 0.0057 x 5 = 106.23 mmol/L

Detect human serum (diluted 5 times), human milk (diluted 5 times), mouse serum (diluted 5 times) and 10% mouse liver tissue homogenate (protein concentration is 7.54 gprot/L, diluted 2 times) according to the protocol, the results are as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.