Technical Manual

Chlorine (Cl) Colorimetric Assay Kit

- **Catalogue Code: MAES0139**
- **Size: 96T**
- **Research Use Only**

1. Key Features and Sample Types:

Detection method:

Colorimetric method

Specification:

96T

Range:

1.0-60 mmol/L

Sensitivity:

1 mmol/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background:

Chlorine ion is the main anion in extracellular fluid. About 70% of the chlorine intake by human body exists in plasma, intercellular fluid and lymph, only a small amount exists in intracellular fluid and cells that secreting Cl^-, and the another part exists in connective tissue and collagen fibers. The main physiological function of chloride ion is basically the same as that of sodium ion, which plays the same role in maintaining electrolyte balance and osmotic pressure balance in the body.

3. Intended Use:

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

4. Detection Principle:

Chloride ion in biological fluids are replaced by the mercury ions in mercury thiocyanate through ion replacement, which resulted in the formation of difficult-to-dissociate mercury chloride. The substituted thiocyanate ions were combined 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.

5. Kit Components & Storage:

Item	Specification	Storage
Standard Solution (100 mmol/L)	1 mL×1 vial	2-8°C, 6 months
Chromogenic Agent A	10 mL × 1 vial	2-8°C, 6 months
Chromogenic Agent B	20 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Chromogenic Agent C	1 mL × 1 vial	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (440-480 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water

6. Assay Notes:

1. Prevent the formation of bubbles when adding the liquid to the microplate.

2. It is recommended to use double distilled water instead of normal saline or phosphate buffered solution to prepare tissue homogenates and prevent chlorine ion pollution.

7. Reagent preparation:

1. Bring 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:** Mix the chromogenic agent A: chromogenic agent B: chromogenic agent C at the ratio of 50: 100: 3 fully. Prepare the fresh solution before use.

8. Sample Preparation:

1. Serum sample:

Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) and preserve on ice before detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:

Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) and preserve on ice before detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. Tissue sample:

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Use filter paper to absorb excess water and weigh. Homogenize at the ratio of the volume of double distilled water (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (1.0-60 mmol/L).

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

Sample Type:	Dilution Factor:
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;

9. Assay Protocol:

Ambient Temperature: 25-30°C

Optimum detection wavelength: 460 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

10. Operation Steps:

The preparation of standard curve

Dilute 100 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 20, 30, 40, 50, 60 mmol/L.

The measurement of samples

1. **Standard well:** Take 10 μL of standard solution with different concentration to the corresponding well.
   Sample well: Take 10 μL of sample to the corresponding well.

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.

Operation Table

	Standard well	Sample well
Standard solution with different concentration (μL)	10	
Sample (μL)		10
Working solution (μL)	250	250
Stand at room temperature for 5 min, and measure the OD value of each well at 460 nm with microplate reader.		

11. Calculations:

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

1. Liquid sample:

Chlorine ion content (mmol/L) =

$(\Delta A_{460} - b) \div a \times f$

2. Tissue sample:

Chlorine ion content (mmol/gprot) =

$(\Delta A_{450} - b) \div a \times f \div C_{pr}$

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0).
x: The concentration of standard.
a: The slope of standard curve .
b: The intercept of standard curve
f: Dilution factor of sample before test
ΔA_{460}: $OD_{Sample} - OD_{Blank}$
C_{pr}: Concentration of protein in sample (gprot/L)

12. Performance Characteristics:

Detection Range	1.0-60 mmol/L
Sensitivity	1 mmol/L
Average recovery rate (%)	105
Average inter-assay CV (%)	6.4
Average intra-assay CV (%)	3.6

Analysis

For human serum, take 10 μL of diluted sample with double distilled water for 5 times, then carry the assay according to the operation table.

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 × 5

= 106.23 mmol/L

Safety Notes

Some of the reagents in the kit contain dangerous substances. Avoid touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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