



Chlorine Assay Kit

(Cat/No.:BC066 Size:96T)

1. Principle of the Assay (Microplate method)

Chloride ions react with mercuric thiocyanate to form a colored complex, and the color intensity is proportional to the chloride ion concentration.

2. Kit Components(The kit is valid for 6 months)

Reagent	Volume	Component	Storage
Reagent 1	0.1 mL ×1 vial	100 mmol/L (355 mg/dL) chloride standard solution	2–8°C
Reagent 2	30 mL ×1 bottle	Working solution	2–8°C, protected from light

3. Sample Requirements

1. Samples should be collected and processed according to routine laboratory procedures. Samples may include serum, plasma, cerebrospinal fluid, tissue (cell/bacteria) homogenates, and urine.
2. Samples are stable for 7 days at 2–8°C and for several months below –20°C.

4. Required Instruments and Reagents

Microplate reader adjustable to 436 nm (or within 426–446 nm) with 96-well plate (one plate provided), deionized water, 37°C water bath or incubator.

5. Assay Procedure

(I). Sample Pretreatment:

Serum (plasma) or other liquid samples: use directly (if solid residues are present, centrifuge at 4000 rpm for 5 min and use the supernatant).

Tissue samples: accurately weigh tissue and add **deionized water** at a ratio of weight (g):volume (mL) = 1:9, homogenize in an ice-water bath, centrifuge at 4000–8000 rpm for 10 min, and collect the supernatant for assay (**protein concentration of animal tissue homogenate supernatant should be determined**).

Bacteria/cell samples: collect bacteria or cells into centrifuge tubes (remove culture medium), add 0.5 mL deionized water per 5×10^6 bacteria or cells, resuspend, sonicate on ice (power 20% or 200 W, 5 s on, 15 s off, repeat 5–10 times), centrifuge at 4000–8000 rpm for 10 min, and collect the supernatant for assay (**protein concentration can be measured if cell counting was performed previously, protein determination may be omitted**).

(II). Operation Table:



	Blank Well	Standard Well	Sample Well
Deionized water (μL)	2.5		
100 mmol/L chloride standard solution (μL)		2.5	
Sample (μL)			2.5
Reagent 2 (μL)	250	250	250
Gently shake the plate to mix, incubate at 37°C for 5 min, then measure absorbance (A) of each well at 436 nm using a microplate reader.			

6. Calculation Formula

1. Calculation for serum (plasma) and other liquid samples:

$$\text{Serum (plasma) chloride content (mmol/L)} = \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{standard}} - A_{\text{blank}}} \times C_{\text{standard}} \times N$$

2. Calculation formula for animal tissue (or cells/bacteria):

$$\text{Chloride ion content (mmol/gprot)} = \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{standard}} - A_{\text{blank}}} \times C_{\text{standard}} \div \text{Cpr}$$

3. Calculation formula for plant tissue:

$$\text{Chloride ion content (mmol/g Tissue)} = \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{standard}} - A_{\text{blank}}} \times C_{\text{standard}} \div \frac{W}{V_{\text{sample total}}}$$

4. Calculation formula for bacteria/cell samples:

$$\text{Chloride ion content (mmol/10}^4\text{ Cell)} = \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{standard}} - A_{\text{blank}}} \times C_{\text{standard}} \div \frac{\text{Total cell number}}{V_{\text{sample total}}}$$

In the above formulas:

C_{standard}: concentration of standard solution, 100 mmol/L;

N: dilution factor before sample testing;

Cpr: protein concentration of tissue homogenate, g_prot/L (prot refers to protein);

W: tissue weight, g;

Total cell number: number of cells before sonication, 10⁴ cells;

V_{sample total}: total volume of extraction solution added during sample pretreatment (homogenization/lysis), L.

7. Technical Parameters

Parameter	Specification	Parameter	Specification
Wavelength range	426–446 nm	Wavelength range	426–446 nm

8. Precautions

1. Avoid chloride contamination during the experiment and pay attention to the laboratory environment.

2. When preparing tissue homogenates, use **deionized water** as the homogenization medium



to avoid chloride contamination. **Do not** use normal saline or other chloride-containing media.

3. When measuring chloride content in animal tissues or cells, it is recommended to determine total protein concentration simultaneously.

4. If sample concentration is too high (>120 mmol/L), dilute the sample with deionized water and re-measure.

5. Avoid introducing air bubbles when adding samples to the plate.

6. This kit is for research use only.