（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K189-M

Specification：96T(80 samples)

Measuring instrument：Microplate reader（440-480 nm）

Detection range：1.0-60 mmol/L

Elabscience® Chlorine (Cl) Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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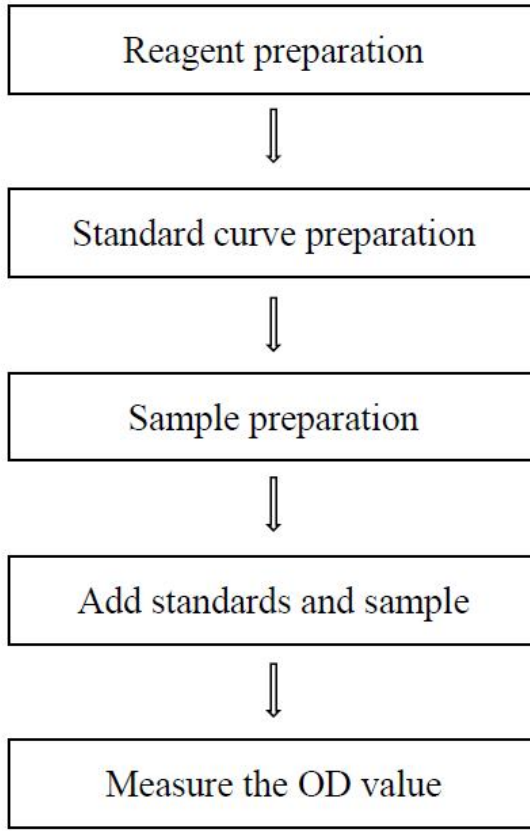
Assay summary



Intended use

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

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.

Kit components & storage

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

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

Double distilled water

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② Preheat chromogenic agent B for 2-3 min in 90-95°C water bath before use.

③ The preparation of working solution：

Before testing, please 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 on spot.

④ The 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 diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 20, 30, 40, 50, 60 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**5**	**10**	**20**	**30**	**40**	**50**	**60**
100 mmol/L standard (μL)	0	10	20	40	60	80	100	120
Double distilled water (μL)	200	190	180	160	140	120	100	80

Sample preparation

① 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 10000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① Prevent the formulation 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 homogenate and avoid chlorine ion pollution.

Operating steps

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.

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 absoluted OD value.

3. Plot the standard curve by using absoluted 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:

$$\text{Chlorine ion content (mmol/L)} = (\Delta A_{460} - b) \div a \times f$$

2. Tissue sample:

$$\text{Chlorine ion content (mmol/gprot)} = (\Delta A_{450} - b) \div a \times f \div C_{pr}$$

[Note]

f: Dilution factor of sample before test.

ΔA_{460}: $OD_{Sample} - OD_{Blank}$.

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

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)

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	15.50	34.80	50.50
%CV	3.9	3.6	3.3

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	15.50	34.80	50.50
%CV	6.1	6.7	6.4

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 105%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	8.4	22	45
Observed Conc. (mmol/L)	8.5	23.8	47.7
Recovery rate (%)	101	108	106

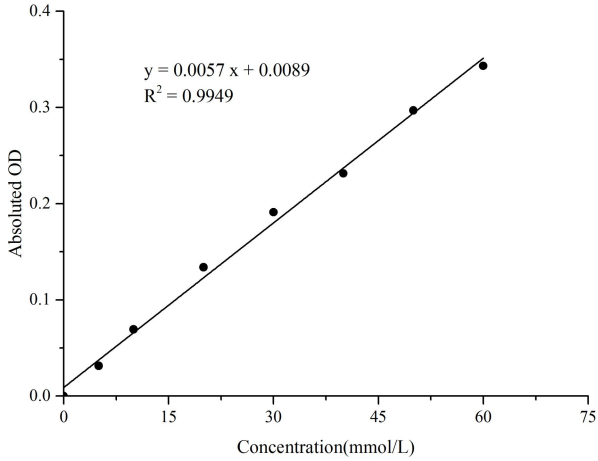
Sensitivity

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.

2. 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), so the standard curve and data are provided as below for reference only.

Concentration (mmol/L)	0	5	10	20	30	40	50	60
Average OD	0.064	0.096	0.134	0.198	0.255	0.296	0.361	0.408
Absoluted OD	0.000	0.032	0.069	0.134	0.191	0.232	0.297	0.343



Appendix II Example Analysis

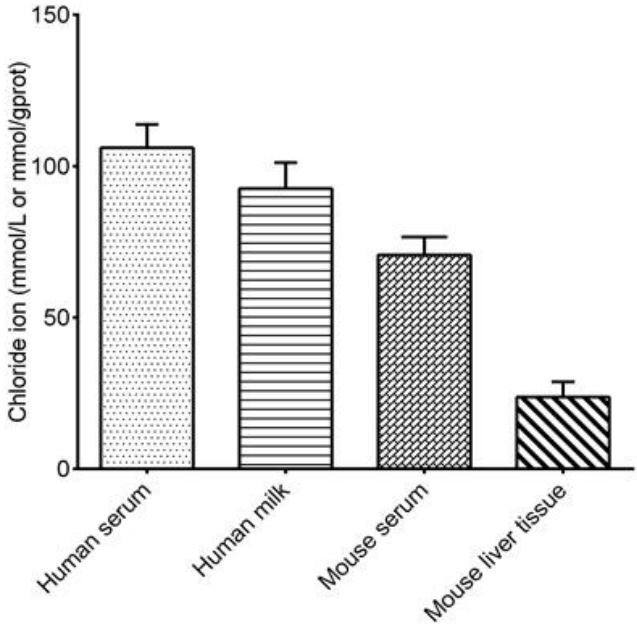
Example analysis：

For human serum, take 10 μL of diluted sample with double distilled water for 5 times, and carry 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 × 5 = 106.23 mmol/L

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



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.