PRODUCT INFORMATION & MANUAL

Chlorine (Cl) Assay Kit (Colorimetric)

NBP3-25924

For research use only.
Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Chlorine (Cl) Colorimetric Assay Kit

Catalog No: NBP3-25924

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 1 mmol/L

Detection range: 1.0-60 mmol/L

Average intra-assay CV (%): 3.6

Average inter-assay CV (%): 6.4

Average recovery rate (%): 105

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

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

▲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.

▲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	Specification	Storage
Reagent 1	100 mmol/L Standard Solution	1 mL×1 vial	2- 8℃ , 12 months
Reagent 2	Chromogenic Agent A	10 mL × 1 vial	2- 8℃ , 12 months
Reagent 3	Chromogenic Agent B	20 mL × 1 vial	2- 8℃ , 12 months, shading light
Reagent 4	Chromogenic Agent C	1 mL × 1 vial	2- 8℃ , 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.			

▲Materials prepared by users

Instruments

Microplate reader (440- 480nm), Micropipettor, Incubator, Vortex mixer, Centrifuge

Reagents

Double distilled water

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

⚠ Precautions

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

▲ 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.

Pre-assay preparation

▲Reagent preparation

1. Bring all the reagents to room temperature before use.
2. Preheat reagent 3 for 2-3 min in 90-95 °C water bath before use.
3. Preparation of working solution:

   Mix the reagent 2: reagent 3: reagent 4 at the ratio of 50: 100: 3 fully.

   Prepare the fresh solution before use.

▲Sample preparation

1. Serum sample:

   Fresh blood was collected and placed at 25℃ for 30 min to clot the blood. Centrifuge the sample at 4℃ for 15 min at 2000 g, the upper yellowish clear liquid was taken as serum. Place the serum on ice for detection. If not detected on the same day, stored the serum at -80℃ , which can be stored for a month.

2. Plasma sample:

   The fresh blood was added into the test tube containing anticoagulant and mixed upside down. Centrifuge the sample at 4℃ for 10 min at 700~1000 g, the upper yellowish transparent liquid was taken as the plasma, and the middle white interference layer (white blood cells and platelets) could not be absorbed. Place the plasma on ice for detection. If not detected on the same day, stored the serum at -80℃ , which can be stored for a month.

3. 10% tissue homogenate sample:

   Accurately weigh the tissue sample, add 9 times the volume of double distilled water according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and 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.

Assay protocol

▲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.

▲Detailed operating steps

1. 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. Reference is as follows:

Number	Standard concentrations (mmol/L)	100 mmol/L Standard (μL)	Double distilled water (μL)
A	0	0	200
B	5	10	190
C	10	20	180
D	20	40	160
E	30	60	140
F	40	80	120
G	50	100	100
H	60	120	80

2. 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.

▲Summary 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 at 460 nm.		

▲Calculation

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.

liquid sample:

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

Tissue sample:

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

Note:

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).

Appendix I Data

▲Example analysis

For human serum, take 10 μL of sample diluted with double distilled water for 5 times, and 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

Appendix II References

1. Hamilton R H . A direct photometric method for chloride in biological fluids, employing mercuric thiocyanate and perchloric acid[J]. Clinical Chemistry, 1966, 12(1): 1-17.
2. Merchant M . Miniaturization of a chloride ion assay for use in a microtiter format[J]. Microchemical Journal, 2009, 92(1): 80-82.