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

Catalog No：E-BC-K940-M

Specification：48T (32 samples)/96T (80 samples)

Measuring instrument：Microplate reader(535-550 nm)

Detection range：0.2-100 μmol/L

Elabscience® Hexavalent Chromium Ion (Cr^{6+}) Colorimetric Assay Kit

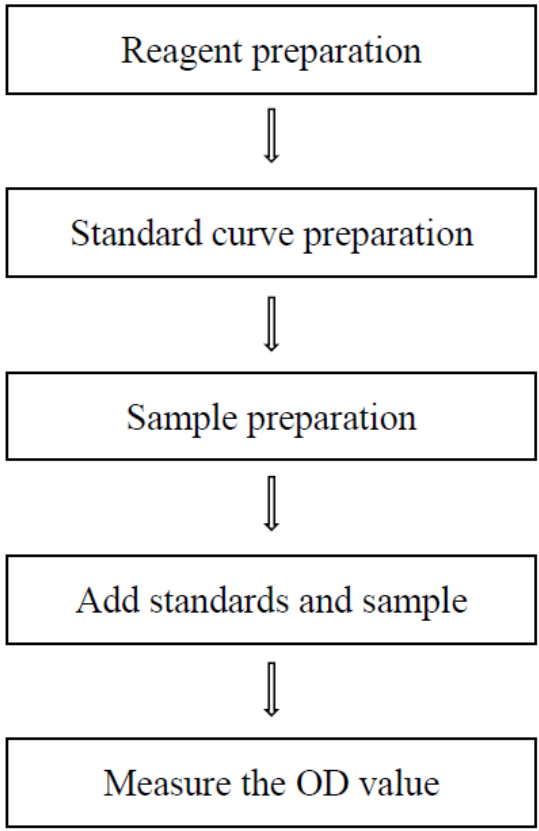
This manual must be read attentively and completely before using this product. If you have any problem, please contact our Technical Service Center for help：

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.

Table of contents

Assay summary



Intended use

This kit can be used to measure hexavalent chromium ion (Cr^{6+}) content in water samples.

Detection principle

Hexavalent chromium ion (Cr^{6+}) is a highly toxic and harmful substance, which is a great threat to human health and environmental pollution. The Cr^{6+} pollution in the environment mainly comes from the wastewater and waste gas discharged by industries such as electroplating, leather, and metallurgy. Substances containing Cr^{6+} have a strong irritant effect and high permeability on the skin, which can cause damage to the cardiovascular and hematopoietic functions of the human body, and lead to diseases in organs such as the liver, kidney and lung.

The detection principle of this kit is as follows: Cr^{6+} can react with the chromogenic agent under acidic conditions to produce a purple-red substance, with the maximum absorption at a wavelength of 540 nm. The content of Cr^{6+} in the water sample is calculated by measuring the OD value at 540 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Acid Reagent	1.5 mL × 1 vial	3 mL × 1 vial	-20℃, 12 months
Reagent 2	Chromogenic Agent	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months, shading light
Reagent 3	Standard	0.2 mL × 1 vial	0.4 mL × 1 vial	-20℃, 12 months, shading light
	Microplate	48 wells	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 (535-550 nm, optimum wavelength: 540 nm), Incubator

Reagents:

Absolute ethanol

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② The preparation of chromogenic solution:
Dissolve one vial of chromogenic agent with 2 mL of absolute ethanol, mix well to dissolve (If it does not dissolve, ultrasonicate for 2-5 min). Store at -20°C for 1 month protected from light.

③ The preparation of chromogenic working solution:
For each well, prepare 50 μL of chromogenic working solution (mix well 10 μL of chromogenic solution and 40 μL of double distilled water). The chromogenic working solution should be prepared on spot and used up within 8 hours.

④ The preparation of 100 μmol/L standard solution:
Before testing, please prepare sufficient 100 μmol/L standard solution. For example, prepare 1700 μL of 100 μmol/L standard solution (mix well 5 μL of standard and 1695 μL of double distilled water). Store at -20°C for 7 days protected from light.

⑤ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 100 μmol/L standard solution with double distilled water to a serial concentration, the recommended dilution gradient is as follows: 0, 5, 10, 20, 40, 60, 80, 100 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**5**	**10**	**20**	**40**	**60**	**80**	**100**
100 μmol/L standard (μL)	0	25	50	100	200	300	400	500
Double distilled water (μL)	500	475	450	400	300	200	100	0

Sample preparation

① Sample preparation

Water samples: detect directly. The water sample is required to be clarified.

② Dilution of sample

Note: The diluent is double distilled water. For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: Add 20 μL of acid reagent into wells.
Sample well: Add 20 μL of acid reagent into wells.

② Add 200 μL of standard solution with different concentrations into standard wells. Add 200 μL of sample into sample wells.

③ Add 50 μL of chromogenic working solution into each well.

④ Mix fully for 5 s with microplate reader and incubate at 37°C for 10 min protected from light. Measure the OD value of each well at 540 nm.

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:

Water samples:

$$\underset{(\mu mol/L)}{Cr^{6+}\ content} = \frac{\Delta A - b}{a} \times f$$

[Note]

ΔA: $\Delta A = OD_{sample} - OD_{blank}$.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three water 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 (μmol/L）	34.00	62.00	80.00
%CV	0.4	1.7	0.8

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	34.00	62.00	80.00
%CV	2.6	4.5	3.5

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 101.7%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (μmol/L)	34	62	80
Observed Conc. (μmol/L)	32.98	65.10	82.40
Recovery rate (%)	97	105	103

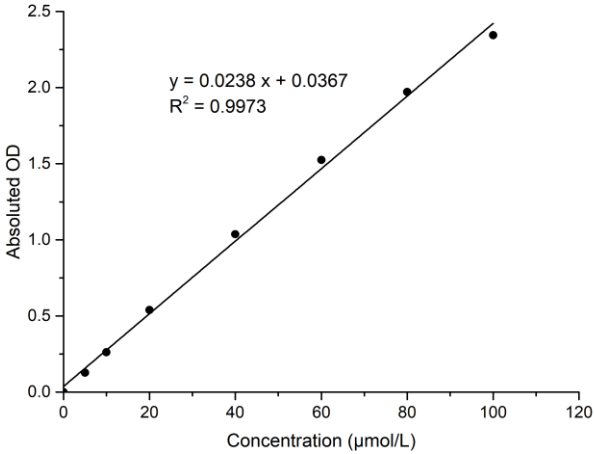
Sensitivity

The analytical sensitivity of the assay is 0.2 μmol/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 (μmol/L)	0	5	10	20	40	60	80	100
OD value	0.038	0.164	0.302	0.578	1.072	1.562	2.016	2.423
	0.040	0.168	0.300	0.576	1.081	1.565	2.006	2.343
Average OD value	0.039	0.166	0.301	0.577	1.077	1.564	2.011	2.383
Absolute OD value	0	0.127	0.262	0.538	1.038	1.525	1.972	2.340



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.