TECHNICAL MANUAL

Hexavalent Chromium Ion (Cr6+) Colorimetric Assay Kit

- **SKU CODE:** MAES0531
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add acid reagent and standards/samples
- Add chromogenic working solution
- Incubate and measure OD value

2. Intended use

This kit can be used to measure hexavalent chromium ion (Cr6+) content in water samples.

3. Detection principle

Hexavalent chromium ion (Cr6+) is a highly toxic and harmful substance that poses a significant threat to human health and environmental pollution. Environmental Cr6+ pollution primarily originates from wastewater and waste gases discharged by industries such as electroplating, leather processing, and metallurgy. Substances containing Cr6+ have strong irritant effects and high permeability on skin, which can cause damage to cardiovascular and hematopoietic functions of the human body, leading to diseases in organs such as the liver, kidney, and lungs.

The detection principle of this kit is as follows: Cr6+ can react with the chromogenic agent under acidic conditions to produce a purple-red substance with maximum absorption at a wavelength of 540 nm. The content of Cr6+ in water samples is calculated by measuring the OD value at 540 nm.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Acid Reagent	1.5 mL x 1 vial	3 mL x 1 vial	-20 °C, 12 months
Reagent 2	Chromogenic Agent	Powder x 1 vial	Powder x 2 vials	-20 °C, 12 months, shading light

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 3	Standard	0.2 mL x 1 vial	0.4 mL x 1 vial	-20 °C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (535-550 nm, optimum wavelength: 540 nm), Incubator

Reagents:

Absolute ethanol

6. Reagent preparation

1. Equilibrate all reagents to 25 °C before use.
2. **Preparation of chromogenic solution:** Dissolve one vial of chromogenic agent with 2 mL of absolute ethanol and mix well to dissolve completely (if it does not dissolve, ultrasonicate for 2-5 min). Store at -20 °C for 1 month protected from light.
3. **Preparation of chromogenic working solution:** For each well, prepare 50 µL of chromogenic working solution by mixing 10 µL of chromogenic solution and 40 µL of double distilled water. The chromogenic working solution should be prepared fresh and used within 8 hours.
4. **Preparation of 100 µmol/L standard solution:** Before testing, prepare sufficient 100 µmol/L standard solution. For example, prepare 1700 µL of 100 µmol/L standard solution by mixing 5 µL of standard with 1695 µL of double distilled water. Store at -20 °C for 7 days protected from light.
5. **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 serial concentrations. The recommended dilution gradient is as follows: 0, 5, 10, 20, 40, 60, 80, 100 µmol/L.

7. Sample preparation

Sample preparation:

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

Dilution of sample:

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

8. 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.

9. Calculation

The standard curve:

1. Average the duplicate readings for each standard.

2. Subtract the mean OD value of the blank (Standard #1) from all standard readings. This is the absolute OD value.

3. Plot the standard curve using absolute OD value of standards and corresponding concentrations as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or Excel).

The sample:

Water samples:

Cr6+ content (µmol/L) = (ΔA - b) / a × f

[Note]

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

f: Dilution factor of sample before test

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (µmol/L)	34.00	62.00	80.00

Parameters	Sample 1	Sample 2	Sample 3
%CV	0.4	1.7	0.8

Inter-assay Precision

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

Recovery

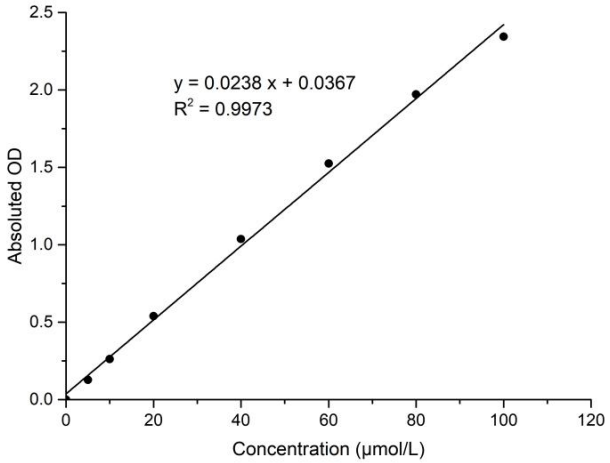
Sample	Sample 1	Sample 2	Sample 3
Expected Conc. (µmol/L)	34	62	80
Observed Conc. (µmol/L)	32.98	65.10	82.40
Recovery rate (%)	97	105	103

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.

Standard curve data

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), the standard curve and data are provided below for reference only:



10. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.