



Chromium Assay Kit (Colorimetric)

Chromium Assay Kit (Colorimetric) is a detection kit for the quantification of Chromium in serum, plasma, environmental, food and beverage.

Catalog number: ARG82145

Package: 250 tests

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Chromium is a chemical element with the symbol Cr and atomic number 24. It is the first element in group 6. It is a steely-grey, lustrous, hard, and brittle transition metal. Chromium is the main additive in stainless steel, to which it adds anti-corrosive properties. Chromium is also highly valued as a metal that is able to be highly polished while resisting tarnishing. Polished chromium reflects almost 70% of the visible spectrum, with almost 90% of infrared light being reflected. The name of the element is derived from the Greek word χρῶμα, chrōma, meaning color, because many chromium compounds are intensely colored. [Provide by Wikipedia: Chromium]

PRINCIPLE OF THE ASSAY

This Chromium Assay Kit (Colorimetric) is a simple one-step colorimetric assay that measures the amount of Chromium present in samples. In the assay, Cr (VI) forms a stable complex with a specific chromogenic dye. The optical density at O.D. 480 nm is directly proportionate to the Cr (VI) concentration in the sample. Cr (III) can be converted to Cr (VI) with nitric acid/hydrochloric acid, thus allowing the determination of Cr (III) or total Cr [Cr (III) + Cr (VI)] in the sample.

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MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped at room temperature. Store the Reagent A at -20°C. And other reagents at 2-8°C. Shelf life: 6 months after receipt.

Component	Quantity	Storage information
Reagent A	300 µL	-20°C
Reagent B	20 mL	4°C
Standard, Cr (VI) 40 mg/L	300 µL	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 430-505 nm
- Centrifuge
- Chemical hood
- Clear flat-bottom 96 well microplate
- Deionized or Distilled water
- Concentrated HNO₃ / concentrated HCl / ammonia
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- Samples should be clear, colorless and free from particles or precipitates. Substances that may potentially interfere with the assay include: azide, Ba^{2+} , Pb^{2+} , Fe^{3+} , Gold (III), Sn (II), Ti (IV).
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C. Collect the serum and assay directly.

Plasma: Collect blood with heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Collect the plasma layer and assay directly.

General Sample Treatment Procedures:

The following procedure converts Cr (III) in a sample to Cr (VI) by oxidation with nitric acid. This experiment should be performed with special care in a chemical hood. Weigh 0.5 g solid sample (e.g. alloy, food, hair), or transfer 1-2 mL blood or serum samples, into a 50 mL beaker. Add 10 mL of concentrated HNO₃ and 1 mL of concentrated HCl. Cover with a watch glass until the initial brisk reaction is subsided. Add another 5 mL of concentrated HNO₃ and heat the solution gently until all carbides are decomposed. After cooling down to room temperature, neutralize the solution with 3% ammonia. Filter the solution with Whatman No. 42 and use the filtrate for assay.

Note:

- Samples should be clear, colorless and free from particles or precipitates. Substances that may potentially interfere with the assay include: azide, Ba²⁺, Pb²⁺, Fe³⁺, Gold (III), Sn (II), Ti (IV).
- If necessary, water samples can be concentrated by evaporation. If determination of Cr (III) or total Cr [Cr (III) + Cr (VI)] is desired, please refer to the General Sample Treatment Procedure.

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REAGENT PREPARATION

- **Working Reagent:** for each well, mix 1 μL of Reagent A and 55 μL of Reagent B.
- **Standard:** Prepare 600 μL of 2000 $\mu\text{g/L}$ Cr (VI) Standard Premix by mixing 30 μL of Standard and 570 μL of deionized water (>18 megaohm). Dilute standard as follows.

Standard tube	Standard ($\mu\text{g/L}$)	Distilled water (μL)	2000 $\mu\text{g/L}$ Standard (μL)
S1	2000	0	300
S2	1000	150	150
S3	500	225	75
S4	0	300	0

ASSAY PROCEDURE

Equilibrate reagents to room temperature. Briefly centrifuge tubes before use. Unused Reagent A should be stored at -20°C .

96-WELL ASSAY PROCEDURE

1. Add 250 μL of diluted **Standards** and **samples** into wells of clear bottom 96-well microplate.
2. Add 50 μL of **Working Reagent** to each well. Tap lightly to mix.
3. Incubate for **20 minutes** at **room temperature** and read the absorbance at **O.D. 480 nm (430-505 nm)**.

CUVETTE ASSAY PROCEDURE

The cuvette assay procedure is essentially the same as the 96-well plate assay. The Working Reagent is prepared by mixing 4 μL of **Reagent A** and 220 μL of

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Reagent B. Assay by mixing **1000 µL** of **Standard** or **Sample** with **200 µL** of **Working Reagent**.

CALCULATION OF RESULTS

1. Subtract blank OD (distilled water, S4) from the OD values of standards and plot the Standard Curve and determine its Slope. Cr(VI) concentration of a Sample is calculated as,

$$\text{Cr (VI) } (\mu\text{g/L}) = [(\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}) / \text{Slope}] \times n$$

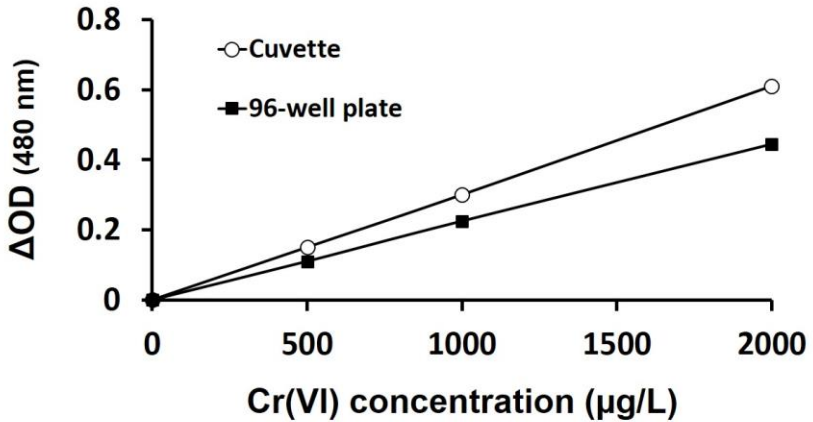
Note:

- $\text{OD}_{\text{SAMPLE}}$, OD_{BLANK} : the O.D. 480 nm values of the sample and blank (S4).
 - If the Sample Cr (VI) concentration is higher than 2000 µg/L, dilute sample in deionized water and repeat the assay. Multiply result by the dilution factor n.
2. Conversion factor: 1000 µg/L chromium is equivalent to 19.2 µM or 1 ppm.

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EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Chromium Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

20 μg/L (0.38 μM, 0.02 ppb)