

Version 1c Last updated 10 March 2026

ab272522

Chromium Assay Kit

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Chromium Assay Kit datasheet:

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For quantitative determination of Chromium in biological, environmental, food and beverage samples.

This product is for research use only and is not intended for diagnostic use.

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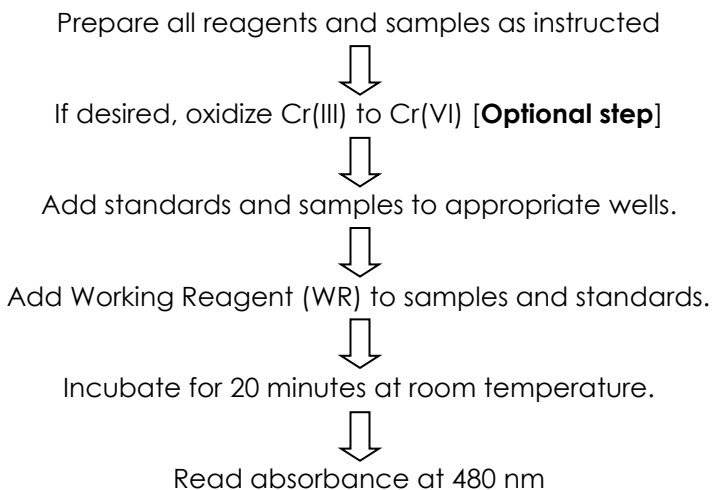
1. Overview

Chromium Assay Kit (ab272522) provides a simple one-step colorimetric means to directly measure Cr(VI) in a sample. In the assay, Cr(VI) forms a stable complex with a specific chromogenic dye. The optical density at 480nm 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. The assay is sensitive with a detection limit of 20 µg/L Cr.

Sensitive and accurate. Linear detection range of 20 - 2000 µg/L Chromium.

Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. No wash and reagent transfer steps are involved. Can be readily automated for processing thousands of samples per day.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website www.abcam.com.

4. Storage and Stability

Store kit at 4°C immediately upon receipt, apart from Reagent A, which should be stored at -20°C. Avoid multiple freeze-thaw cycles. Kit has a storage time of 6 months from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Reagent A	300 µL	-20°C
Reagent B	20 mL	+4°C
Cr(VI) Standard 40 mg/L	300 µL	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Spectrophotometer
- Concentrated HNO_3 , concentrated HCl , ammonia.
- Chemical fume hood
- 1.5 mL tubes
- Pipetting devices
- Centrifuge
- 96-well clear plate with flat bottom (alternatively, 1 mL cuvettes may be used)
- Standard microplate reader - capable of reading absorbance at 430-505 nm (peak absorbance is at 480 nm).

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent.
- Pipette standards and samples to the bottom of the wells.
- Add the reagents to the side of the tube to avoid contamination.
- Some Solutions supplied in this kit are caustic; care should be taken with their use.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 250 assays.

All reagents are supplied ready to use.

Δ Note: Unused Reagent A should be stored at -20°C.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Prepare serially diluted standards immediately prior to use.

10.1 Prepare 600 μL of 2000 $\mu\text{g/L}$ Premix by mixing 30 μL Cr(VI) Standard and 570 μL deionized water (>18 mega Ohm).

10.2 Dilute the 2000 $\mu\text{g/L}$ Premix as shown in the table.

Standard #	2000 $\mu\text{g/mL}$ Premix (μL)	H ₂ O (μL)	Standard ($\mu\text{g/L}$)
1	300	0	2000
2	150	150	1000
3	75	225	500
4	0	300	0

10.2 Transfer 250 μL of each Standard into separate wells of the plate.

11. Sample Preparation

The kit may be used with biological (serum, plasma etc), environmental (water, soil etc), food and beverage samples.

Samples should be clear, colorless and free from particles or precipitates.

Δ Note: Substances that may potentially interfere with the assay include: azide, Ba^{2+} , Pb^{2+} , Fe^{3+} , Gold(III), Sn(II) , Ti(IV) .

Δ Note: If necessary, water samples can be concentrated by evaporation.

Oxidation of Cr(III) to Cr(VI):

The following procedure converts Cr(III) in a sample to Cr(VI) by oxidation with nitric acid.

Δ Note: This experiment should be performed with special care in a chemical fume hood.

- 11.1** 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.
- 11.2** Add 10 mL concentrated HNO_3 and 1 mL concentrated HCl . Cover with a watch glass until the initial brisk reaction is subsided.
- 11.3** Add another 5 mL concentrated HNO_3 and heat the solution gently until all carbides are decomposed.
- 11.4** After cooling down to room temperature, neutralize the solution with 3% ammonia.
- 11.5** Filter the solution with Whatman No. 42 and use the filtrate for assay.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

Immediately prior to starting the reaction, prepare enough Working Reagent (WR) for all samples and standards by mixing per reaction tube: 1 μ L Reagent A, 55 μ L Reagent B.

Component	Working Reagent (μ L/reaction)
Reagent A	1
Reagent B	55

12.1 96-well plate procedure:

- 12.1.1 Transfer 250 μ L Samples or Standards to appropriate wells.
- 12.1.2 Add 50 μ L Working Reagent to each well. Tap plate to mix.
- 12.1.3 Incubate for 20 min at room temperature.
- 12.1.4 Read optical density at 480 nm (430-505nm).

12.2 Cuvette procedure:

- 12.2.1 The cuvette assay procedure is essentially the same as the 96-well plate assay.
- 12.2.2 The Working Reagent is prepared by mixing 4 μ L Reagent A and 220 μ L Reagent B.
- 12.2.3 Assay by mixing 1000 μ L Standard or Sample with 200 μ L Working Reagent.

13. Calculations

- 13.1** Subtract blank OD (Standard #4) from the standard OD values and plot the OD against standard concentrations.
- 13.2** Determine the slope using linear regression fitting.
- 13.3** The Cr(VI) concentration of the Sample is calculated as follows:

$$[\text{Cr(VI)}] \text{ in } \mu\text{g/L} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}})}{\text{Slope}} \times n$$

$\text{OD}_{\text{Sample}}$ = OD 480 value of the sample

OD_{Blank} = OD 480 value of water (Standard #4)

n = Dilution factor

Δ Note: if the Sample Cr(VI) concentration is higher than 2000 $\mu\text{g/L}$, dilute sample in deionized water and repeat the assay. Multiply result by the dilution factor n .

Conversion factor: 1000 $\mu\text{g/L}$ chromium is equivalent to 19.2 μM or 1 ppm.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

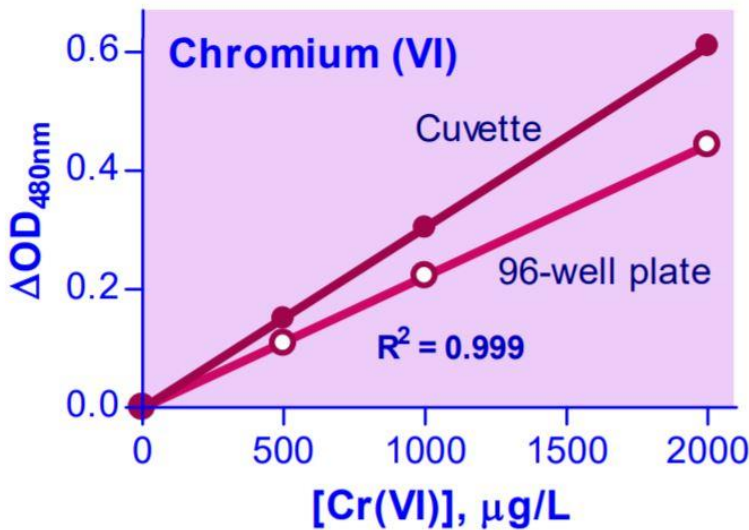


Figure 1. Example of Chromium Assay Kit standard curves.

15. Notes

Technical Support

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