QuantiChrom™ Chromium Assay Kit (DCRM-250)

Quantitative Colorimetric Determination of Chromium (VI)

DESCRIPTION

CHROMIUM is widely used in various industries such as electroplating, leather tanning, chrome paint, dying, hardened steel, ceramic and glass industry. Chromium exists in two stable oxidation states, hexavalent Cr(VI) and trivalent Cr(III). Cr(VI) is produced solely by industrial processes, whereas in nature, chromium exists in its trivalent form. Cr(III) is generally regarded as nontoxic due to poor absorption. Cr(VI) is considered a pulmonary carcinogen and has tested positive in genotoxicity tests. It is one of the most serious pollutants in many water streams due to its carcinogenic potential. Most countries apply a legal limit of 50-100 µg/L Cr in drinking water.

BioAssay Systems' Chromium Assay Kit 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.

KEY FEATURES

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.

APPLICATIONS

Determination of chromium in biological (serum, plasma etc), environmental (water, soil etc), food and beverage samples.

KIT CONTENTS (sufficient for 250 tests in 96-well format)

Reagent A: 300 µL **Reagent B:** 20 mL
Cr(VI) Standard: 300 µL 40 mg/L

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

Precautions: 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.

96-WELL ASSAY PROCEDURE

Use clear flat-bottom plates. Prior to assay, bring all reagents to room temperature. Unused Reagent A should be stored at -20°C.

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).

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.

1. *Standards*. Prepare 600 µL 2000 µg/L Cr(VI) Standard Premix by mixing 30 µL Standard and 570 µL deionized water dH_2O (>18 megaohm). Dilute standard as follows.

No	Premix + dH_2O	Standard (µg/L)
1	300 µL + 0 µL	2000
2	150 µL + 150 µL	1000
3	75 µL + 225 µL	500
4	0 µL + 300 µL	0

Transfer 250 µL standards into separate wells of the plate.

Samples: transfer 250 µL of each sample into separate wells of the plate.

2. *Assay*. Prepare enough Working Reagent, for each well, by mixing 1 µL Reagent A and 55 µL Reagent B. Add 50 µL Working Reagent to each well. Tap plate to mix. Incubate for 20 min at room temperature.

Read optical density at 480 nm (430-505nm).

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 Reagent A and 220 µL Reagent B. Assay by mixing 1000 µL Standard or Sample with 200 µL Working Reagent.

CALCULATION

Subtract the blank control OD (#4) from the OD values of the standards. Plot the Standard Curve and determine its Slope. Cr(VI) concentration of a Sample is calculated as,

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

where OD_{SAMPLE} and OD_{BLANK} are the OD_{480nm} values of the Sample and Blank Control (#4), respectively. Slope is the slope of the standard curve.

Note: 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*.

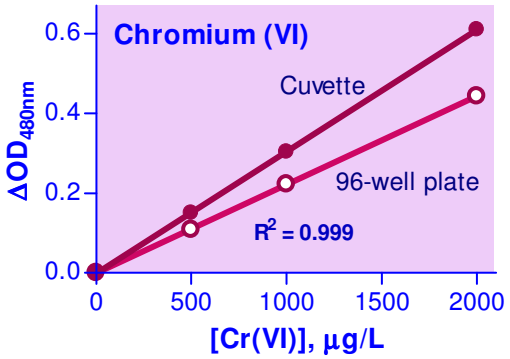
Conversion factor: 1000 µg/L chromium is equivalent to 19.2 µM or 1 ppm.

GENERAL SAMPLE TREATMENT PRECEDURE

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 fume 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 concentrated HNO_3 and 1 mL concentrated HCl. Cover with a watch glass until the initial brisk reaction is subsided. Add another 5 mL concentrated HNO_3 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.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat bottom 96-well plates, plate reader, cuvette, spectrophotometer, concentrated HNO_3, concentrated HCl, ammonia and chemical hume hood.



LITERATURE

1. Barceloux, DG (1999). Chromium. J Toxicol Clin Toxicol. 37(2):173-94.

2. Greenberg, AE, Clesceri, LS, Trussell, RR, Eds. (1995) Standard Methods for the Examination of Water and Wastewater, American Public Health Association, 19th ed., Washington, DC, 3-59.

3. De, AK (2000). Environmental Chemistry, 4th ed.; New Age International, New Delhi, 229, 2000.