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Product Information

Chromium Assay Kit

Catalog Number **MAK130**

TECHNICAL BULLETIN

Product Description

Chromium, an essential trace element, is a transition metal that primarily exists in two stable oxidation states, hexavalent [Cr(VI)] and trivalent [Cr(III)]. Chromium is widely used in various industries such as electroplating, leather tanning, chrome paint, dying, hardened steel, ceramic, and glass. Cr(VI) is produced almost exclusively by industrial processes; whereas, in nature chromium primarily exists in its trivalent form. Cr(III) is generally regarded as nontoxic due to its poor absorption. Cr(VI) is considered a pulmonary carcinogen and has tested positive in genotoxicity tests. Cr(VI) is considered a serious pollutant in many water streams due to its carcinogenic potential. In trace amounts, Cr(III) may be required for glucose and lipid metabolism.

This kit is suitable for the detection of chromium in biological, environmental, food, and beverage samples.

In this kit, chromium is determined in a simple, one-step assay in which Cr(VI) forms a specific complex with a chromogenic dye, resulting in a colorimetric (480 nm) product, proportional to the chromium present. Cr(III) can be converted to Cr(VI) with nitric acid/hydrochloric acid, allowing the determination of Cr(III) and/or total chromium [Cr(III) +Cr(VI)].

Components

The kit is sufficient for 250 assays in 96-well plates.

Reagent A Catalog Number MAK130A	300 µL
Reagent B Catalog Number MAK130B	20 mL
Cr(VI) Standard, 40 mg/L Catalog Number MAK130C	300 µL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader
- Concentrated Nitric Acid (HNO_3, Catalog Number 78005 or equivalent)
- Concentrated (37% or ~12 M) Hydrochloric Acid (HCl, Catalog Number 320331 or equivalent)
- 3% Ammonia solution
- Whatman® No. 42 filter paper (Catalog Number Z740497 or equivalent)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped at room temperature. Store Reagent A at –20 °C. Store all other reagents at 2–8 °C.

Procedure

Bring all reagents to room temperature prior to use. Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents.

For best results, it is recommended to run samples and standards in duplicate.

Sample Preparation

Samples should be clear, colorless, and free from particles or precipitates. Substances that may potentially interfere with the assay include: azide, Ba(II), Pb(II), Fe(III), Gold(III), Sn(II), and 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, the Cr (III) in a sample can be converted to Cr(VI) by oxidation with nitric acid. Perform oxidation reaction with special care in a chemical fume hood. Weigh 0.5 g of solid sample (e.g., alloy, food, or hair) or transfer 1–2 mL of liquid (e.g., blood or serum) into a 50 mL beaker. Add 10 mL of concentrated nitric acid (HNO_3) and 1 mL of concentrated hydrochloric acid (HCl). Cover with a watch glass until the initial brisk reaction is subsided. Add another 5 mL of concentrated nitric acid and heat the solution gently until all carbides are decomposed. After cooling down to room temperature, neutralize the solution with 3% ammonia solution. Filter the solution with Whatman No. 42 filter paper and use the filtrate for assay.

Cr(VI) Standards for Colorimetric Detection

Dilute 30 μL of the Cr(VI) Standard with 570 μL of water to create a 2,000 μg/L Cr(VI) Standard Solution. Dilute the 2,000 μg/L Cr(VI) Standard Solution as indicated in Table 1.

Table 1.
Colorimetric Standards

Number	Standard	Water	Concentration of Cr(VI)
1	300 μL	0 μL	2,000 μg/L
2	150 μL	150 μL	1,000 μg/L
3	75 μL	225 μL	500 μg/L
4	0 μL	300 μL	0 μg/L

Assay Reaction

1. Transfer 250 μL of the appropriate standards and 250 μL of samples into separate wells of a 96 well plate.

2. Set up the Master Reaction Mix according to the scheme in Table 2. Prepare enough of the Master Reaction Mix for each of the sample and standard wells. 50 μL of the Master Reaction Mix is required for each reaction (well). The Master Reaction Mix should be prepared fresh each time the reaction is run.

Table 2.
Master Reaction Mix

Reagent	Volume
Reagent A	1 μL
Reagent B	55 μL

3. Add 50 μL of the Master Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 20 minutes at room temperature.

4. Measure the absorbance at 480 nm (A_{480}). If the sample absorbance is higher than the standard absorbance at 2,000 μg/L, dilute the sample in water and repeat the assay. Multiply the results by the dilution factor.

These assays can be adapted for use in standard 1 mL cuvettes. To adapt to cuvettes follow protocol as for 96 well plates but prepare Master Mix with 1 μL of Reagent A and 220 μL of Reagent B. Mix 1,000 μL of standard or sample with 200 μL of Master Mix.

Results

Calculations

Subtract the absorbance value for the 0 μg/L standard (blank) from the absorbance value of the rest of the standards. Use the corrected values obtained from the appropriate standards to plot a standard curve and determine the slope using linear regression fitting.

Note: A new standard curve must be set up each time the assay is run.

Cr(VI) concentration

The Cr(VI) concentration in the sample can be calculated using the following equation:

$$\text{Cr(VI) } (\mu g/L) = \frac{(A_{480})_{sample} - (A_{480})_{blank}}{\text{Slope}} \times n$$

where:

$(A_{480})_{sample}$ = Absorbance of sample

$(A_{480})_{blank}$ = Absorbance of assay blank (0 μg/L standard)

n = dilution factor

Conversion factors for chromium:

1,000 μg/L = 19.2 μM or 1 ppm

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at temperature indicated in bulletin
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument. The absorbance is typically read at 480nm but can be read between 430–505 nm.
	Type of 96 well plate used	For colorimetric assays, use clear plates
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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