Chromium (Cr) Assay Kit

Catalog No.: abx298895

Size: 100 Assays

Storage: Store all components at 4°C.

Application: For quantitative detection of Chromium concentrations in serum, plasma, water, soil, beverage samples, and other biological fluids.

Detection Range: 20 ng/ml – 2000 ng/ml

Introduction: Chromium exists in two stable oxidation states, Cr(VI) and Cr(III). Hexavalent chromium is found naturally in the environment and is produced solely by industrial processes. In biological systems, chromium exists in its trivalent form. Cr(III) is generally regarded as non-toxic due to poor absorption. Cr(VI) is considered a pulmonary carcinogen and is a contaminant that is routinely tested and monitored in many water streams due to its carcinogenic potential.

Abbexa's Chromium Assay Kit is a sensitive colorimetric assay that can directly measure Cr(VI) in a sample. In the assay, Cr(III) can be converted to Cr(VI) with nitric acid and hydrochloric acid, thus allowing the determination of Cr(III) or total Cr [Cr(III) + Cr(VI)] in the sample. Cr(VI) forms a stable complex with a chromogenic dye, which has an absorption maxima at 540 nm. The optical density at 540 nm is directly proportionate to the Cr(VI) concentration in the sample.

Kit components

1. 96 well microplate
2. Reaction Buffer: 1 ml
3. Enhancing Reagent: 2 × 1 ml
4. Dye Reagent 2 × 1 ml
5. Standard (2000 ng/ml): 1 ml

Materials Required But Not Provided

1. Microplate reader (540 nm)
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Timer
6. Mortar
7. HNO_3
8. HCl
9. Ammonia
10. Filter paper

Protocol

Sample preparation

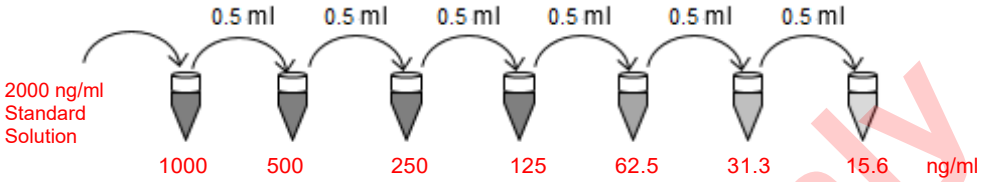
The following procedure converts Cr(III) in a sample to Cr(VI) by oxidation with nitric acid. This experiment should be performed with special care in a chemical fume hood.

1. Add 0.5 g solid sample (e.g. alloy, food, hair) or 1-2 ml blood or serum samples, into a 50 ml beaker.
2. Add 10 ml concentrated HNO_3 and 1 ml concentrated HCl. Cover with a watch glass until the initial brisk reaction has subsided.
3. Add another 5 ml concentrated HNO_3 and heat the solution gently until all carbides have decomposed.
4. Cool down to room temperature, then neutralize the solution with 3% ammonia. Filter the solution with filter paper and use the filtrate for the assay.

A. Assay Procedure

If the expected concentration is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured concentration within the detection range of the kit.

1. Label 7 tubes with 1000 ng/ml, 500 ng/ml, 250 ng/ml, 125 ng/ml, 62.5 ng/ml, 31.3 ng/ml, and 15.6 ng/ml. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 2000 ng/ml Standard Solution to the 1st tube and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Set the sample, standard and blank wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 150 µl of sample to the sample wells.
4. Add 150 µl of prepared standards to the standard wells.
5. Add 150 µl of distilled water to the blank wells.
6. Add 10 µl of Reaction Buffer to all wells.
7. Add 20 µl of Enhancing Reagent to all wells.
8. Add 20 ul of Dye Reagent to all wells.
9. Tap the plate gently to mix. Allow to stand for 5 minutes. Read and record absorbance at 540 nm.

B. Calculations

Chromium mass (ng) per ml of sample:

$$\mathbf{Chromium\ (ng/ml)} = \frac{\mathbf{C_{Standard}} \times \mathbf{V_{Standard}}}{\mathbf{V_{Sample}}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}} = \mathbf{2000} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}}$$

where:

$\mathbf{C_{Standard}}$ Concentration of highest standard (2000 ng/ml)

$\mathbf{V_{Sample}}$ Volume of sample (0.15 ml)

$\mathbf{V_{Standard}}$ Volume of standard (0.15 ml)