Chromium Assay Kit

Catalog No.: abx298895

Size: 96 tests

Storage: Store all components at 4°C.

Application: For quantitative detection of Chromium concentration in serum, plasma, water, soil, beverages, and other biological fluids.

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

Introduction and Principle of the Assay

Chromium exists in two stable oxidation states, Cr(VI) and Cr(III). In biological systems, chromium exists in its trivalent form; Cr(VI) is not found naturally in the environment, and is instead introduced by industrial processes. Cr(III) is generally regarded as non-toxic due to poor absorption. In contrast, Cr(VI) is considered a pulmonary carcinogen and as such is routinely tested and monitored for 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 proportional to the Cr(VI) concentration in the processed 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. Double-distilled water
5. Timer
6. Mortar
7. Concentrated HNO_3
8. Concentrated HCl
9. Ammonia
10. Filter paper

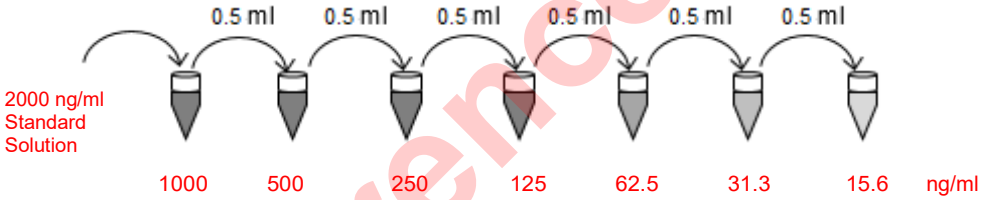
A. Sample Preparation

Caution: This procedure involves hazardous reagents and must be performed entirely in a chemical fume hood.

1. Add 0.5 g of solid sample (e.g. alloy, food, hair), or 1 – 2 ml of liquid sample (e.g. blood, serum, water), into a 50 ml beaker.
2. Add 10 ml concentrated HNO_3 and 1 ml concentrated HCl. Immediately cover with a watch glass until the initial vigorous reaction has subsided.
3. Add another 5 ml concentrated HNO_3, then gently heat the solution until all carbides have decomposed.
4. Cool the solution to room temperature, then neutralize with 3% ammonia.
5. Filter the solution with filter paper and collect the filtrate for detection in the assay.

B. Assay Procedure

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 double-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. Mark positions for the samples, standard and blank wells on the 96 well microplate. *It is recommended to test all wells 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 µl of Dye Reagent to all wells.
9. Tap the plate gently to mix, then allow the plate to stand for 5 minutes.
10. Measure and record the OD values at 540 nm.

Note:

- 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.

C. Calculations

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. This standard curve can be used to calculate the concentration of Chromium in each sample.

Alternatively, based on this curve, the concentration of Chromium in each sample well can be derived with the formula:

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

where:

$\mathbf{OD_{Sample}}$	The absorbance value of the sample well
$\mathbf{OD_{Blank}}$	The absorbance value of the blank well
$\mathbf{OD_{Standard}}$	The absorbance value of the top (2000 ng/ml) standard well
$\mathbf{C_{Standard}}$	The concentration of the top standard (2000 ng/ml)
$\mathbf{V_{Standard}}$	The volume of the top standard (0.15 ml)
$\mathbf{V_{Sample}}$	The volume of the sample (0.15 ml)