Chromium Microplate Assay Kit

User Manual

Catalog # FTA0146

(Version 1.2A)

Detection and Quantification of Chromium (Cr) Content in Serum, Plasma, Other biological fluids, Water, Soil, Food and Beverage Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

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.

Chromium Microplate Assay Kit provides a sensitive colorimetric means to directly measure Cr(VI) in a sample. In the assay, 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. Cr(VI) forms a stable complex with a specific chromogenic dye. The optical density at 540nm is directly proportionate to the Cr(VI) concentration in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Reaction Buffer	1 ml x 1	4 °C
Enhancer	1 ml x 2	4 °C
Dye Reagent	1 ml x 2	4 °C
Standard (2000 ng/ml)	1 ml x 1	4 °C
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 540 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. HNO3
9. HCl
10. Ammonia

IV. 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. 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 HNO3 and 1 mL concentrated HCl. Cover with a watch glass until the initial brisk reaction is subsided. Add another 5 mL concentrated HNO3 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 and use the filtrate for assay.

V. ASSAY PROCEDURE

Add following reagents in the microplate:

Reagent	Sample	Standard	Blank
Sample	150 µl	--	--
Standard	--	150 µl	--
Distilled water	--	--	150 µl
Reaction Buffer	10 µl	10 µl	10 µl
Enhancer	20 µl	20 µl	20 µl
Dye Reagent	20 µl	20 µl	20 µl
Mix, wait for 5 minutes, then record absorbance measured at 540 nm.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

VI. CALCULATION

1. According to the volume of sample

$Cr\ (ng/ml) = C_{Standard} \times V_{Standard} \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$

$= 2000 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$

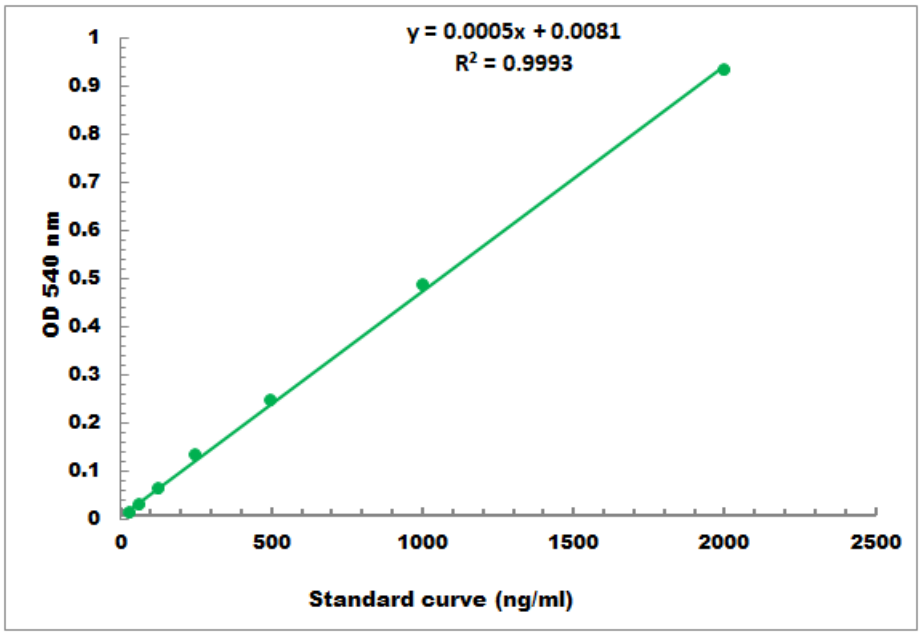
$C_{Standard}$: the standard concentration, 2000 ng/ml;

$V_{Standard}$: the volume of standard, 0.15 ml;

V_{Sample}: the volume of sample, 0.15 ml.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES