



# Chromium Microplate Assay Kit

**Catalog # AS0131**

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

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic procedures.

Contact information:

Tel:+1 (301) 446-2499 Fax:+1 (301) 446-2413

Email:[techcn@signalwayantibody.com](mailto:techcn@signalwayantibody.com) Web:[www.sabbiotech.com](http://www.sabbiotech.com)

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## 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	2 ml x 1	4 °C
Dye Reagent	2 ml x 1	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
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. HNO<sub>3</sub>
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 HNO<sub>3</sub> and 1 mL concentrated HCl. Cover with a watch glass until the initial brisk reaction is subsided. Add another 5 mL concentrated HNO<sub>3</sub> 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 $\mu$ l	--	--
Standard	--	150 $\mu$ l	--
Distilled water	--	--	150 $\mu$ l
Reaction Buffer	10 $\mu$ l	10 $\mu$ l	10 $\mu$ l
Enhancer	20 $\mu$ l	20 $\mu$ l	20 $\mu$ l
Dye Reagent	20 $\mu$ l	20 $\mu$ l	20 $\mu$ l
Mix, wait for 5 minutes, then record absorbance measured at 540 nm.			

## VI. CALCULATION

1. According to the volume of sample

$$\begin{aligned} Cr \text{ (ng/ml)} &= C_{\text{Standard}} \times V_{\text{Standard}} \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / V_{\text{Sample}} \\ &= 2000 \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) \end{aligned}$$

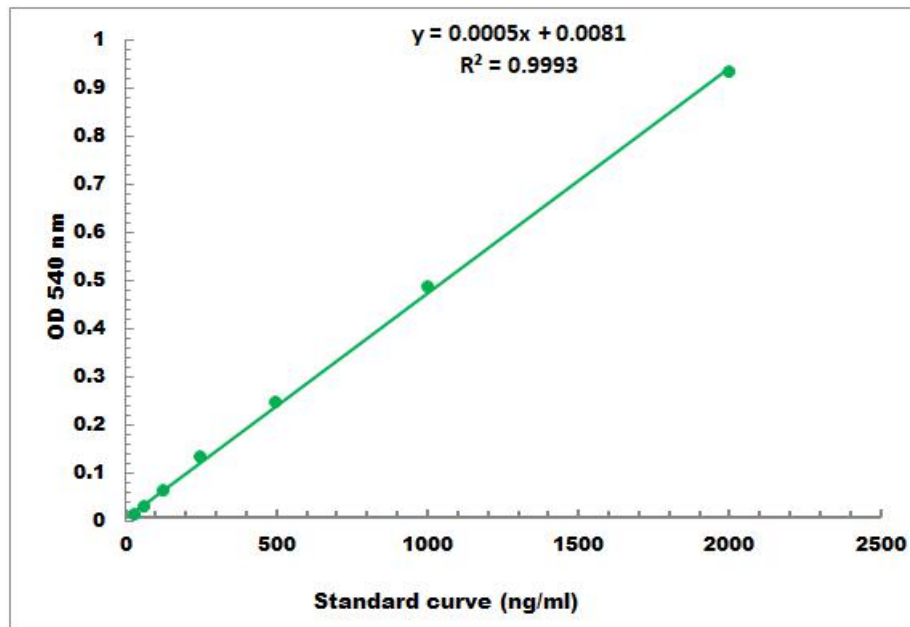
$C_{\text{Standard}}$ : the standard concentration, 2000 ng/ml;

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

$V_{\text{Sample}}$ : 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: 40 ng/ml -4000 ng/ml

## VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to [www.sabbiotech.cn](http://www.sabbiotech.cn) or contact us at [techcn@signalwayantibody.com](mailto:techcn@signalwayantibody.com)

## IX. NOTES