



Chromium Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1162

(Version 1.3A)

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.

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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 Colorimetric 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 mlx 1	4 °C
Enhancer	1 mlx 2	4 °C, keep in dark
Dye Reagent	1 ml x 2	4 °C, keep in dark
Standard (2000 ng/ml)	1 mlx 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. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 2000/1000/500/250/125/62.5/31.25 ng/ml.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. 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.

VI. 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:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C = \frac{(OD_{\text{Sample}} - OD_{\text{Blank}}) - \text{Intercept}}{\text{Slope}} \times n (\text{ng/ml})$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

According to the volume of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

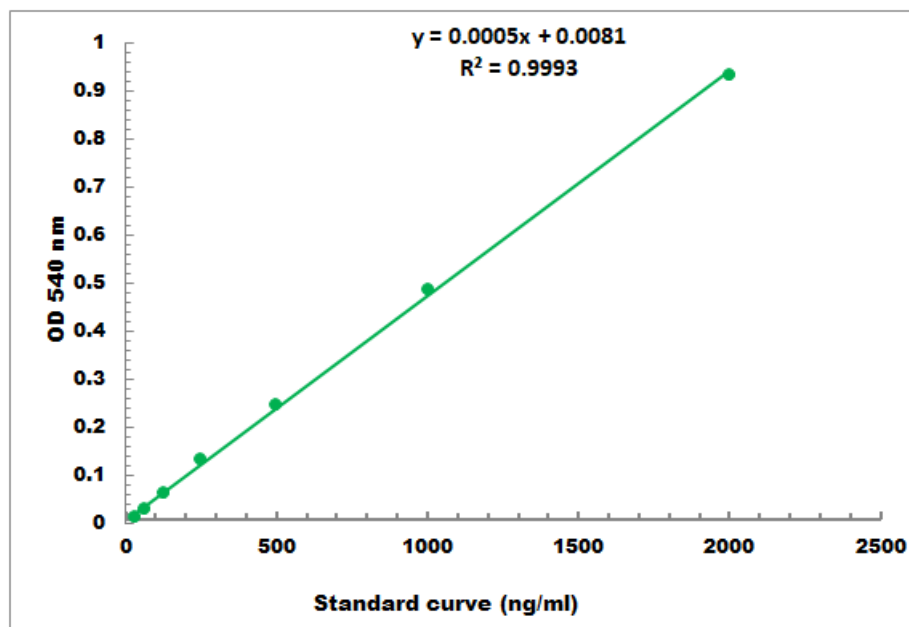
C_{Standard} : the standard concentration, mg/ml

V_{Standard} : the volume of standard in assay procedure, μl

V_{Sample} : the volume of sample in assay procedure, μl

VIII. 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