ab102510

Cobalt Quantification Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Cobalt levels in various samples.

This product is for research use only and is not intended for diagnostic use.

Version 2a

Version 2a

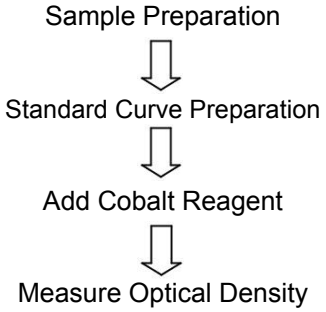
Table of Contents

1. Overview

Cobalt is a hard, lustrous, grey metal, a chemical element (symbol Co, atomic number 27 and atomic mass 58.9). Cobalt is an essential trace-element for all multicellular organisms as the active center of coenzymes called cobalamins. These include vitamin B-12 which is essential for plants and animals. Cobalt is also an active nutrient for bacteria, algae, and fungi, and may be a necessary nutrient for all life. Cobalt, particularly the Co^{2+} species, forms complexes with a large number of organic and inorganic molecules with a range of extinction coefficients in the visible range.

Abcam's Cobalt Quantification Kit takes advantage of the reaction of Co^{2+} with mercaptoethanol in basic media. Under the condition, Cobalt forms a complex with a strong absorbance band at 475 nm. The metal ions Fe^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Mn^{2+}, exhibit less than 10% interference at this wavelength. The kit provides a quick simple accurate method of quantitating Co^{2+} in a variety of samples.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Cobalt Reagent	1 mL
Cobalt Chloride Standard (1 µmol; Lyophilized)	1 vial

* Store kit at room temperature, keep tightly capped.

COBALT REAGENT: Ready to use as supplied. Store at room temperature.

COBALT STANDARD: Dissolve in 1 ml dH_2O to generate a 1 mM solution. Store at room temperature.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader
- 96-well plate
- Orbital shaker

4. Assay Protocol

1. **Sample Preparation:**

   Sample Cobalt concentrations can vary over a rather wide range Take samples between 2-200 µl and adjust the well volume to 200 µl with water.

   For unknown samples, we suggest testing several different amounts of sample to ensure the readings are within the standard curve

2. **Standard Curve Preparation:**

   Add 0, 10, 20, 30, 40, 50 µl of the 1 mM Cobalt Standard to a series of wells. Adjust volume to 200 µl/well with water to generate 0, 10, 20, 30, 40 and 50 nmol per well of the Cobalt Standard.

3. **Development:**

   Add 10 µl of the Cobalt Reagent to each well containing Cobalt Standard or samples. Mix well. Incubate at room temperature for 10 minutes.

4. Measure OD at 475 nm in a microplate reader.

5. Data Analysis

Correct background by subtracting the zero Cobalt Standard from all readings.

Plot standard curve nmol/well vs. standard readings. Apply sample readings to the standard curve to get the amount of Cobalt in the sample wells.

The Cobalt concentration in the test samples:

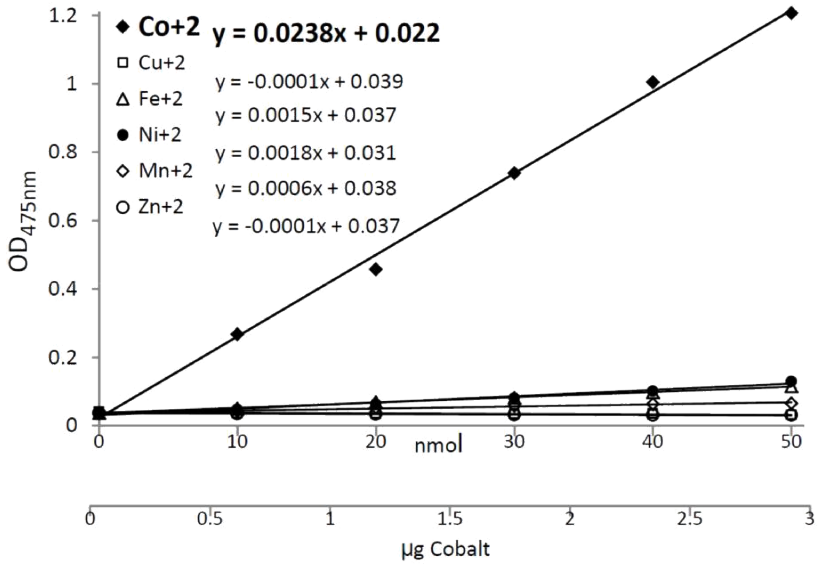
Concentration = Ay / Sv (nmol/ml**, or** μ**M)**

Where:

Ay is the amount of Cobalt (nmol) in sample well from the standard curve.

Sv is the sample volume (ml) added to the sample well.

Cobalt molecular weight: 58.9 g/mol.



Cobalt Standard Curve: Assays were performed following the kit protocol.

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

UK, EU and ROW
Email: technical@abcam.com
Tel: +44 (0)1223 696000
www.abcam.com

US, Canada and Latin America
Email: us.technical@abcam.com
Tel: 888-77-ABCAM (22226)
www.abcam.com

China and Asia Pacific
Email: hk.technical@abcam.com
www.abcam.cn

Japan
Email: technical@abcam.co.jp
Tel: +81-(0)3-6231-0940
www.abcam.co.jp