ABS_Bio™ Copper Colorimetric Detection Kit

(Cat# K330-200; 200 assays; store kit at 4℃)

Introduction

As one of the heavy metals, a high concentration of Cu^{2+} in biological or environmental systems is quite toxic to human health. As an essential micronutrient element for human life, Cu^{2+} is a necessary cofactor or structural component of numerous enzymes needed in metabolic processes. Determination of copper in serum is important in the diagnosis of an inborn error of metabolism, Wilson's disease, and various kinds of anemias.

The ABS_Bio™ Copper Colorimetric Detection Kit provides a simple, sensitive, one-step colorimetric assay to detect copper in various samples. In this assay, Cu^{2+} in a solution containing bicinchoninic acid is chemically reduced by the assayed substance to form a purple colored complex. The intensity of the color, measured at 360 nm, is directly proportional to the amount of copper in the sample. The kit is supplied with sufficient reagents for 200 tests in 96-well plate assay and has a linear detection range of 1.6-500 µg/dL (0.25-77.5 µM). It could easily be modified for use in a 384-well or a high-throughput assay.

Kit Components (200 tests)

TCA: 12 mL Reagent A: 22 mL Reagent B: 1.5 mL Copper Standard (0.5 mg/dL): 2 x 2 mL

Storage and Handling: Store kit at 4℃. Shelf Life: 12 months after receipt. Warm up Reagents to room temperature before use.

Protocol

1. Sample & Standard preparation

Serum, Plasma (EDTA-plasma cannot be used, haemolysed specimens are unacceptable), other bodily fluids, or cell culture supernatant can be measured directly. Urine sample should be acidified to a pH of 2-3 with 6N HCl to assay. Homogenize cell (2 x 10^6) or tissue (20 mg) samples in 200 µL PBS, then centrifuge to collect the supernatant for assay.

Transfer 100 µL of the dH_2O (blank), standard and sample into separate Eppendorf tubes, add 50 µL of the TCA solution to each tube and mix by vortexing. Centrifuge tubes at 14,000 rpm for 4 min. Collect the supernatant and use it for assay.

2. Reaction

Add 100 µL of the dH_2O (blank), standard and sample into separate wells of a clear flat-bottom 96-well plate.
Prepare enough working reagent by mix 100 µL of reagent A and 5 µL of reagent B for each reaction.
Transfer 100 µL working reagent into each reaction well. Tap plate to mix well. Incubate 5 min. at room temperature.

3. Measurement

Read the optical density at 360 nm (350-370 nm).

4. Calculation

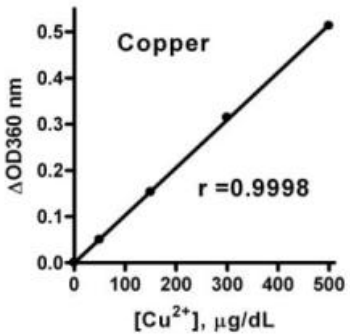
Calculate the copper concentration of the samples.

[Copper]= (OD_{sample}-OD_{blank})/ ($OD_{standard}$-OD_{blank}) x 500 x *n* (µg/dL)

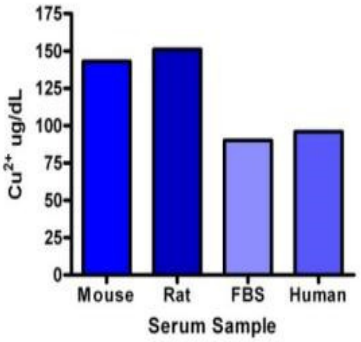
OD_{sample}, $OD_{standard}$ and OD_{blank} are the optical densities of the sample, standard and dH_2O. 500 is the concentration of copper standard (500 µg/dL), *n* is the sample dilution factor.

Conversions: 1 µg/dL copper equals 0.155 µM.

Typical Standard Curve



Copper standard in 96 wells-plate assay.



Serum sample in 96 wells-plate assay.

Sensitivity and Limit of Detection

The Limit of Detection was determined as 1.6 µg/dL (0.25 µM), with a linear detection range of up to 500 µg/dL (77.5 µM) in the 96-well plate colorimetric assay. Sensitivity was determined as 0.5 µg/dL. Samples with values above 500 µg/dL should be dilute with dH_2O, re-assayed, and multiplied by the dilution factor.

Interferences

EDTA inhibits copper to chromogenic system. The test is not affected by presence of bilirubin up to 40 mg/dL, hemoglobin up to 0.2 g/dL.

References

Brenner, AJ. et al. 1995. Analytical Biochemistry. 226:80-84
Braun, RD. et al. 1989, Analytica Chimica Acta 221: 223-238.

Related Products:

Triglyceride Detection Kit (#K316-100)
ALP Fluorimetric Detection Kit (#K110-200)
NAD/NADH Detection Kit (#K120-100)
Creatinine Detection Kit (#K148-200)
Glucose Detection Kit (#K188-100)
Glutathione Detection Kit (#K140-100)
Glutathione Peroxidase Detection Kit (#K143-100)
Glutathione Reductase Detection Kit (#K146-100)
HRP Fluorimetric Detection Kit (#K210-100)
Xanthine Colorimetric Detection Kit (#K133-100)
ATP Detection Kit (#K135-100)
Acetate Detection Kit (#K308-100)
Ammonia Detection Kit (#K103-200)
Magnesium Colorimetric Detection Kit (#K332-200)
Zinc Colorimetric Detection Kit (#K333-200)
Heme Detection Kit (#K169-200)
Lactate Detection Kit (#K207-100)
NADP/NADPH Detection Kit (#K123-100)
Nitric Oxide Detection Kit (#K116-100)
Phosphatase Detection Kit (#K196-500)
Urea Detection Kit (#K158-200)
Choline Detection Kit (#K310-100)
TBARS Detection Kit (#K145-100)
Pyruvate Detection Kit(#K150-100)
Hemoglobin Detection Kit (#K168-200)
ADP Detection Kit (#K134-100)
Phosphate Detection Kit (#K198-500)
Sulfate Detection Kit (#K149-200)
Iron Colorimetric Detection Kit (#K329-200)
Calcium Colorimetric Detection Kit (#K301-200)