Copper Assay Kit

Catalog No.: abx298883

Specification: 96 tests

Detection Range: 1.84 µmol/L - 60 µmol/L

Sensitivity: 1.84 µmol/L

Storage: Store all components at 2-8°C in the dark.

Application: For detection and quantification of copper in serum, plasma and tissue homogenates.

Introduction

Copper is an essential dietary mineral to all living organisms due to its importance in the respiratory enzyme complex Cytochrome C oxidase. Copper is a component of the blood pigment hemocyanin in crustaceans and mollusks, and a component of iron-complexed hemoglobin in fish and vertebrates. In humans, copper is predominately found in liver, muscle and bone. An adult body contains between 1.4-2.1 mg of copper per kilogram of body weight. Copper is important in several biological processes, including immune function, antioxidant defense, iron metabolism, enzyme reaction and nucleic acid synthesis. A deficiency of copper can affect bone and cholesterol metabolism, causing cardiovascular disease. An excess of copper can cause lung, kidney, and liver damage.

Abbexa's Copper Assay Kit is a quick, convenient, and sensitive method for measuring and calculating copper ion concentration. The copper ion in the sample will react with 3,5-DiBr-PAESA to form a purple complex which has a maximum absorption peak at 580 nm. The copper ion content can be calculated indirectly by measuring the OD value at 580nm.

Kit components

1. 96-well microplate
2. Detection Reagent A: 35 ml
3. Detection Reagent B: 2 vials
4. Copper Standard (100 µmol/L): 1 ml
5. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (580 nm)
2. Distilled water
3. Pipette and pipette tips
4. Vials/tubes
5. Incubator

Protocol

A. Preparation of samples and reagents

1. Reagents

- **Detection Reagent B solution**: Dissolve a vial of reagent 2 powder with 1.25 ml distilled water and mix. The prepared solution can be stored for 2-8°C for up to 5 days.
- **Detection Reagent Working Solution**: Mix Detection Reagent A and Detection Reagent B at a ratio of 14:1. Prepare the solution immediately before use.

2. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Fresh **s**amples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 25°C or at room temperature for up to 30 mins. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Chelating agents such as EDTA and citrate should not be used. Centrifuge for 10 mins at 700-1000 × g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue homogenates:** Weigh 0.02-1 g of tissue and wash with pre-chilled PBS at 2-8°C. For each 1 g of tissue, add 9 ml of pre-chilled normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 10000 x g at 4°C for 10 min. Collect the supernatant and preserve it on ice for detection or do the assay immediately. The protein concentration in the supernatant should be determined separately.

Samples should not contain detergents such as SDS, Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment.

The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human Serum	1
Human Plasma	1
Dog Serum	1
Rat Serum	1
Rabbit Serum	1
Porcine Serum	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for tissue homogenates.

B. Assay Procedure

1. Set sample, standard and blank wells on the microplate and record their positions. *Add the solution to the bottom of each well without touching the side walls. Pipette samples up and down to mix before adding to wells. Avoid foaming or bubbles.*
2. Label 8 tubes A-H.
3. Dilute the 100 µmol/L copper standard with distilled water to create a serial dilution. The recommended dilution gradient is as follows: 0, 5, 10, 20, 30, 40, 50, and 60 µmol/L. See the table for serial dilution reference.

Tube	Standard concentration (µmol/L)	Copper standard (µl)	Distilled water (µl)
A	0	100	0
B	5	95	5
C	10	90	10
D	20	80	20
E	30	70	30
F	40	60	40
G	50	50	50
H	60	40	60

4. Add 20 µl of each standard solution to the standard wells.
5. Add 20 µl of sample to the sample wells.
6. Add 300 µl of Detection Reagent Working Solution into all wells.
7. Cover the plate with a plate sealer and incubate at 37°C for 5 minutes
8. Measure the OD value of each well with a microplate reader at 580nm.

C. Calculation of Results

Plot the standard curve by using the OD value of the standard and correspondent concentration on the y-axis and x-axis respectively. Create the standard curve with graph software. The concentration of the sample can be calculated according to the formula based on the OD value of the sample. The standard curve is: y = ax + b.

1. Serum or plasma sample:

$$\text{Copper ion content } (\mu\text{mol/l}) = \frac{\Delta A_{580} - b}{a} \times f$$

2. Tissue samples:

$$\text{Copper ion content } (\mu\text{mol/g}) = \frac{\Delta A_{580} - b}{a} \times \frac{f}{C_{pr}}$$

where:

y	$OD_{Standard} - OD_{Blank}$
x	The concentration of the standard curve
a	The slope of the standard curve
b	The intercept of the standard curve
ΔA_{580}	$OD_{Sample} - OD_{Blank}$
C_{pr}	Concentration of the protein in sample (g/L)
f	Dilution factor of sample before test