

PRODUCT INFORMATION & MANUAL

Copper Assay Kit (Colorimetric)

NBP3-24482

For research use only.

Not for diagnostic or therapeutic procedures.

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Copper Assay Kit (Colorimetric)

Catalog No: NBP3-24482

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Microplate reader

Sensitivity: 1.84 µmol/L

Detection range: 1.84-60 µmol/L

Average intra-assay CV (%): 3.0

Average inter-assay CV (%): 3.1

Average recovery rate (%): 105

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲ Intended use

This kit can be used to measure Copper (Cu) concentration in serum, plasma and animal tissue sample.

▲ Background

Copper is essential to all living organisms as a trace dietary mineral because it is a key constituent of the respiratory enzyme complex cytochrome c oxidase. In molluscs and crustaceans, copper is a constituent of the blood pigment hemocyanin, replaced by the iron-complexed hemoglobin in fish and other vertebrates. In humans, copper is found mainly in the liver, muscle, and bone. The adult body contains between 1.4 and 2.1 mg of copper per kilogram of body weight.

Copper is involved in many biological processes, including enzyme reaction, nucleic acid synthesis, antioxidant defense, iron metabolism and immune function. Copper deficiency can affect the metabolism of bone and cholesterol and cause cardiovascular disease. Excess copper is associated with the damage of lung, kidney and liver.

▲ Detection principle

In acidic condition, the copper ion in the sample react with 3,5-DiBr-PAESA to form a purple complex which has a maximum absorption peak at 580 nm. And copper ion content can be calculated indirectly by measuring the OD value at 580 nm.

▲ Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Chromogenic Agent A	35 mL × 1 vial	2-8℃, 12 months, shading light
Reagent 2	Chromogenic Agent B	Powder × 2 vials	2-8°C , 12 months, shading light
Reagent 3	100 µmol/L Copper Standard	1 mL × 1 vial	2-8°C , 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.

▲ Materials prepared by users



1 Instruments

Microplate reader (575-585 nm), Micropipettor, Incubator



Reagents:

Double distilled water

▲ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

- 1. Prevent the formulation of bubbles in the microplate, or the result will be affected when measuring the OD value.
- 2. Serum and plasma samples should be clarified and avoid hemolysis. Heparin is recommended as anticoagulant.
- 3. Carry the assay in a ventilated place.

Pre-assay preparation

▲ Reagent preparation

- Preparation of reagent 2 application solution:
 Dissolve a vial of reagent 2 powder with 1.25 mL double distilled water and mix fully.
 The prepared solution can be stored at 2-8°C for 5 days.
- Preparation of chromogenic agent:
 Mix reagent 1 (mL) and reagent 2 application solution (mL) at a ratio of 14:1 fully. Prepare the fresh solution before use.

▲ Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

▲ Dilution of sample

It is recommended to take $2\sim3$ samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (1.84-60 µmol/L).

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: The diluent is double distilled water.

▲ Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
Α	Α	Α	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
В	В	В	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
С	С	С	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
Е	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
Н	Н	Н	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A, blank wells; B-H, standard wells; S1-S80, sample wells.

▲ Detailed operating steps

The preparation of standard curve

Dilute 100 μ mol/L copper standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 20, 30, 40, 50, 60 μ mol/L. Reference is as follows:

Number	Standard concentrations (µmol/L)	100 μmol/L Standard(μL)	Double distilled water (µL)
Α	0	100	0
В	5	95	5
С	10	90	10
D	20	80	20
Е	30	70	30
F	40	60	40
G	50	50	50
Н	60	40	60

The measurement of samples

(1) Standard well: Take 20 µL of standard solution with different concentrations into the wells.

Sample well: Take 20 µL of tested sample into the wells.

- (2) Add 300 µL of chromogenic agent into each tube of Step 1.
- (3) Cover the plate with sealer and incubate at 37°C for 5 min.
- (4) Measure the OD value at 580 nm with microplate reader.

▲ Summary operation table

	Standard well	Sample well			
Standard solution with different concentrations (µL)	20				
Sample (µL)		20			
Chromogenic agent (µL)	300	300			
Cover the plate with sealer and incubate at 37°C for 5 min and measure the					

OD value at 580 nm.

▲ Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample.

The standard curve is: y= ax + b.

1. For serum or plasma sample:

Copper ion content (μ mol/L) = (ΔA_{580} - b) ÷ a × f

2. For tissue sample:

Copper ion content (μ mol/gprot) = (ΔA_{580} - b) \div a \div C_{pr} × f

Note:

- y: $OD_{Standard} OD_{Blank}$. (OD_{Blank} is the OD value when the standard concentration is 0).
- x: The concentration of standard.
- a: The slope of standard curve.
- b: The intercept of standard curve.

 $\Delta A_{580}\text{: }OD_{\text{Sample}}-OD_{\text{Blank.}}$

C_{pr}: Concentration of protein in sample, gprot/L

f: Dilution factor of sample before test.

Appendix I Data

▲ Example analysis

Take 20 μL of sample, carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.0045 x + 0.0011, the average OD value of the sample well is 0.170, the average OD value of the blank well is 0.099, the calculation result is:

Cu content (μ mol/L)= (0.170-0.099-0.0011)÷ 0.0045=15.51 (μ mol/L)

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Collect fresh blood and stand at 25° C for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4° C. Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80 $^{\circ}$ C for a month.

▲ Plasma

Take fresh blood into the tube which has anticoagulant (Chelating agents such as EDTA and citrate should not be used as anticoagulants in plasma samples, heparin is recommended.), centrifuge at 700-1000 g for 10 min at 4° C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

▲ Tissue sample

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8 $^{\circ}$ C . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of double distilled water (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4 $^{\circ}$ C . Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant.

Appendix III References

- 1. Bost M, Houdart S, Oberli M, et al. Dietary Copper and human health: current evidence and unresolved issues[J]. Journal of Trace Elements in Medicine & Biology, 2016, 35:107-115.
- 2. Klevay L M. IHD from copper deficiency: a unified theory[J]. Nutrition Research Reviews, 2016, 29(2):172-179.
- 3. Esfahani S T, Hamidian M R, Madani A, et al. Serum zinc and copper levels in children with chronic renal failure.[J]. Pediatric Nephrology, 2006, 21(8):1153-1156.
- Skoczyńska A, Gruszczyński L, Wojakowska A, et al. Association between the Type of Workplace and Lung Function in Copper Miners[J]. Biomed Research International, 2016, 2016(3):1-15.