Copper (Cu) Colorimetric Assay Kit

Catalog No: E-BC-K300-M

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

- 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 or plasma 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℃ , 6 months, shading light
Reagent 2	Chromogenic Agent B	Powder × 2 vials	2-8℃ , 6 months, shading light
Reagent 3	100 μmol/L Copper Standard	1 mL × 1 vial	2-8℃ , 6 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

Instruments

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

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL)

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

1. 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℃ for 5 days.
2. 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 2~3 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.

Assay protocol	
Ambient temperature	25-30℃
Optimum detection wavelength	580 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	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
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

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

▲Operating steps

The preparation of standard curve

Dilute 100 μmol/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 μmol/L.

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℃ for 5 min.
(4) Measure the OD value at 580 nm with microplate reader.

▲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℃ for 5 min and measure the OD value at 580 nm with microplate reader.		

▲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: $y = ax + b$.

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

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.

ΔA_{580}: $OD_{Sample} - OD_{Blank}$.

f: Dilution factor of sample before test.

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	1.84-60 μmol/L	Average intra-assay CV (%)	3
Sensitivity	1.84 μmol/L	Average inter-assay CV (%)	3.1
Average recovery rate (%)	105		

▲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 (μmol/L)= (0.170-0.099-0.0011)÷ 0.0045=15.51 (μmol/L)