（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K300-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（575-585 nm）

Detection range：1.84-60 μmol/L

Elabscience® Copper (Cu) Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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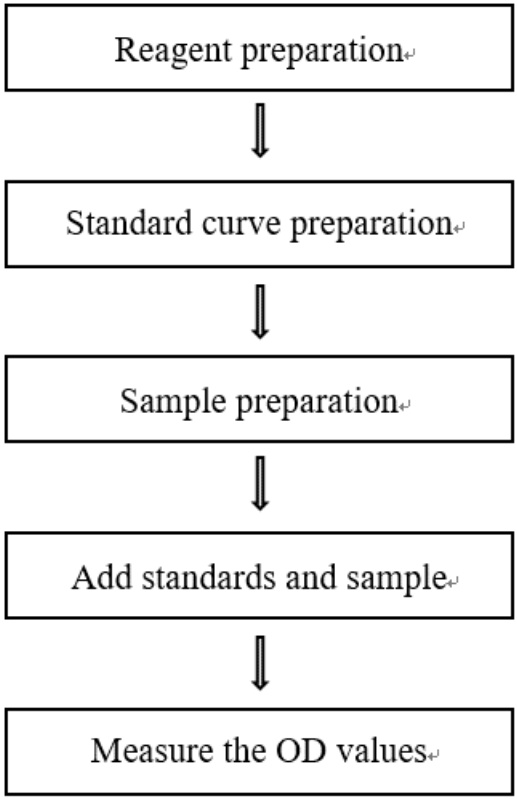
Assay summary



Intended use

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

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	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Chromogenic Agent A	17.5 mL ×1 vial	35 mL ×1 vial	2-8℃, 12 months shading light
Reagent 2	Chromogenic Agent B	Powder ×1 vial	Powder ×2 vials	2-8℃, 12 months shading light
Reagent 3	100 μmol/L Copper Standard	1 mL ×1 vial	1 mL ×1 vial	2-8℃, 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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader(575-585 nm), Vortex mixer, Micropipettor, Water bath

Reagents:

Double distilled water

Reagent preparation

① The preparation of chromogenic agent B application solution:

Dissolve a vial of chromogenic agent B with 1.25 mL double distilled water and mix fully. Aliquoted store at -20℃ for 1 month.

② The preparation of chromogenic agent:

For each tube, prepare 300 μL of chromogenic agent (mix well 280 μL of chromogenic agent A and 20 μL of chromogenic agent B application solution). The chromogenic agent should be prepared on spot.

③ The preparation of standard curve:

Always prepare a fresh set of standards. Discard working standard dilutions after use.

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. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**5**	**10**	**20**	**30**	**40**	**50**	**60**
100 μmol/L copper standard (μL)	0	5	10	20	30	40	50	60
Double distilled water (μL)	100	95	90	80	70	60	50	40

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL double distilled water with a dounce homogenizer at 4℃.

④ Centrifuge at 10000 × g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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. For the dilution of other sample types, please do pretest to confirm the dilution factor.

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.

Operating steps

① Standard tube: Take 15 μL of standard solution with different concentrations into tubes.

Sample tube: Take 15 μL of tested sample into tubes.

② Add 230 μL of chromogenic agent into each tube.

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.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

$$\text{Cu content (μmol/L)} = (\Delta A - b) \div a \times f$$

2. Tissue sample:

$$\text{Cu content (μmol/gprot)} = (\Delta A - b) \div a \times f \div C_{pr}$$

[Note]

f: Dilution factor of sample before test.

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

ΔA: Absolute OD (OD_{Sample} - OD_{Blank}).

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	4.80	26.70	52.00
%CV	3.5	3.0	2.5

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	4.80	26.70	52.00
%CV	2.8	3.2	3.3

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 105%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/L)	7.5	28.5	48.2
Observed Conc. (μmol/L)	7.6	30.5	51.6
Recovery rate (%)	101	107	107

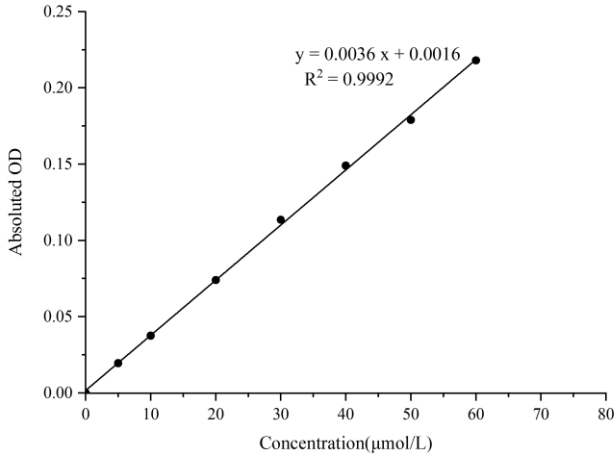
Sensitivity

The analytical sensitivity of the assay is 1.84 μmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (μmol/L)	**0**	**5**	**10**	**20**	**30**	**40**	**50**	**60**
Average OD	0.102	0.122	0.147	0.196	0.241	0.281	0.331	0.365
Absoluted OD	0	0.020	0.046	0.094	0.139	0.179	0.230	0.264



Appendix II Example Analysis

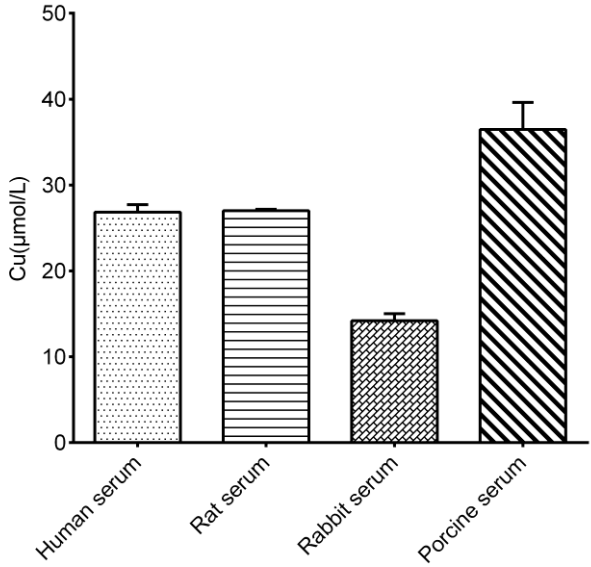
Example analysis:

Take 15 μL of rat serum, carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.0036 x + 0.0016, the average OD value of the sample well is 0.170, the average OD value of the blank well is 0.074, and the calculation result is:

$$\text{Cu content (μmol/L)} = (0.170 - 0.074 - 0.0016) \div 0.0036 = 26.22 \ \text{μmol/L}$$

Detect human serum, rat serum, rabbit serum and porcine serum, according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.