Technical Manual

Copper (Cu) Colorimetric Assay Kit

- **Catalogue Code: MAES0175**
- **Size: 96T**
- **Research Use Only**

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

1.84-60 μmol/L

Sensitivity:

1.84 μmol/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. 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.

3. Intended Use

This kit can be used to measure copper (Cu) concentration in serum or plasma sample.

4. 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.

5. Kit Components & Storage

Item	Specification	Storage
Chromogenic Agent A	35 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Chromogenic Agent B	Lyophilized × 2 vials	2-8°C, 6 months, avoid direct sunlight
Copper Standard (100 μmol/L)	1 mL × 1 vial	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (575-585 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water

6. Assay Notes:

1. Prevent the formation of bubbles in the microplate, or the result will be affected when measuring the OD value.

2. Serum and plasma samples should be clarified to prevent hemolysis. Heparin is recommended as an anticoagulant.

3. Perform the assay in a well ventilated place.

7. Reagent Preparation:

1. Bring all reagents to room temperature before use, pre-heat chromogenic agent A at 37°C until clear.

2. Preparation of **chromogenic agent B application solution:** Dissolve a vial of chromogenic agent B powder with 1.25 mL double distilled water and mix fully. The prepared solution can be stored at 2-8°C for 5 days.

3. **Preparation of chromogenic agent:** Mix chromogenic agent A (mL) and chromogenic agent B application solution (mL) at a ratio of 14:1 fully. Prepare the fresh solution before use.

8. Sample Preparation

1. Serum sample:
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) and preserve on ice before detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
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) and preserve on ice before detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. 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;

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 580 nm

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-H, standard wells; S1-S80, sample wells.

10. Operation 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°C 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°C for 5 min and measure the OD value at 580 nm with Microplate Reader.		

11. Calculations

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.

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

y: The absolute OD value of standard ($OD_{Standard} - OD_{Blank}$)

x: The concentration of Standard

a: The slope of standard curve

b: The intercept of standard curve

ΔA_{580}: Absolute OD ($OD_{Sample} - OD_{Blank}$)

f: Dilution factor of sample before test

12. Performance Characteristics

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

Analysis

Take 20 μL of rat serum, 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, and the calculation result is:

$$\text{Cu content (μmol/L)} = (0.170-0.099-0.0011) \div 0.0045$$

$$= 15.51\ (\mu mol/L)$$

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

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

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