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

Catalog No：E-BC-K775-M

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

Measuring instrument：Microplate reader（575-585 nm）

Detection range：0.18-5 μmol/L

Elabscience®Cell Copper (Cu^{2+}) Colorimetric Assay Kit (Complexing Method)

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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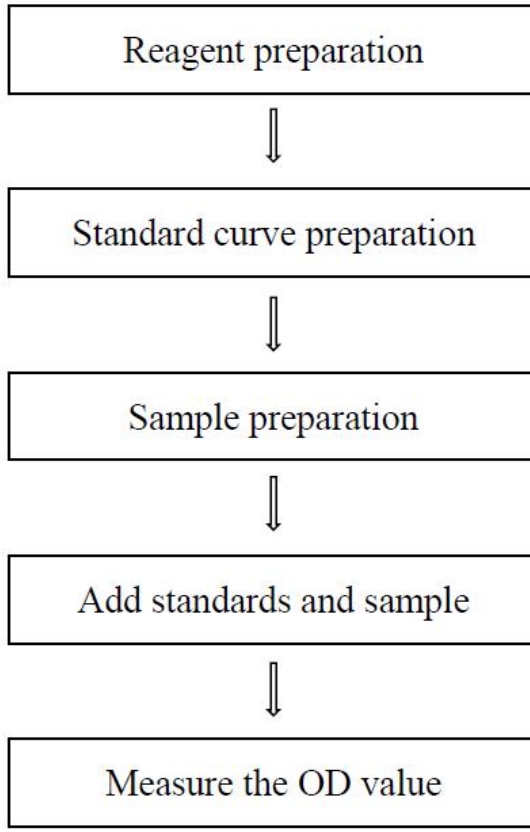
Assay summary



Intended use

This kit is used to measure copper ion content in cell sample.

Detection principle

In acidic condition, the copper ion in the sample react with complexing agent 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	3.5 mL × 1 vial	7 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	5 μmol/L Copper Standard	5 mL × 1 vial	5 mL × 1 vial	2-8℃, 12 months
Reagent 4	Lysis Buffer	12 mL × 1 vial	24 mL × 1 vial	2-8℃, 12 months, shading light
	Microplate	48 wells	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, optimum wavelength: 580 nm), Micropipettor, Vortex mixer, Incubator

Reagent preparation

① Incubate chromogenic agent A at 37°C until clarified and equilibrate other reagents to room temperature before use.

② Preparation of chromogenic agent B working solution：

Dissolve one vial of chromogenic agent B with 250 μL of double distilled water, mix well to dissolve. Aliquoted store at -20°C for 1 month.

③ Preparation of chromogenic solution：

Before testing, please prepare sufficient chromogenic solution according to the test wells. For example, prepare 75 μL of chromogenic solution (mix well 70 μL of chromogenic agent A and 5 μL of chromogenic agent B working solution, mix well. The chromogenic solution should be prepared on spot.

④ The preparation of standard curve：

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

Dilute 5 μmol/L Copper Standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.5, 1, 2, 2.5, 3, 4, 5 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**0.5**	**1**	**2**	**2.5**	**3**	**4**	**5**
5 μmol/L Copper Standard (μL)	0	40	80	160	200	240	320	400
Double distilled water (μL)	400	360	320	240	200	160	80	0

Sample preparation

① Sample preparation

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 2×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Lyse 2×10^6 cells with 200 μL lysis buffer. Place on the ice box and mix well every 5 min, lyse for 10 min.

④ Centrifuge at 12000×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
293T cell	1
Molt-4 cell	1
Jurkat cell	1
Hela cell	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 Use fresh cell samples for experiments.

② Avoid bubbles when adding liquid to the plate.

③ Carry the assay in a ventilated place.

Operating steps

① Standard well: Take 100 μL of standard solution with different concentrations into the wells.

Sample well: Take 100 μL of sample into the wells.

② Add 50 μL of Chromogenic solution into each tube.

③ Cover the plate with sealer and incubate at 37℃ 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:

$$\text{Cu content (μmol/gprot)} = (\Delta A_{580}\text{-b}) \div a \times f \div C_{pr}$$

[Note]

f: Dilution factor of sample before tested.

ΔA_{580}: OD_{Sample} – OD_{Blank}(OD_{Blank} is the OD value when the standard concentration is 0).

C_{pr}: The concentration of protein in sample, gprot/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three 239T cell 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)	1.70	2.60	4.30
%CV	3.2	3.1	2.7

Inter-assay Precision

Three 239T cell samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	1.70	2.60	4.30
%CV	2.8	3.0	3.5

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)	0.8	2.2	3.6
Observed Conc. (μmol/L)	0.8	2.3	3.9
Recovery rate(%)	101	106	108

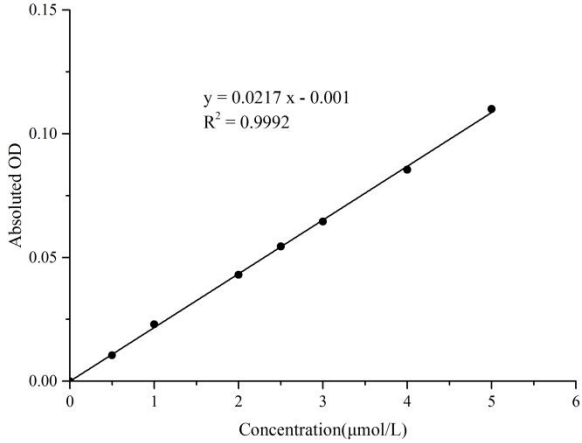
Sensitivity

The analytical sensitivity of the assay is 0.18 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**	**0.5**	**1**	**2**	**2.5**	**3**	**4**	**5**
Average OD	0.049	0.059	0.072	0.092	0.103	0.113	0.134	0.159
Absoluted OD	0.000	0.011	0.023	0.043	0.055	0.065	0.086	0.110



Appendix II Example Analysis

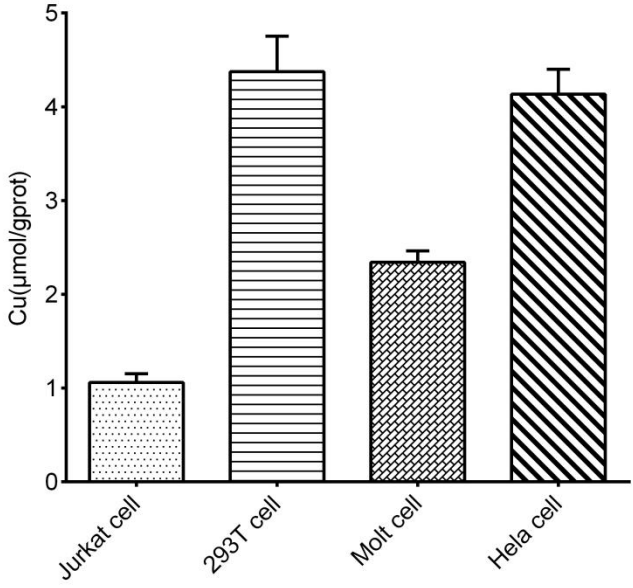
Example analysis:

For Molt-4 cells, take 2×10^6 Molt-4 cells, add 0.2 mL lysis buffer, process sample and take 100 μL cell homogenate supernatant, carry the assay according to the operation table. The results are as follows:

standard curve: y = 0.0217 x - 0.001, the OD value of the sample is 0.086, the OD value of the blank is 0.049, the concentration of protein in sample is 0.70 gprot/L, and the calculation result is:

$$\text{Cu content (μmol/gprot)} = (0.086 - 0.049 + 0.001) \div 0.0217 \div 0.70 = 2.50 \text{ μmol/gprot}$$

Detect Molt-4 cells (the number of cells is 2×10^6), Jurkat cells (the number of cells is 2×10^6), 293T cells (the number of cells is 2×10^6) and Hela cells (the number of cells is 2×10^6) 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.