PRODUCT INFORMATION & MANUAL

Cell Copper (Cu) Assay Kit (Colorimetric)

NBP3-25914

For research use only.
Not for diagnostic or therapeutic procedures.

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Cell Copper (Cu) Assay Kit (Colorimetric)

Catalog NBP3-25914

Method: Colorimetric method

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

Instrument: Microplate reader

Sensitivity: 0.18 μmol/L

Detection range: 0.18-5 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 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	Specification	Storage
Reagent 1	Chromogenic Agent A	7 mL × 1 vial	2-8℃ , 12 months, shading light
Reagent 2	Chromogenic Agent B	Powder × 2 vials	2-8℃ , 12 months, shading light
Reagent 3	5 μmol/L Copper Standard	2 mL × 1 vial	2-8℃ , 12 months
Reagent 4	Lysis Buffer	24 mL × 1 vial	2-8℃ , 12 months, shading light
	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, Vortex mixer, Incubator.

⚠ 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. Use fresh cell samples for experiments.
2. Avoid bubbles when adding liquid to the plate.
3. Carry the assay in a ventilated place.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use and incubate reagent 1 at 37°C until clarified.
2. Preparation of reagent 2 application solution:
   Dissolve a vial of reagent 2 powder with 0.25 mL double distilled water and mix fully.The prepared solution can be stored at -20°C for one month with shading light.
3. Preparation of chromogenic solution:
   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

Cell sample:

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add reagent 4 at a ratio of cell number (10^6): reagent 4 (mL) =2: 0.2. Place on the ice box and crack for 10 min. Centrifuge at 12000 g for 10 min. then take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant.

▲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 (0.18-5 μmol/L).

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.

Assay protocol

▲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.

▲Detailed operation steps

1. The preparation of standard curve

Dilute 5 μmol/L Copper ion 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:

Number	Standard concentrations (μmol/L)	5 μmol/L Copper ion standard (μL)	Double distilled water (μL)
A	0	0	400
B	0.5	40	360
C	1	80	320
D	2	160	240
E	2.5	200	200
F	3	240	160
G	4	320	80
H	5	400	0

2. The measurement of samples

(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 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.

▲Summary operation table

	Standard well	Sample well
Copper ion standard with different concentrations (μL)	100	
Sample (μL)		100
Chromogenic solution (μL)	50	50
Cover the plate and incubate at 37℃ for 5 min and measure the OD value.		

▲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.

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

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.

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 Data

▲Example analysis

For Molt-4 cells, take 2×10^6 Molt-4 cells, add 0.2 mL reagent 4, 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:

Cu content (μmol/gprot) = (0.086 - 0.049 + 0.001) ÷ 0.0217 ÷ 0.70 = 2.50 μmol/gprot