Cell Copper (Cu) Colorimetric Assay Kit (Complexing Method)

Catalog Number EEA007 (96 tests)

Rev 2.0

For safety and biohazard guidelines, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

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

In acidic conditions, copper ions in the sample react with a complexing agent to form a purple complex which has a maximum absorption peak at 580 nm. The copper ion content can be calculated indirectly by measuring the OD value at 580 nm.

Contents and storage

Kit and components are shipped at 2-8 ℃. An unopened kit can be stored at 2-8 ℃ for 12 months.

Components	Quantity (96 tests)
Chromogenic Agent A	7 mL
Chromogenic Agent B	Powder ×2 vials
5 μmol/L Copper Standard	5 mL
Lysis Buffer	24 mL
Microplate	1 plate
Plate Sealer	2 pieces

Required materials

- Distilled or deionized water
- Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)
- Microtiter plate reader with software capable of measurement at or near 580 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution
- Incubator capable of maintaining 37 ℃.

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Sample preparation guidelines

Sample requirements

- Use fresh cell samples for experiments.

Cells:

- 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 lysis buffer at a ratio of cell number (10^6): lysis buffer (mL) =2: 0.2.
- Place on the ice box and wait for 10 min. Centrifuge at 12000 g for 10 min., then take the supernatant for detection.

Meanwhile, determine the protein concentration of supernatant (Catalog No. 23227).
If not detected on the same day, the cells sample (without homogenization) can be stored at -80 ℃ for 1 month.

Prepare samples

It is recommended to take 2~3 samples with expected large difference to do a 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).

Note: Use all samples within 2 hours of dilution

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
HEL Cell	1

Note: the diluent is double distilled water

Bring all reagents to room temperature before use and incubate Chromogenic Agent A at 37 ℃ until clarified.

Preparation of chromogenic agent B application solution

Dissolve a vial of chromogenic agent B powder with 0.25 mL double distilled water and mix fully.

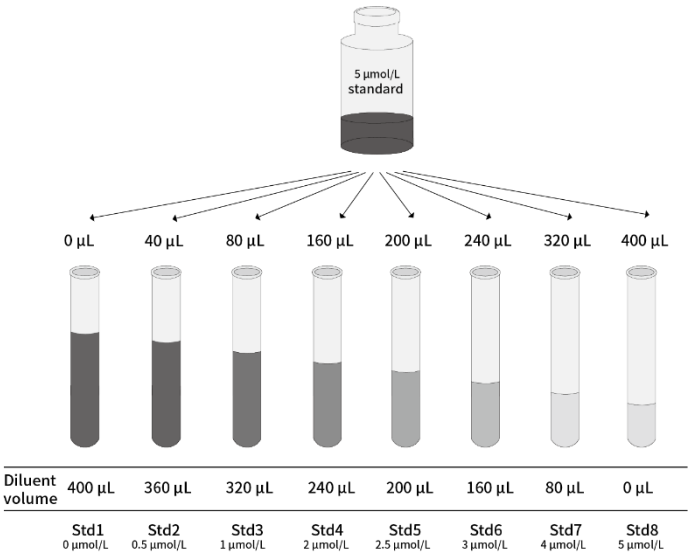
Preparation of chromogenic solution

Mix 14 parts of the chromogenic agent A (mL) with 1 part of the chromogenic agent B application solution (mL). Prepare the fresh solution before use.

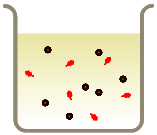
Prepare diluted standards

Note: Use glass or plastic tubes for diluting standards.

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.



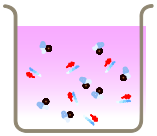
Assay procedure



1. Add sample

a. Take 100 μL of standard solution with different concentrations into the wells.

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



2. Add substrate

a. Add 50 μL of chromogenic solution into each tube of Step 1.

b. Cover the plate with sealer and incubate at 37 ℃ for 5 min.

c. 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 (Std1) 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).

Cell sample:

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

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.
1000*: 1 L=1000 mL

To easy calculate the test results, refer to the calculation file available on the webpage.

Example analysis

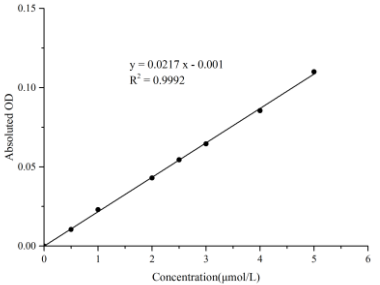
For Molt-4 cells, take 2×10^6 Molt-4 cells, add 0.2 mL lysis buffer, process sample and take 100 µL cell homogenate supernatant, carry the assay according to the operation table. The results are as follows:
standard curve: y = 0.0217 x - 0.001, the OD value of the sample is 0.086, the OD value of the blank is 0.049, the concentration of protein in sample is 0.70 gprot/L, and the calculation result is:

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

Performance characteristics

- Standard curve (example)

The following data were obtained for the various standards over the range of 0–5 µmol/L standard.

Concentration (mmol/L)	OD at 580 nm	Standard Curve
5.00	0.159	
4.00	0.134	
3.00	0.113	
2.50	0.103	
2.00	0.092	
1.00	0.072	
0.50	0.059	
0.00	0.049	

- Inter-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	0.75	1.50	3.50
%CV	3.2	3.1	3.0

CV = Coefficient of Variation

- Intra-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)	0.75	1.50	3.50
%CV	3.0	3.0	2.9

CV = Coefficient of Variation

- Expected values

This assay was tested with cell samples without dilutions.

Sample Type	Range (**μmol/gprot**)	Average (**μmol/gprot**)
293T cell（2×10^6）	3-5	4.79
jurkat cell（2×10^6）	0.7-1.5	1.09

▪ 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 98%.

	Sample 1 (low conc.)	Sample 2 (middle conc.)	Sample 3 (high conc.)
Expected Conc. (μmol/L)	0.75	1.50	3.50
Observed Conc. (μmol/L)	0.74	1.49	3.40
Recovery rate (%)	98	99	97

▪ Recommended 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.												

- 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.

Limited product warranty

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