



ProFoldin

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INSTRUCTIONS

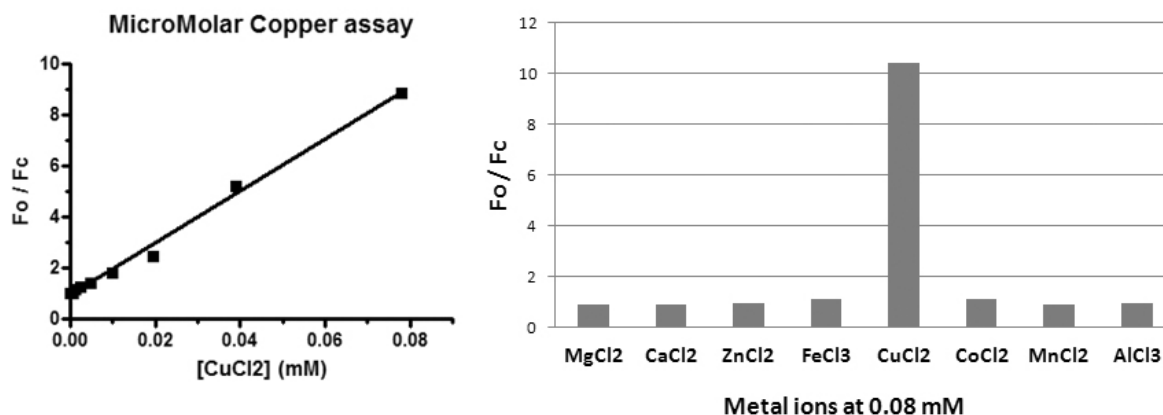
ProFoldin MicroMolar Copper Assay Kit

CATALOG NUMBER MCA1000

INTRODUCTION

Copper (Cu^{2+}) is an essential metal ion in biological systems. Many enzymes such as amine oxidase and galactose oxidase require copper for their biological functions. Low levels of copper are associated with disorders including mental retardation, depigmentation, anaemia, hypotonia and scorbutic changes in bone. Levels of copper are key diagnostic indicator of diseases such as Wilson's disease, microcytic hypochromic anaemia and bone disease.

The MicroMolar Copper Assay Kit is for measurement of micromolar concentrations of free copper ion Cu^{2+} (0.001 mM – 0.050 mM). The assay is based on the principle that binding the fluorescence dye MCA selectively with copper results in decrease of the fluorescence intensity (emission 535 nm, excitation 485 nm). The assay is compatible with 1 mM Mg^{2+} , Ca^{2+} , Zn^{2+} , Mn^{2+} and Al^{3+} , 0.1 mM Co^{2+} and Ni^{2+} . The Cu^{2+} sample should be diluted in water or 10 mM HEPES buffer, pH 7.4. It is not compatible with Tris buffer. EDTA, thiols, ammonia and amines strongly binds Cu^{2+} and should be avoided in the assay.



The assay kit can be used for high-throughput measurements of copper concentrations in biological samples or environmental water samples. The kit can also be used for biochemical assays of enzyme assays associated with copper metabolism.

The kit includes 100 μl of 100 x MCA dye and 30 μl of 1 mM CuCl_2 . It is for 200 assays using 96-well plates (100 μl of sample volume). Cuvettes may also be used for measurements. The kit also provides a 1 mM CuCl_2 solution for standard curve.



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PROTOCOL

The following assay protocol is based on using a 96-well plate the measurement. The sample volume is 100 μ l and the final assay volume is 150 μ l. For 384-well plate assays, the sample volume is 30 μ l and the final assay volume is 45 μ l. For assays using cuvette, the sample volume is 660 μ l and the final assay volume is 990 μ l.

STANDARD CURVE

- Sample preparation:** Prepare 100 μ l of CuCl_2 solutions with a two-fold serial dilution from 0.05 mM to zero in water or a 10 mM HEPES, pH 7.4 buffer. For 10 samples, dilute 7 μ l of the 100 x MCA dye 100-fold with water to make 700 μ l of 1 x MCA dye.
- Detection:** Mix 100 μ l of the CuCl_2 sample with 50 μ l of the 1x MCA dye solution for 5 min. Read the fluorescence intensity (**Fc**) at 535 nm (excitation 485 nm). The fluorescence without copper is **Fo**.
- Data Analysis:** Calculate the **Fo / Fc** ratio values and plot the correlation between the **Fo / Fc** ratio values and the copper concentrations to generate the linear standard curve.

$$\mathbf{Fo / Fc = a [Copper] + b}$$

Where the **Fo / Fc** values are from experimental data, the **a** and **b** values are from the linear fitting between the **Fo / Fc** values and the copper concentrations.

UNKNOWN SAMPLES

Follow the same procedure to get the **Fo / Fc** ratio values from the unknown samples. Calculate the copper concentrations in the unknown samples using the **Fo / Fc** ratio values from the unknown samples and the **a** and **b** values from the standard curve.

$$\mathbf{[Copper] = (Fo / Fc - b) / a}$$

RELATED PRODUCTS

| | |
|---------|--|
| NZA1000 | NanoMolar Zinc Assay Kit |
| NMA1000 | NanoMolar Nickel / Cobalt Assay Kit |
| DMA200 | MicroMolar Calcium / Magnesium Assay Kit |
| NPA1000 | NanoMolar Phosphate Assay Kit |
| PPD1000 | MicroMolar Polyphosphate Assay Kit |
| HIS200 | MicroMolar Histidine Assay Kit |
| CYS200 | MicroMolar Cysteine Assay kit |
| EDTA200 | MicroMolar EDTA Assay kit |
| DTT200 | MicroMolar DTT Assay kit |
| DAK1000 | Detergent assay kit |
| SDS200 | NanoGram SDS Assay Kit |
| CMC1000 | Detergent Critical Micelle Concentration (CMC) Assay Kit |
| LIP1000 | MicroGram Lipid Assay Kit |
| CLA100 | MicroMolar Chloride Assay Kit |
| MSA200 | MicroMolar Sulfate Assay Kit |

For more concentration assays or enzyme activity assays, please visit our website at www.profoldin.com.