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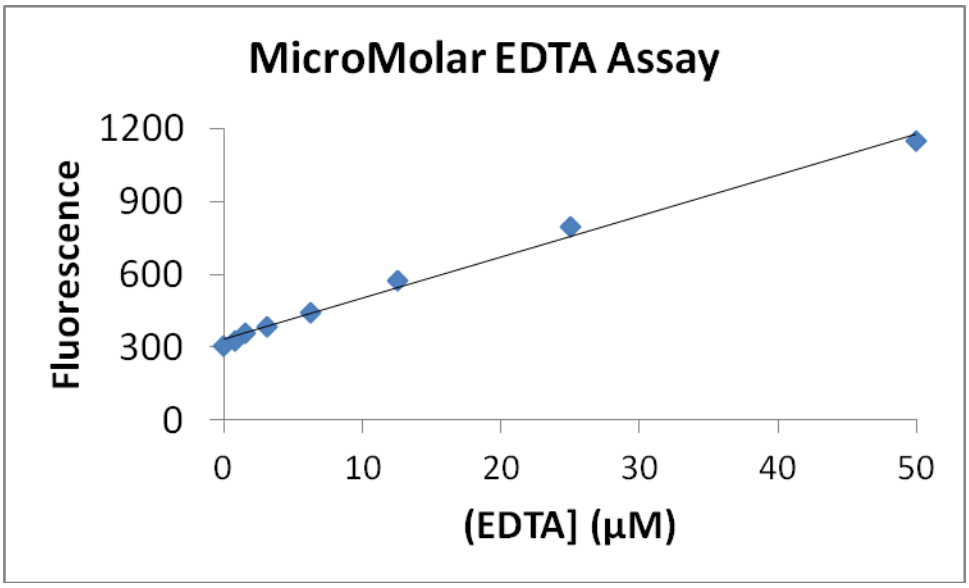
INSTRUCTIONS

ProFoldin
MicroMolar EDTA Assay Kit

CATALOG NUMBER **EDTA200**

INTRODUCTION

EDTA (Ethylenediaminetetraacetic acid) is a common chelating agent in biochemistry. EDTA should be avoided in protein purification with a Ni-column. Free EDTA should not be included in many enzyme reactions where divalent cations such magnesium, calcium and zinc are required for the enzyme activity. The MicroMolar EDTA Assay Kit (Catalog number EDTA200) is designed for measurement of micromolar concentrations of EDTA. The assay is based on increase of fluorescence at 535 nm of the dye C56 in the presence of EDTA. The assay kit can be used for measurements EDTA concentrations in biological samples, biochemical reactions and environmental water samples. The assay is compatible with HEPES buffer, low concentrations of non-ionic detergent (<0.01%), Tris-HCl (<10 mM), and phosphate (< 1 mM). It is not compatible with thiol compounds such as DTT, 2-mercaptoethanol or cysteine.



The MicroMolar EDTA Assay Kit (catalog number EDTA200) includes 200 µl of 100 x C56 dye and 1000 µl of 1 mM EDTA. It is for measurement of 200 samples using 96-well plates. Cuvettes may also be used for measurements.

ASSAY PROTOCOL

The following assay protocol is based on using a 96-well plate for the measurement. The sample volume is 100 µl and the final assay volume is 200 µl. For 384-well plate assays, the sample volume is 40 µl and

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the final assay volume is 80 µl. For assays using cuvette, the sample volume is 500 µl and the final assay volume is 1000 µl.

STANDARD CURVE

1. **Dye dilution:** For 10 samples, dilute 0.011 ml of the 100 x C56 dye 100-fold with water to make 1.1 ml of 1 x C56 dye. Keep the solution at room temperature for 5 min.

2. **Sample preparation**: Prepare 100 µl of EDTA solutions in the wells of a black 96-well plate with a two-fold serial dilution from 0.1 mM to zero in 10 mM HEPES, 0.1 M NaCl, pH 7.4 buffer.

3. **Detection:** Mix 100 µl of 1 x dye C56 with 100 µl of the EDTA solutions for 5 min and read the fluorescence at 535 nm (excitation at 485 nm).

   Note: The assay sensitivity is higher when the incubation time after dye addition is longer. The assay linearity will be also affected by the incubation time.

4. **Data Analysis**: Plot the fluorescence intensity **Fc** and the EDTA concentration **[EDTA]** to generate the linear standard curve.

$$\mathbf{Fc = a\ [EDTA] + b}$$

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

UNKNOWN SAMPLES

Follow the same procedure to measure the fluorescence intensity **Fc** values from the unknown samples. Calculate the EDTA concentrations in the unknown samples using the **Fc** values from the unknown samples and the **a** and **b** values from the standard curve.

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

RELATED PRODUCTS

HIS200	MicroMolar Histidine Assay Kit
CYS200	MicroMolar Cysteine Assay kit
PEP200	Peptide Assay Kit
DAK1000	Detergent assay kit
LIP1000	MicroGram Lipid Assay Kit
DDT200	MicroMolar DTT Assay kit

For more concentration assays of various biochemical molecules and inorganic ions, please visit our website at www.profoldin.com.