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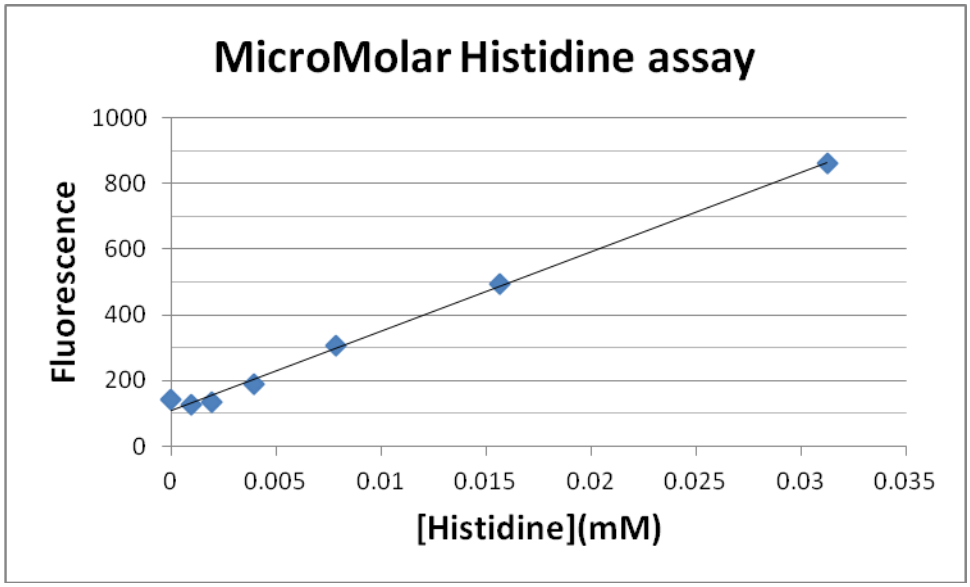
INSTRUCTIONS

ProFoldin
MicroMolar Histidine Assay Kit

CATALOG NUMBER **HIS200**

INTRODUCTION

Histidine is an important molecule in biology and pharmaceutical science. Metabolic block of histidine results in increased concentrations of histidine in blood, urine, and cerebrospinal fluid. Disorders of histidine metabolism is related to diseases such as histidinemia and urocanic aciduria. Histidine is a common ingredient of pharmaceutical products. The MicroMolar Histidine Assay Kit is designed for measurement of micromolar concentrations of histidine. The assay is based on increase of fluorescence at 535 nm of the dye C53 in the presence of histidine. The assay kit can be used for measurements histidine concentrations in biological samples, biochemical reactions, pharmaceutical products and environmental water samples. The assay is compatible with HEPES buffer, low concentrations of non-ionic detergent (<0.01%), $MgCl_2$ (< 5 mM), $CaCl_2$ (<5 mM), EDTA (< 1 mM) and phosphate (< 1 mM). It is not compatible with thiol compounds such as DTT. For measurement of other amino acids, see the information of Amino Acid Assay Kit (Catalog Number AAK1000).



The MicroMolar Histidine Assay Kit (catalog number HIS200) includes 750 µl 10 x C53 Dye and 50 µl of 10 mM Histidine. It is for measurement of 200 samples using 96-well plates. Cuvettes may also be used for measurements.

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ASSAY PROTOCOL

The following assay protocol is based on using a 96-well plate for the measurement. The sample volume is 150 µl and the final assay volume is 188 µl. For 384-well plate assays, the sample volume is 60 µl and the final assay volume is 75 µl. For assays using cuvette, the sample volume is 800 µl and the final assay volume is 1000 µl.

STANDARD CURVE

1. **Sample preparation:** Prepare 150 µl of histidine solutions in the wells of a black 96-well plate with a two-fold serial dilution from 0.050 mM to zero in a 10 mM HEPES, pH 7.4 buffer. For 10 samples, dilute 40 µl of the 10 x C53 dye 10-fold with water to make 400 µl of 1 x C53 dye.

2. **Detection:** Mix 37.5 µl of 1 x dye C53 with 150 µl of the histidine solutions for 5 min and read the fluorescence at 535 nm (excitation at 485 nm).

3. **Data Analysis**: Plot the fluorescence intensity **Fc** and the histidine concentration **[Histidine]** to generate the linear standard curve.

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

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

UNKNOWN SAMPLES

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

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

RELATED PRODUCTS

MicroMolar Cysteine Assay Kit	Catalog number: CYS200
MicroMolar Primary Amine Assay Kit	Catalog number: PAA100K
MicroMolar EDTA Assay Kit	Catalog number: EDTA200
MicroGram Lipid Assay Kit	Catalog number: LIP1000

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