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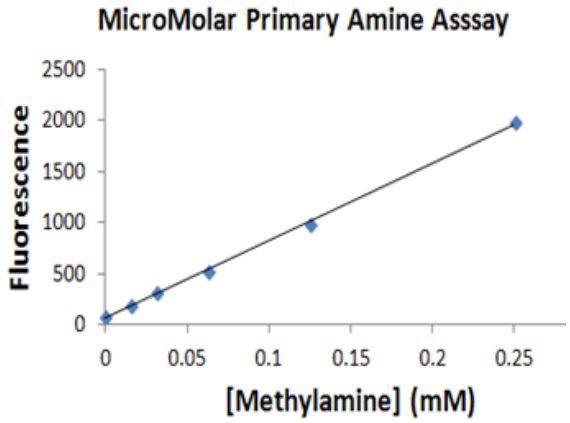
INSTRUCTIONS

ProFoldin
MicroMolar Primary Amine Assay Kit

CATALOG NUMBER **PAA100K**

INTRODUCTION

The MicroMolar Primary Amine Assay Kit is designed for measurement of primary amines or ammonia or their salts at micromolar concentrations. Amino acids, peptides and many drug molecules contain primary amine groups can be quantified using this kit. Protonated primary amines or ammonium salts release the free primary amine or ammonia in the kit assay buffer. Primary amines or ammonia interact with the assay reagent PAA dye to form fluorescent products with excitation at 390 nm and emission at 470 nm. The kit can be generally used for measurements of micromolar concentrations of primary amines or ammonia or their salts in biological samples, biochemical reactions, pharmaceutical products and environmental water samples. The assay is not compatible with amine-based buffers such as Tris buffer. It is compatible with thiol compounds such as DTT.



The Primary Amine Assay Kit (Catalog number PAA100K) includes 0.5 ml of 10 x PAA dye, 5.5 ml of Assay buffer, 5 ml of reagent A and 50 µl of 10 mM Tris-HCl. It is for measurement of 100 samples using 96-well plates. Cuvettes may also be used for measurements.

ASSAY PROTOCOL

The following assay protocol is based on assays using a 96-well plate for the measurement. The sample volume is 50 µl and the final assay volume is 100 µl. For 384-well plate assays, the sample volume is 30 µl and the final assay volume is 60 µl. For assays using cuvette, the sample volume is 500 µl and the final assay volume is 1000 µl.

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STANDARD CURVE

1. **Sample preparation:** Prepare 50 µl of primary amine or its salt solutions in the wells of a black 96-well plate with a two-fold serial dilution from 1 mM to zero in Assay Buffer. For 10 samples, dilute 0.05 ml of the 10 x PAA dye 10-fold with 0.45 ml of Reagent A to make 0.5 ml of 1 x PAA dye.

2. **Detection:** Mix 50 µl of 1 x PAA dye with 50 µl of the amine solutions for 5 min and read the fluorescence at 470 nm (excitation at 390 nm).

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

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

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

UNKNOWN SAMPLES

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

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

RELATED PRODUCTS

NPA1000	NanoMolar Phosphate Assay Kit
HIS200	MicroMolar Histidine Assay Kit
CYS200	MicroMolar Cysteine Assay kit
CAK1000	Coenzyme A 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
MAD100K	MicroMolar ADP Assay kit
MCA1000	MicroMolar Copper Assay Kit
NZA1000	NanoMolar Zinc Assay Kit
MSA200	MicroMolar Sulfate Assay Kit
PMX200	MicroGram Polymyxin Assay Kit

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