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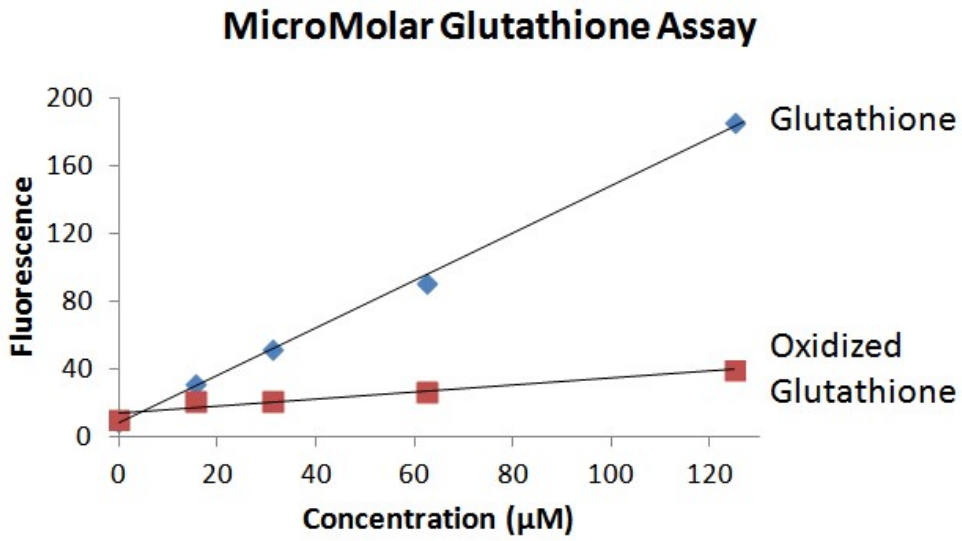
INSTRUCTIONS

ProFoldin
MicroMolar Glutathione Assay Kit

CATALOG NUMBER MGA200

INTRODUCTION

Glutathione is an essential antioxidant in the cells that prevents damage of biomolecules due to oxidation. Glutathione is also a popular ingredient in pharmaceutical products. The MicroMolar Glutathione Assay Kit is for measurement of micromolar concentrations of glutathione. The assay is based on increase of the fluorescence intensity (emission 535 nm, excitation 485 nm) of the kit fluorescence dye MAA upon binding to glutathione. The assay detects reduced glutathione (GSH) at a much higher sensitivity than the oxidized form (GSSG).



The assay kit can be used for measurements glutathione concentrations in pharmaceutical products, biochemical reactions or other 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 and cysteine. It is not compatible with samples with histidine.

The MicroMolar Glutathione Assay Kit (catalog number MGA200) includes 500 µl of 10 x MAA dye. It is for 200 assays using 96-well plates or 500 samples using 384-well plates. Cuvettes may also be used for measurements. For 96-well plate assays, the sample volume is 100 µl and the final assay volume is 125 µl. For 384-well plate assays, the sample volume is 40 µl and the final assay volume is 50 µl. For assays using cuvette, the sample volume is 800 µl and the final assay volume is 1000 µl.

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PROTOCOL

The following assay protocol is based on the assay format using a 96-well plate.

STANDARD CURVE

1. **Sample preparation:** Freshly prepare 100 µl of glutathione solutions in a 96-well black plate with a two-fold serial dilution from 0.2 mM to zero in water or a 10 mM HEPES, pH 7.4 buffer. For 10 samples, dilute 26 µl of the 10 x MAA dye 10-fold to make 260 µl of 1 x MAA dye.

2. **Detection:** Mix 25 µl of 1 x MAA dye with 100 µl of the glutathione solutions and immediately read the fluorescence at 535 nm with excitation at 485 nm.

> *Note:* A longer incubation after addition of the dye may increase the detection sensitivity of the oxidized form of glutathione GSSG and other amino acids or peptides.

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

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

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

UNKNOWN SAMPLES

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

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

RELATED PRODUCTS

MicroMolar Cysteine Assay Kit	Catalog number: CYS200
MicroMolar Primary Amine Assay Kit	Catalog number: PAA100K
MicroMolar UDP Assay Kit	Catalog number: MUD100K
MicroMolar EDTA Assay Kit	Catalog number: EDTA200
NanoMolar Zinc Assay Kit	Catalog number: NZA1000
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.