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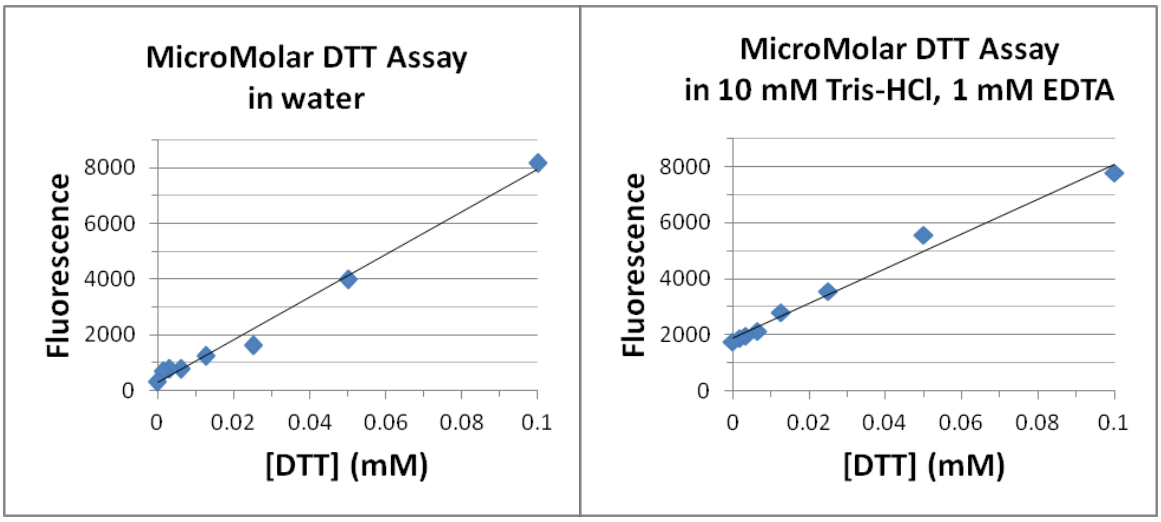
INSTRUCTIONS

ProFoldin
MicroMolar DTT Assay Kit

CATALOG NUMBER **DTT200**

INTRODUCTION

DTT (Dithiothreitol) is a common reducing agent in biochemistry. Removal of DTT is required for Cys-based protein labeling and for disulfide bond formation in proteins. The MicroMolar DTT Assay Kit (Catalog number DTT200) is designed for measurement of micromolar concentrations of DTT. The assay is based on increase of fluorescence at 535 nm of the dye C55 in the presence of DTT. The assay kit can be used for measurements DTT 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%), $MgCl_2$ (< 5 mM), $CaCl_2$ (<5 mM), Tris-HCl (<10 mM), EDTA (< 1mM) and phosphate (< 1 mM). It is not compatible with thiol compounds such as 2-mercaptoethanol or cysteine.



The MicroMolar DTT Assay Kit (catalog number DTT200) includes 5 ml of Dye C55. 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 125 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.

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STANDARD CURVE

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

2. **Detection:** Mix 25 µl of Dye C55 with 100 µl of the DTT solutions for 10 min and read the fluorescence at 535 nm (excitation at 485 nm).

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

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

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

UNKNOWN SAMPLES

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

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

RELATED PRODUCTS

DTT100C	Colorimetric DTT Assay Kit
PPD1000	MicroMolar Polyphosphate Assay Kit
HIS200	MicroMolar Histidine Assay Kit
CYS200	MicroMolar Cysteine Assay kit
PEP200	Peptide Assay Kit
PAA100K	MicroMolar Primary Amine Assay Kit
CAK1000	Coenzyme A Assay Kit
EDTA200	MicroMolar EDTA Assay kit
DAK1000	Detergent Assay Kit
CMC1000	Detergent Critical Micelle Concentration (CMC) Assay Kit
LIP1000	MicroGram Lipid Assay Kit
NPA1000	NanoMolar Phosphate Assay Kit
MAD100K	MicroMolar ADP Assay kit
MUD100K	MicroMolar UDP assay kit
MCA1000	MicroMolar Copper Assay Kit
NZA1000	NanoMolar Zinc Assay Kit
MSA200	MicroMolar Sulfate Assay Kit

For more information of concentration assays, please visit www.profoldin.com.