ProFoldin
10 Technology Drive, Suite 40, Number 188
Hudson, MA 01749-2791 USA
Phone: (508) 735-2539 **FAX: (508) 845-9258**
www.profoldin.com info@profoldin.com

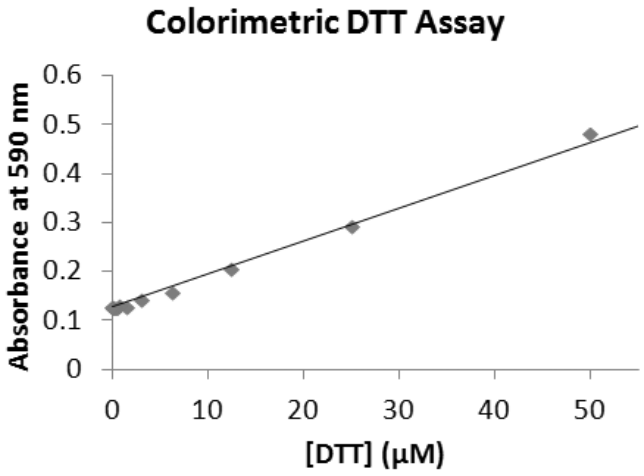
INSTRUCTIONS

ProFoldin
Colorimetric DTT Assay Kit

CATALOG NUMBER **DTT100C**

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 Colorimetric DTT Assay Kit (Catalog number DTT100C) is designed for measurement of micromolar concentrations of DTT. The assay is based on measurement of light absorbance at 495 nm. The assay kit can be used for measurements DTT concentrations in biological samples, biochemical reactions and environmental water samples. The assay is compatible with common buffers. It is not compatible with NADH or NADPH.



The Colorimetric DTT Assay Kit (catalog number DTT100C) includes 1 ml of 10 x Buffer, 0.2 ml of 10 x Reagent A, 0.2 ml of 10 x Reagent B and 1 ml of 10 x Reagent C. 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 using a 96-well plate for the measurement. The sample volume is 100 µl and the final assay volume is 190 µl. For 384-well plate assays, the sample volume is 40 µl and the final assay volume is 76 µl. For assays using cuvette, the sample volume is 500 µl and the final assay volume is 950 µl.

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STANDARD CURVE

1. **Sample preparation:** Prepare 100 µl of DTT solutions in the wells of a clear 96-well plate with a two-fold serial dilution from 0.1 mM to zero in water or a 20 mM Tris-HCl, pH 8.0 buffer. The 10 x Reagent stock solutions are diluted 10 fold to make the 1 x Reagent solutions.

2. **Detection:** Add 20 µl of 1x Reagent A and 20 ul of 1 x Reagent B and incubate the mixture for 10 min. The add 50 µl of 1x Reagent C and read the light absorbance at 590 nm ($\mathbf{A_{590}}$)

3. Data Analysis: Plot the A_{590} values and the DTT concentration **[DTT]** to generate the linear standard curve.

$$\mathbf{A_{590} = a\ [DTT] + b}$$

Where the $\mathbf{A_{590}}$ values are from experimental data, the **a** and **b** values are from the linear fitting between the $\mathbf{A_{590}}$ values and the DTT concentrations.

UNKNOWN SAMPLES

Follow the same procedure to measure the light absorbance at 590 nm ($\mathbf{A_{590}}$) values from the unknown samples. Calculate the DTT concentrations in the unknown samples using the $\mathbf{A_{590}}$ values from the unknown samples and the **a** and **b** values from the standard curve.

$$\mathbf{[DTT] = (A_{590} - b) / a}$$

RELATED PRODUCTS

DTT200	MicroMolar DTT Assay Kit (a fluorescence assay)
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.