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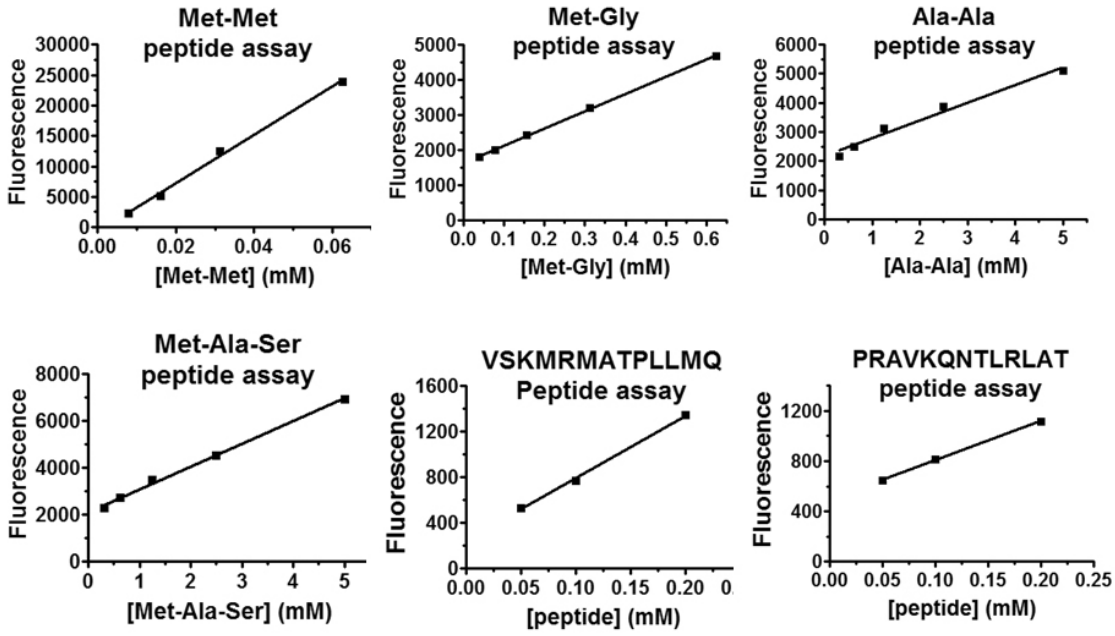
INSTRUCTIONS

ProFoldin
Peptide Assay Kit

CATALOG NUMBER **PEP200**

INTRODUCTION

Short amino acid chains or peptides play key roles in many biological functions. Peptides from antigens are responsible for immune responses. Many biologically secreted and synthetic peptides have been used or being developed as drugs in various therapeutic areas including treatment of infection, cancer, diabetes, and cardiovascular diseases. The MicroMolar Peptide Assay Kit (Catalog number PEP200) is designed for concentration measurement of various peptides. The assay is based on increase of fluorescence at 535 nm of the dye C57 in the presence of peptides. The assay kit can be used for measurements peptide concentrations in synthetic or biochemical reactions, pharmaceutical products and environmental water samples. The assay sensitivity varies from micromolar to millimolar concentrations depending on the nature of peptides. 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.



The MicroMolar Peptide Assay Kit (catalog number PEP200) includes 0.5 ml of 10 x Dye C57. It is for 200 assays 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 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.

STANDARD CURVE

The assay sensitivity greatly depends on the nature of the peptide.

1. **Sample preparation:** Prepare 100 µl of peptide solutions in the wells of a black 96-well plate with a two-fold serial dilution from 5 mM to zero in a 10 mM HEPES, pH 7.4 buffer. For 10 samples, dilute 27 µl of the 10 x C57 dye 10-fold with water to make 270 µl of 1 x C57 dye.

2. **Detection:** Mix 25 µl of 1 x dye C57 with 100 µl of the peptide solutions for 15 min and read the fluorescence at 535 nm (excitation at 485 nm).

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

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

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

UNKNOWN SAMPLES

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

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

RELATED PRODUCTS

HPEP200	Hydrophobic Peptide Assay Kit (for water-insoluble peptides)
PEPD200	Peptide Derivative Assay Kit
HIS200	MicroMolar Histidine Assay Kit
CYS200	MicroMolar Cysteine Assay kit
PAA100K	MicroMolar Primary Amine Assay Kit
EPA001	Easy Protein Assay Reagent
DAK1000	Detergent assay kit
LIP1000	MicroGram Lipid Assay Kit

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