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

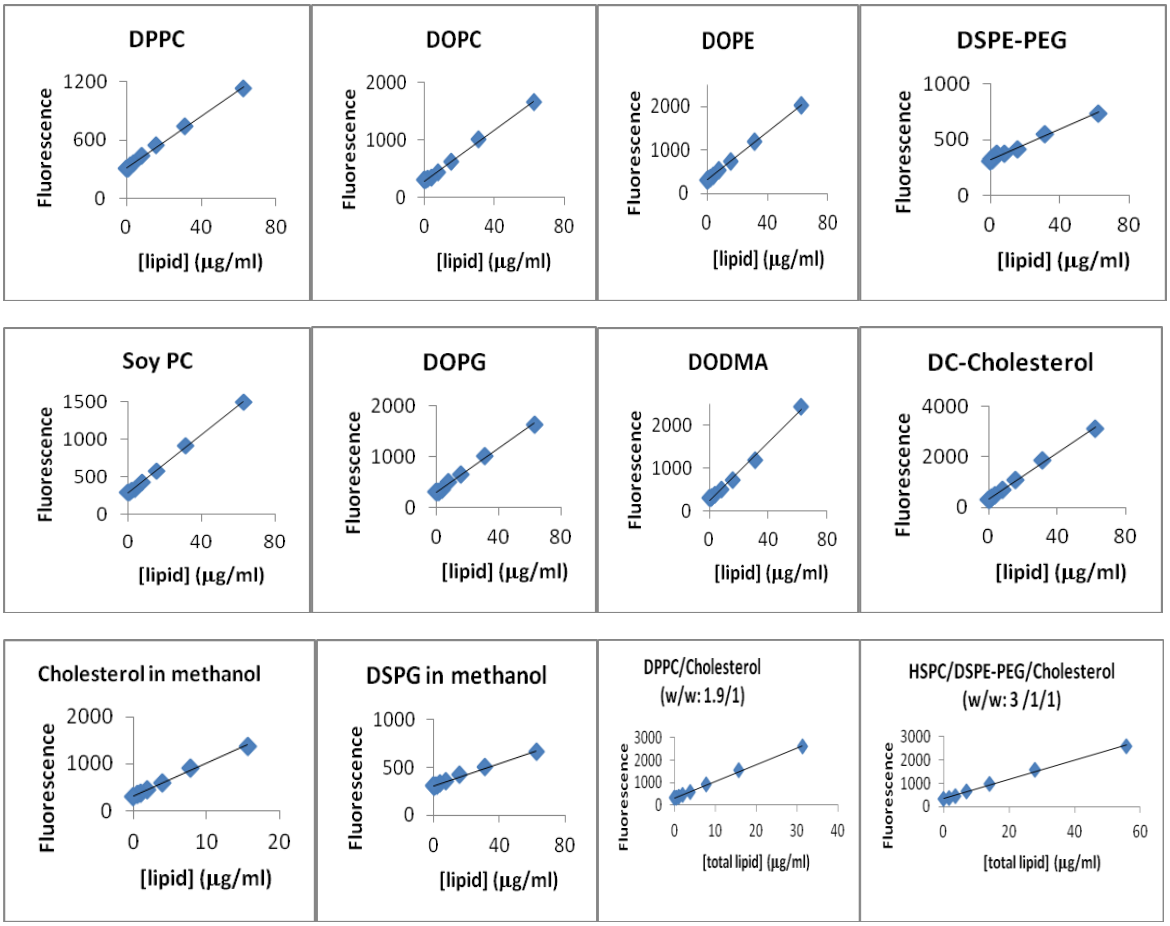
ProFoldin
MicroGram Lipid Assay Kit

CATALOG NUMBER **LIP1000**

INTRODUCTION

Lipids are essential components of cell membranes. Synthetic lipids or lipids isolated from the nature are used for constructions of bilayer membranes for various applications such as membrane protein reconstitution and liposomal drug formulations. The MicroGram Lipid Assay Kit (Catalog number LIP1000) is designed for measurement of various lipids at concentrations of microgram per milliliter. The assay is based on measurement of fluorescence at 465 nm (excitation at 360 nm). It can be used to measure concentrations of various purified lipids or lipid mixtures such as cell membrane lipids or liposomes.

The MicroGram Lipid Assay Kit (Catalog number LIP1000) includes 0.1 ml of 1000 x LIP 10 Dye. It is for measurement of 1000 samples 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.

Protocol 1

Most lipids can be measured using protocol1.

Add 100 µl of 0.1 M NaCl into 12 wells of a black 96-well plate. Mix100 µl of 1 mg/ml lipid in ethanol or methanol with the first well containing 100 µl of 0.1 M NaCl. Perform a two-fold serial dilution in the other wells containing 100 µl of 0.1 M NaCl and leave the last well for zero control. For each 20 samples, dilute 2 µl of 1000 x LIP10 Dye with 2 ml of water to make the 1 x LIP10 Dye. Mix 100 µl of LIP10 Dye with 100 µl of the lipid solutions and incubate the mixture for 5 min. Read the fluorescence at 465 nm (excitation at 360 nm).

Protocol 2

Some lipids should be measured using protocol 2 due to its insolubility in an aqueous solution.

Add 100 µl of methanol into 12 wells of a black 96-well plate. Mix100 µl of 1 mg/ml lipid in ethanol or methanol with the first well containing methanol. Perform a two-fold serial dilution in the other wells containing 100 µl of methanol and leave the last well for zero control. For each 20 samples, dilute 2 µl of 1000 x LIP10 Dye with 4 ml of water to make the 0.5 x LIP10 Dye. Mix 200 µl of the 0.5 x LIP10 Dye with 100 µl of the lipid solutions and incubate the mixture for 5 min. Read the fluorescence at 465 nm (excitation at 360 nm).

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

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

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

UNKNOWN SAMPLES

Dissolve the lipid sample in ethanol or methanol. Follow the same procedure to measure the fluorescence intensity **Fc** values from the unknown samples. Calculate the lipid concentrations in the unknown samples using the **Fc** values from the unknown samples and the **a** and **b** values from the standard curve.

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

For more concentration assays of various biochemical molecules and inorganic ions, please visit our website at www.profoldin.com.