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INSTRUCTIONS

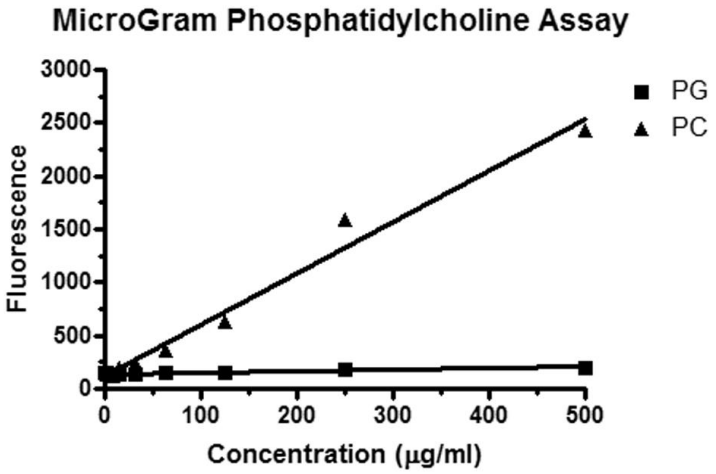
ProFoldin
MicroGram Phosphatidylcholine Assay Kit

CATALOG NUMBER **MPC200K**

INTRODUCTION

The MicroGram Phosphatidylcholine Assay Kit is for measurement of L-α–phosphatidylcholine (PC) at concentrations of 0.005% (0.05 mg/ml) or higher. The assay is based on the principle that phosphatidylcholine interacts with the MPC reagent and enhances the fluorescence intensity at 535 nm (excitation at 485 nm). L-α–phosphatidylglycerol (PG) does not interact with the MPC reagent. The assay kit is sufficient for measurement of 200 samples using 96-well plates (Costar 3915 and Greiner 655076).

The assay is compatible with most biochemical assay buffers such as HEPES or Tris-HCl buffers. It is compatible with low concentrations of Ethanol and DMSO. It is not compatible with phosphate buffers. If ATP is used for the biochemical reaction, it is recommended to use ATP concentrations below 200 µM. The assay is not compatible with most detergents.



PG = L-α-Phosphatidylglycerol (from E. coli)
PC = L-α-Phosphatidylcholine (from egg yolk)

The assay kit (Catalog number MPC200K) includes 5 ml of Reagent A, 20 ml of Reagent B, 0.2 ml of 100 x Reagent C and 10 ml Reagent D. It is for 200 assays using 96 well plates.

ASSAY PROTOCOL

The following assay protocol is based on using a 96-well plate for the measurement. The sample volume is 25 µl and the final assay volume is 175 µl. For assays using cuvette, the sample volume is 145 µl and the final assay volume is 1015 µl.

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STANDARD CURVE

1. **Sample preparation:** Prepare 25 µl of L-α–phosphatidylcholine (PC) solutions in the wells of a black 96-well plate with a two-fold serial dilution from 1 mg/ml to zero in methanol. For each 10 samples, dilute 0.01 ml of 100 x Reagent C with 0.99 ml of Reagent B to make Reagent BC.

2. **Detection:** Mix 25 µl of Reagent A with 25 µl of the PC solutions then add 100 µl of Reagent BC. After mixing the solution, add 50 µl of Reagent D and incubate the mixture for 5 min. Read the fluorescence at 535 nm (excitation at 485 nm).

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

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

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

UNKNOWN SAMPLES

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

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

RELATED PRODUCTS

LIP1000	MicroGram Lipid Assay Kit
CMC1000	Detergent Critical Micelle Concentration (CMC) Assay Kit
DAK1000	Detergent assay kit
SDS200	NanoGram SDS Assay Kit
MPA3000	MicroMolar Phosphate Assay Reagent
NPA1000	NanoMolar Phosphate Assay Kit
PPD1000	MicroMolar Polyphosphate Assay Kit
MSA200	MicroMolar Sulfate Assay Kit
EDTA200	MicroMolar EDTA Assay kit
CLA100	MicroMolar Chloride Assay Kit
DTT200	MicroMolar DTT Assay kit
PAA100K	MicroMolar Primary Amine Assay Kit
MPX200	MicroGram Polymyxin Assay Kit
CPT200	MicroMolar Cisplatin Assay Kit
OPT200	MicroMolar Oxaliplatin Assay Kit

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