

# A Novel Enzymatic Assay for Determination of Phosphatidylinositol in Biological Samples

Paul Templeton, Kyle C. Schmitt, Grigoriy Tchaga, and Gordon Yan  
BioVision Inc., Milpitas, California 95035

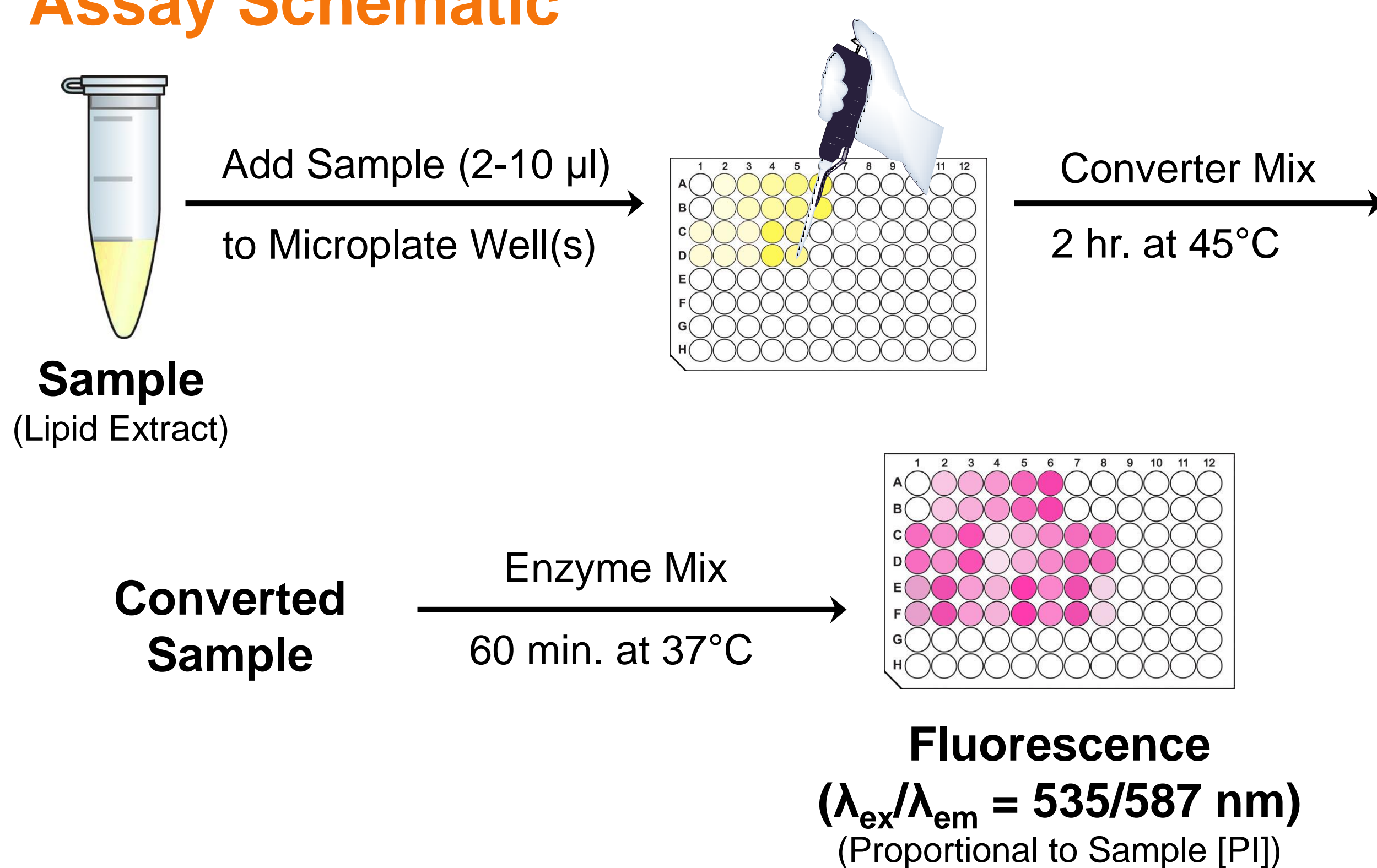


pault@biovision.com

## Introduction

Phosphatidylinositol (PI) is a molecule central to both phospholipid metabolism and eukaryotic signal transduction. This phosphoinositide is a precursor of the important intracellular second messengers diacylglycerol (DAG) and inositol triphosphate (IP<sub>3</sub>). PI and its metabolites are intricately involved in pathways ranging from cell differentiation and growth to apoptosis. As metabolic diseases such as diabetes and non-alcoholic fatty liver disease (NAFLD) become more prominent in our society, monitoring levels of the various biologically active lipid molecules becomes ever more critical. Rapid turnaround in detection of certain lipids is also essential to better understand these diseases, as changes in phospholipid and phosphoinositide concentrations can reflect dysregulation and alterations in metabolism. BioVision has developed a novel microplate-based assay for rapid determination of PI in biological samples, including serum and plasma, cultured cell lysates and tissue homogenates. The assay is simple to perform, highly selective and can detect as little as 15 pmole PI in a reaction. Representing the newest addition to our existing suite of lipid quantification assay kits, this assay is available exclusively from BioVision Inc. The phosphatidylinositol assay (Cat. #K750) can be used in conjunction with our other assay kits that detect and quantify phospholipids and their metabolites – such as phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylethanolamine (PE), lysophosphatidylcholine (LPC), and cardiolipin (CL) – to gain an understanding of the lipid profile in a variety of sample types.

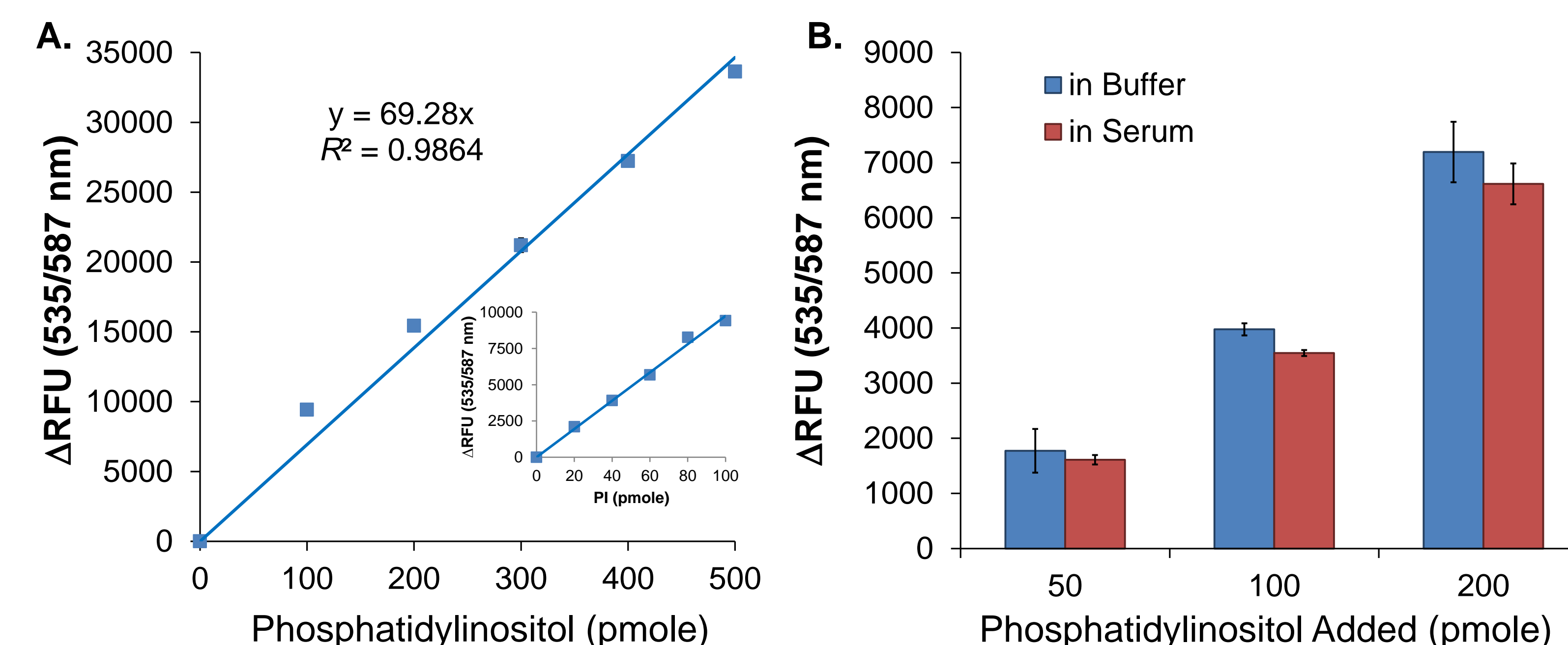
## Assay Schematic



## Assay Features and Benefits

- Simple and Rapid:** Easy to follow protocol with minimal sample processing
- Convenient:** Non-radioactive, no special handling or disposal required
- No Specialized Separation/Detection:** Fluorometric output, compatible with common microplate spectrofluorometers (Ex/Em = 535/587 nm)
- Extremely Stable Reagents:** Long shelf life
- Accurate:** Reproducible results with low intra- and inter-assay variability
- Ample Reagents:** Perform 100 assays in 96-well plate format

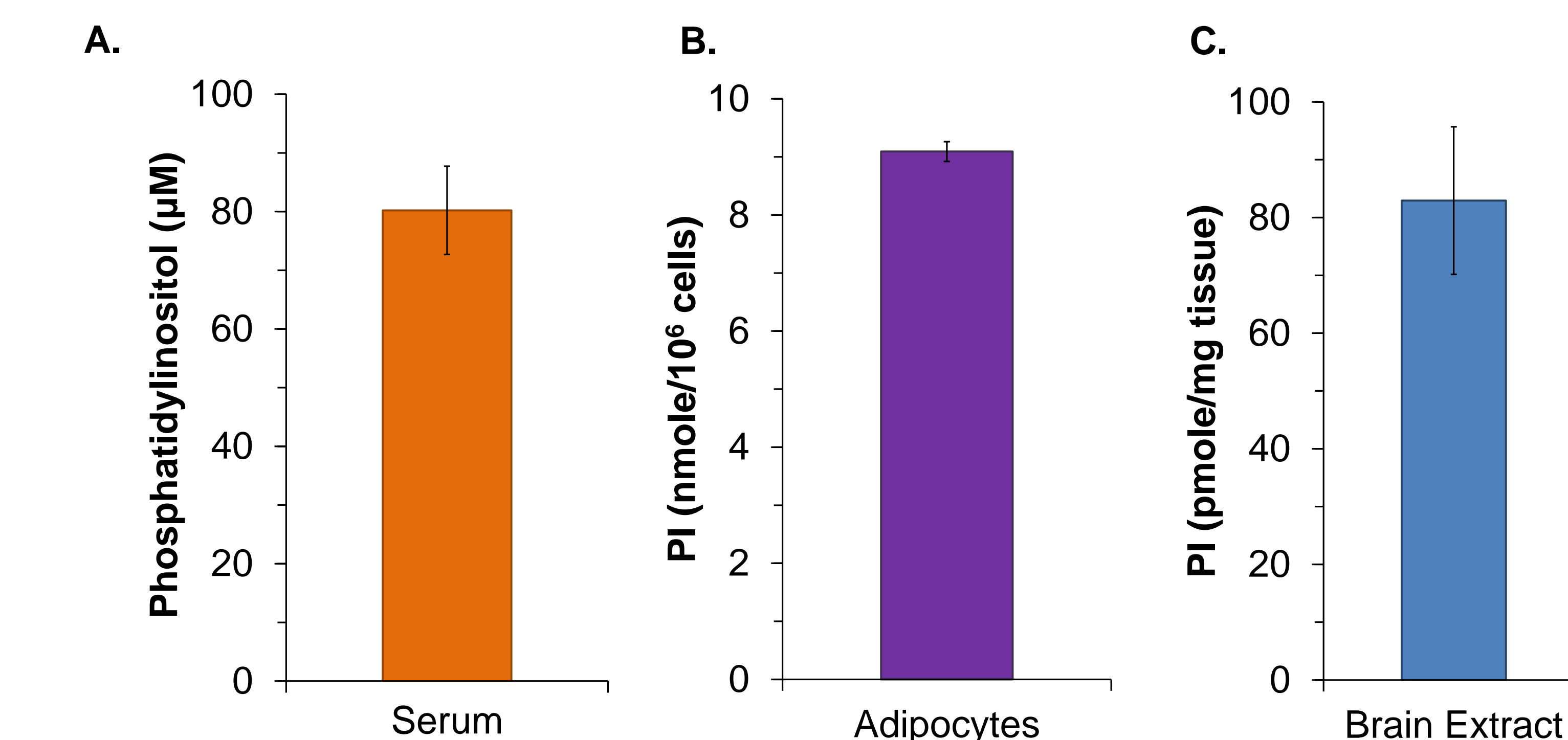
## Determination of Phosphatidylinositol Concentration In Various Biological Samples



**Figure 1. (A)** Phosphatidylinositol standard curve. Inset: lower range of phosphatidylinositol (PI) response shows assay sensitivity. **(B)** Evaluation of potential matrix effects. For validation of assay parallelism, various quantities of PI were spiked into pooled human serum samples prior to extraction and the signal (change in RFU) from each extract was quantified versus PI spiked into Assay Buffer. For each PI concentration, signal recovery in serum versus buffer was  $\geq 90\%$ .

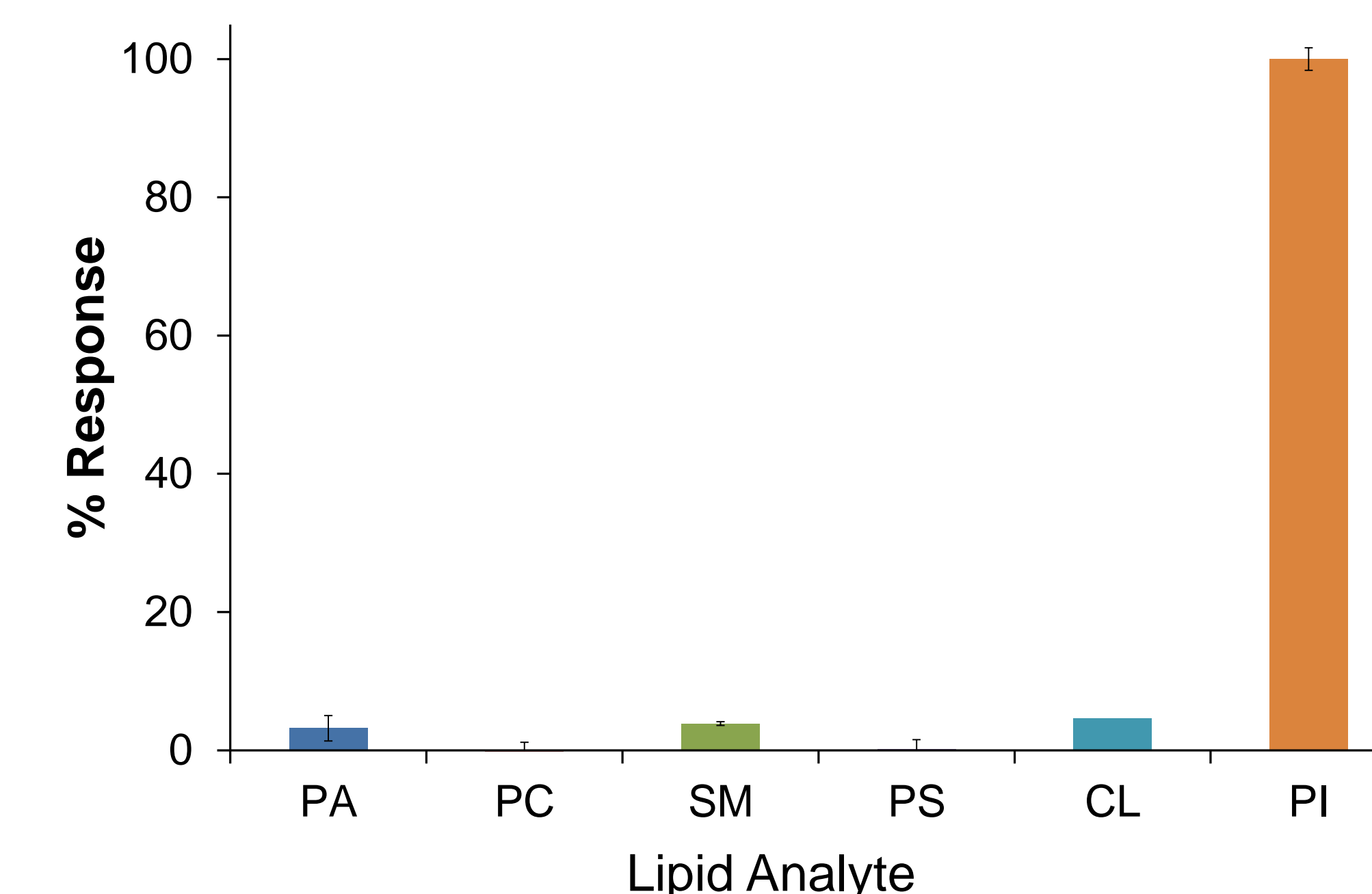
## Multiple Applications of Phosphatidylinositol Assay

- Quantify phosphatidylinositol in lipid extracts from cell lysates or tissue extracts.
- Determine concentration of phosphatidylinositol in blood/serum samples.
- Determine changes in phosphatidylinositol concentrations in tissues, fluids and cells in culture as a response to drugs or inhibitors at various concentrations.

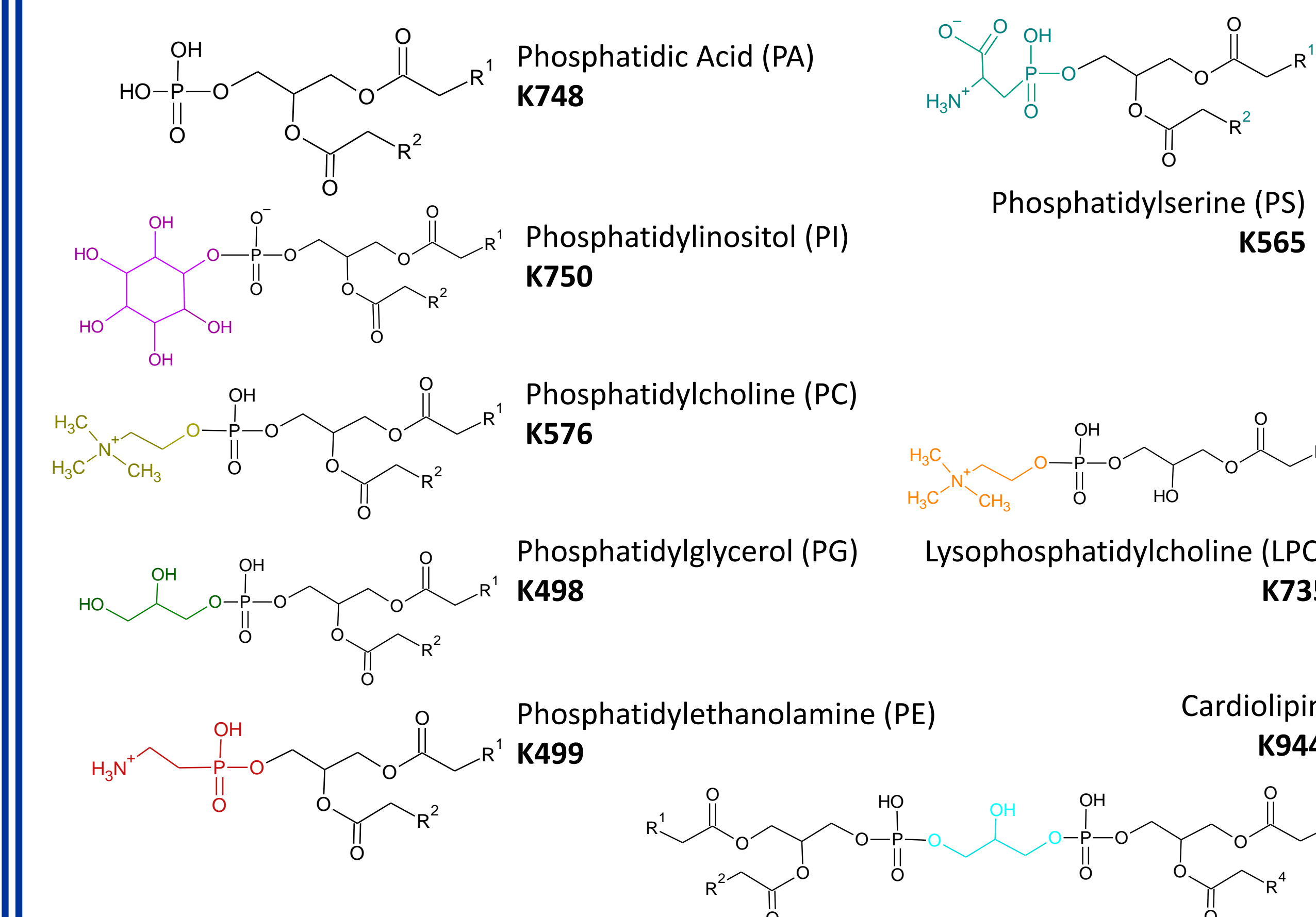


**Figure 2.** Quantification of PI in lipid extracts from **(A)** pooled human serum **(B)** cultured adipocytes differentiated from 3T3-L1 cells and **(C)** rat brain tissue. Serum was pretreated according to the assay kit protocol prior to lipid extraction. Adipocytes and tissue were homogenized using a Dounce homogenizer and clarified by centrifugation in a microfuge at 10000 x g for 10 minutes.

## Specificity of the Assay



**Figure 3.** BioVision's Phosphatidylinositol Assay (K750) was run in the presence of various lipid analytes. Signal generated in the presence of 500 pmole phosphatidylinositol (PI) is considered 100% response. This is compared to 1 nmole phosphatidic acid (PA), 2.5 nmole phosphatidylcholine (PC), 5 nmole phosphatidylserine (PS), 5 nmole sphingomyelin (SM) and 1 nmole cardiolipin (CL) per well.



## Conclusions

- BioVision's PicoProbe™ Phosphatidylinositol Assay Kit (K750) is a high-throughput screening assay for measuring phosphatidylinositol**
- The assay provides a safe, sensitive and reproducible method of measuring phosphatidylinositol in under three hours' time**
- This assay kit can be used with many sample types (cell extracts, tissue lysates and blood samples such as plasma and serum)**
- BioVision's Phosphatidylinositol Assay can be used to better understand lipid metabolism as well as cell signaling**