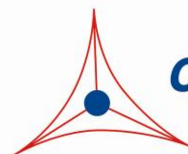
Product Manual

Phospholipid Assay Kit

Catalog Number

MET-5085	96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



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Introduction

Phospholipids are important structural lipids that are the major component of cell membranes and lipid bilayers. They contain a hydrophilic head and a hydrophobic tail which give the molecules their unique amphiphilic characteristics. Most phospholipids contain one diglyceride, a phosphate group, and one choline group (Figure 1). Sphingomyelin, lysophosphatidylcholine (lysolecithin), and phosphatidylcholine (lecithin) are predominantly choline-based phospholipids which account for 95% of phospholipids in human serum and plasma. Phosphatidylcholine is the foremost membrane phospholipid in eukaryotic cell membranes and is present in every cell in the body.

Determining circulatory levels of phospholipids and lipoproteins is critical to the diagnosis of lipid transport disorders and may be used as markers for heart, liver, or lung diseases. Phospholipid biosynthesis and metabolism is important to membrane structure maintenance and function. Many exert considerable influence on lipid homeostasis. They serve as a pool for many lipid messengers and are a source for bioactive lipids such as phosphatidic acid, diacylglycerol, lysophosphatidylcholine, and others. Phospholipids supply choline in the body, which itself and its derivative compounds form cell signaling molecules such as acetylcholine, platelet activating factor and sphingophosphorylcholine. Energy production and storage, prostaglandin production, blood clotting, cholesterol solubility, fat and bile emulsification, and antioxidant activity are pathways involving phospholipid activity. Due to their decreased toxicity, improved bioavailability and excellent permeability, phospholipids are used for liposomal, ethosomal and other nanoparticle formulations used in drug delivery systems.

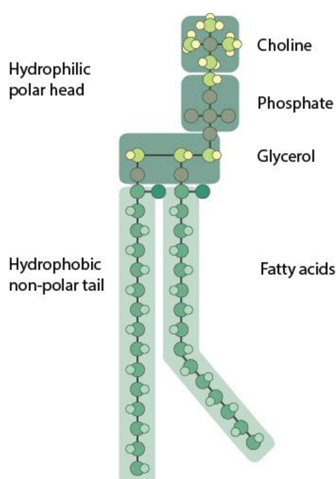

Figure 1: Phospholipid Structure

Cell Biolabs' Phospholipid Assay Kit is a simple fluorometric assay that measures the number of choline-based phospholipids present within plasma, serum, tissue homogenates, or cell suspensions in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 96 assays, including blanks, phospholipid standards and samples. The kit's detection sensitivity limit is approximately 150 nM.

Assay Principle

Cell Biolabs' Phospholipid Assay Kit measures phospholipids through a series of enzyme driven reactions. First, a hydrolyzing enzyme reacts with phospholipids, such as lecithins and sphingomyelins, breaking the phosphodiester bond within their structure. Next, an oxidoreductase reacts to generate a peroxide, which is subsequently detected with a highly specific fluorescence probe. Samples and

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standards are incubated for 30 minutes and then read with a standard 96-well fluorometric plate reader (Ex. 530-570 nm/Em. 590-600 nm). Sample phospholipid levels are determined by comparison with known phospholipid standards.

Related Products

1. STA-337: 8-iso-Prostaglandin F2α ELISA Kit
2. STA-390: Total Cholesterol Assay Kit (Fluorometric)
3. STA-391: HDL and LDL/VLDL Cholesterol Assay Kit
4. STA-397: Serum Triglyceride Quantification Kit (Fluorometric)
5. STA-399: Free Glycerol Assay Kit (Fluorometric)

Kit Components

Box 1 (shipped at room temperature)

1. 96-well Microtiter Plate (Part No. 234501): One 96-well clear bottom black plate.
2. Assay Buffer (10X) (Part No. 260002): One 25 mL bottle.
3. Fluorescence Probe (100X) (Part No. 50854C): One 50 µL tube in DMSO.
4. HRP (Part No. 234402): One 100 µL tube of 100 U/mL HRP solution in glycerol.

Box 2 (shipped on blue ice packs)

1. Phospholipid Standard (Part No. 50851C): One 50 µL amber tube of a 2 mM solution.
2. Hydrolyzing Enzyme (400X) (Part No. 50852D): One 15 µL amber tube.
3. Oxidoreductase (200X) (Part No. 50853C): One 25 µL tube.

Materials Not Supplied

1. Distilled or deionized water
2. (optional) Chloroform
3. (optional) Superoxide dismutase

Storage

Upon receipt, store the Hydrolyzing Enzyme at -80ºC and the Phospholipid Standard, Fluorescence Probe, HRP, and Oxidoreductase at -20ºC. The Fluorescence Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles. Store the remaining kit components at 4ºC.

Preparation of Reagents

- 1X Assay Buffer: Warm the Assay Buffer (10X) to room temperature prior to using. Dilute the Assay Buffer (10X) with deionized water by diluting the 25 mL Buffer with 225 mL deionized water for 250 mL total. Mix to homogeneity. Store the 1X Assay Buffer at 4ºC up to six months.
- Detection Reagent: Prepare a Detection Reagent by diluting the Hydrolyzing Enzyme 1:400, Oxidoreductase 1:200, HRP 1:500, and Fluorescence Probe 1:100 in 1X Assay Buffer. (e.g. for 50

assays, combine 6.3 µL Hydrolyzing Enzyme, 12.5 µL of Oxidoreductase, 5 µL of HRP, and 25 µL Fluorescence Probe, and with 1X Assay Buffer to 2.5 mL total solution). See Table 1 for examples of Detection Reagent preparation based on the number of assays employed. Mix thoroughly and protect the solution from light. For best results, place the Detection Reagent on ice and use within 30 minutes of preparation. Do not store the Detection Reagent solution.

Hydrolyzing Enzyme (µL)	Oxidoreductase (µL)	HRP (µL)	Fluorescence Probe (µL)	1X Assay Buffer (µL)	**Number of Assays (50 µL/well)**
12	24	9.6	48	4,755	**96**
6.3	12.5	5	25	2452	**50**
2.5	5	2	10	981	**20**

Table 1. Preparation of Detection Reagent

Note: The Fluorescence Probe is light sensitive and must be stored accordingly.

Preparation of Samples

Samples should be assayed immediately or stored at -80ºC prior to performing the assay. Optimal experimental conditions for samples must be determined by the investigator. The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design. A set of serial dilutions is recommended for samples to achieve optimal assay results and minimize possible interfering compounds. Run proper controls as necessary. Always run a standard curve with samples.

- Tissues or Cell Suspensions: Homogenize 250 mg of sample (wet tissue or cell pellet) in 4.5 mL of chloroform/methanol (2:1, v/v). Centrifuge to remove debris. After centrifugation, incubate the homogenate at room temperature for 1 hour on an orbital shaker. Induce phase separation by adding 1.25 mL dH_2O. Incubate 10 minutes at room temperature and centrifuge at 1000 x g for 10 minutes. Collect the lower (chloroform) organic phase and re-extract the upper phase with 2 mL of solvent mixture whose composition is $CHCl_3$/MeOH/water (86:14:1, v/v/v). Combine organic phases and dry in a vacuum centrifuge. Dissolve in 200 µL $CHCl_3$/MeOH/water (60:30:4.5, v/v/v) for storage. Before performing the phospholipid assay, samples must be diluted at least 1:50 to 1:400 with Assay Buffer.
- Serum: Collect blood without using an anticoagulant. Allow blood to clot for 30 minutes at room temperature. Centrifuge at 2000 x g and 4ºC for 10 minutes. Remove the serum layer and store on ice. Take care to avoid disturbing the white buffy layer. Aliquot samples for testing and store remaining solution at -80ºC. Perform serum dilutions in 1X Assay Buffer. Serum samples must be diluted at least 1:50 to 1:400 with Assay Buffer. This will provide values within the range of the standard curve.
- Plasma: Collect blood with heparin or citrate and centrifuge at 1000 x g and 4ºC for 10 minutes. Remove the plasma layer and store on ice. Take care to avoid disturbing the white buffy layer. Aliquot samples for testing and store remaining solution at -80ºC. Perform plasma dilutions in 1X Assay Buffer. Plasma samples must be diluted at least 1:50 to 1:400 with Assay Buffer. This will provide values within the range of the standard curve.

Notes:

1. *Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µM will oxidize the probe and could result in erroneous readings. To minimize this interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL.*

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2. *Avoid samples containing DTT or β-mercaptoethanol since the fluorescence probe is not stable in the presence of thiols (above 10 µM).*
3. *Choline can generate high background if present in samples. If choline may be present, run a background control without the Hydrolyzing Enzyme. Subtract this value from sample reading values.*

Preparation of Phospholipid Standard Curve

1. Prepare fresh phospholipid standards by first diluting a portion of the 2 mM Phospholipid Standard stock solution 1:80 in 1X Assay Buffer. (e.g., Add 5 µL of Phospholipid Standard in 395 µL 1X Assay Buffer). Vortex thoroughly. This provides a 25 µM concentration. Use this solution to prepare a series of the remaining phospholipid standards according to Table 2.

Tubes	2mM Phospholipid Standard (µL)	1X Assay Buffer (µL)	Resulting Phospholipid Concentration (µM)
1	5	395	25
2	200 of Tube #1	200	12.5
3	200 of Tube #2	200	6.25
4	200 of Tube #3	200	3.13
5	200 of Tube #4	200	1.56
6	200 of Tube #5	200	0.78
7	200 of Tube #6	200	0.39
8	200 of Tube #7	200	0.20
9	0	500	0

Table 2. Preparation of Phospholipid Standards.

Note: Do not store diluted phospholipid standard solutions.

Assay Protocol

Each phospholipid standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add 50 µL of the diluted phospholipid standards or samples to the 96-well microtiter plate.
2. Add 50 µL of the prepared Detection Reagent to each well and mix the well contents thoroughly.
3. Cover the plate wells to protect the reaction from light. Incubate the plate for 30 minutes at 37ºC.
4. Read the plate with a fluorescence microplate reader equipped for excitation in the 530-570 nm range and for emission in the 590-600 nm range.
5. Calculate the concentration of phospholipid within samples by comparing the sample RFU to the phospholipid standard curve.

Example of Results

The following figures demonstrate typical Phospholipid Assay results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

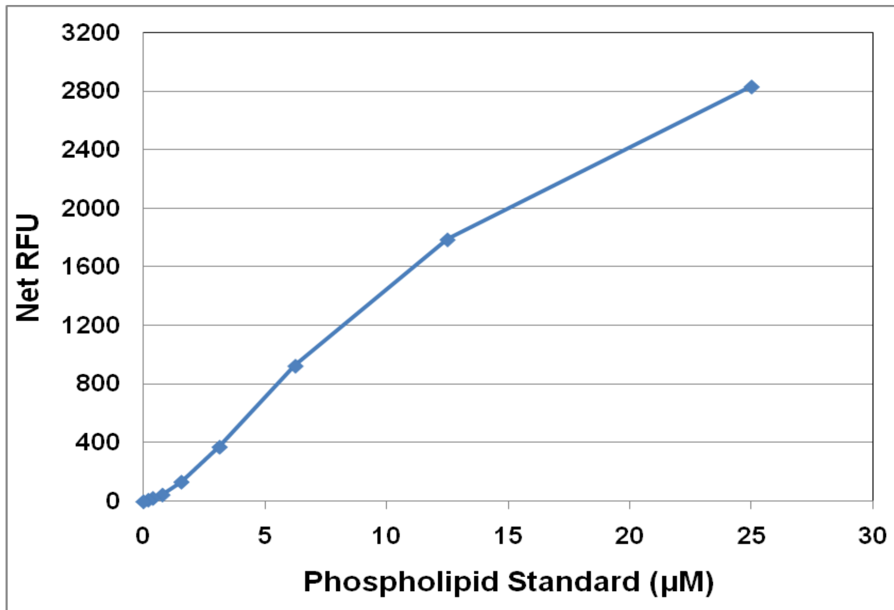

Figure 2: Phospholipid Standard Curve.

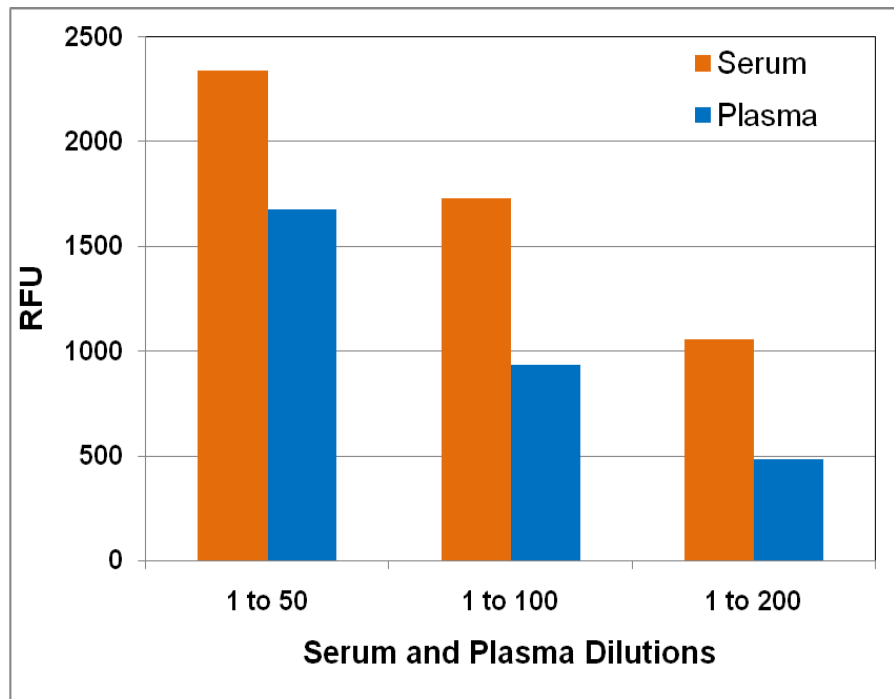

Figure 3: Human Serum and Plasma. Human plasma and serum were both diluted in 1X Assay Buffer and tested according to the assay protocol.

Calculation of Results

1. Calculate the average fluorescence values for every standard, control, and sample. Subtract the average zero standard value from itself and all standard and sample values. This is the corrected fluorescence.
2. Plot the corrected fluorescence for the standards against the final concentration of the phospholipid standards from Table 2 to determine the best curve. See Figure 2 for an example standard curve.

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3. Determine the phospholipid concentration of the samples with the equation obtained from the linear regression analysis of the standard curve. Substitute the corrected fluorescence values for each sample. Remember to account for dilution factors.

$$\textbf{Phospholipid } (\mu M) = \left[\frac{\textbf{Sample corrected fluorescence}}{\textbf{Slope } (\mu M^{-1})} \right] \textbf{ x Sample dilution}$$

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Recent Product Citations

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