Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Phospholipid Assay Kit (Colorimetric/Fluorometric)

03/17

(Catalog # K351-100; 100 assays, Store kit at -20°C)

I. Introduction:

Phospholipids are a major component of lipid bilayer membranes. In addition, phospholipids are biologically important molecules as they are closely involved in signal transduction. Most phospholipids contain one diglyceride, a phosphate group, and one choline moiety. In human serum and plasma, almost 95% of the phospholipids are choline-containing phospholipids such as phosphatidylcholine and sphingomyelin. The remaining 5% is mainly choline-free phospholipids (i.e. phosphatidylethanolamine and phosphatidylserine). Phospholipid concentration is considered a disease biomarker as it can be altered in many lung, liver and heart diseases. BioVision's phospholipid assay kit is simple, accurate and reproducible to measure total choline-containing phospholipids in biological samples such as serum, plasma and exosomes. The signal is directly proportional to the phospholipid concentration of phospholipids in the sample. This kit offers an excellent and efficient method for high-throughput assay that can detect phospholipid in the range of 0.1 to 10 nmol per sample.

II. Applications:

- *In vitro* phospholipid assay of phospholipid concentrations in biological samples
- High-throughput assay of inhibitors/inducers affecting phospholipid metabolism

III. Sample Type:

- Serum, plasma and exosomes

IV. Kit Contents:

Components	K351-100	Cap Code	Part Number
Assay Buffer	25 ml	WM	K351-100-1
Probe Solution	0.2 ml	Red	K351-100-2
Hydrolysis Enzyme	1 Vial	Purple	K351-100-3
Development Mix	1 Vial	Green	K351-100-4
Phospholipid Standard	1 Vial	Yellow	K351-100-5

V. User Supplied Reagents and Equipment:

- 96-well clear flat-bottom or black fluorometric flat-bottom plate
- Multi-well spectrophotometer

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment. Bring all reagents to room temperature before use.

- **Assay Buffer**: Ready to use. Warm to room temperature before use.
- **Probe Solution**: Ready to use. Warm to room temperature before use. Store at -20°C and protect from light. Stable for 2 months.
- **Hydrolysis Enzyme and Development Mix**: Reconstitute each vial with 220 µl of Assay Buffer.
- **Phospholipid Standard**: Dissolve in 200 µl of dH_2O to generate 50 mM phospholipid standard solution. Keep on ice while in use. Store at -20°C.

VII. Phospholipid Assay Protocol:

1. Sample Testing:

Add samples (eg. serum, plasma or exosomes) directly to sample wells in a 96-well plate and bring the volume to 50 µl/well with Assay Buffer. We suggest testing several doses of your sample to make sure the readings are within the standard curve range.

2. Phospholipid Standard Curves:

Colorimetric Assay: Dilute 10 µl of the 50 mM phospholipid standard with 990 µl dH_2O to generate 0.5 mM phospholipid standard. Add 0, 2, 4, 6, 8, 10 µl of the diluted phospholipid standard into a 96-well clear flat-bottom plate to generate 0, 1, 2, 3, 4, 5 nmol standard/well. Bring the volume to 50 µl with Assay Buffer.

Fluorometric Assay: Dilute the 0.5 mM phospholipid standard to 0.05 mM (0.05 nmol/µl) with water. Add 0, 2, 4, 6, 8, 10 µl of the diluted phospholipid standard into a 96-well clear flat-bottom plate to generate 0, 100, 200, 300, 400, 500 pmol standard/well. Bring the volume to 50 µl with Assay Buffer.

3. Reaction Mix:

Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix	Background Control *
Assay Buffer	44 µl	46 µl
Hydrolysis Enzyme	2 µl	---
Development Mix	2 µl	2 µl
Probe Solution	2 µl	2 µl

Add 50 µl of the Reaction Mix to each well containing the phospholipid standard and test samples.

*Endogenous choline present in the samples can generate background. If choline is present in your samples, perform a background control without the Hydrolysis Enzyme and subtract this value from sample readings.

4. **Measurement**: Measure O.D. at 570 nm, or fluorescence at Ex/Em 535/587 nm in a microplate reader at 25°C for 30 min.
5. **Calculation:** Subtract 0 Phospholipid Standard reading from all readings. Plot the Phospholipid Standard Curve. If sample background control is significant, then subtract sample background control reading from sample reading. Apply corrected RFU to Standard Curve to get B nmol Phospholipid in the sample well.

Sample Phospholipid Concentration (C)= B/V X D (nmol/µl, or mM)

Where: **B** is the amount of Phospholipid in the sample well from Standard Curve (nmol)
V is sample volume added into the reaction well (µl)
D is sample Dilution Factor

Note: For spiked samples, correct for any sample interference by using the following equation:

Phospholipid amount in spiked sample well **(B)=** $\frac{RFU_{sample(corrected)}}{RFU_{sample+Phospholipid\ Std\ (corrected)} - RFU_{sample\ (corrected)}} * Phospholipid\ spike\ (nmol)$

Phospholipid Molecular Weight: 770.123

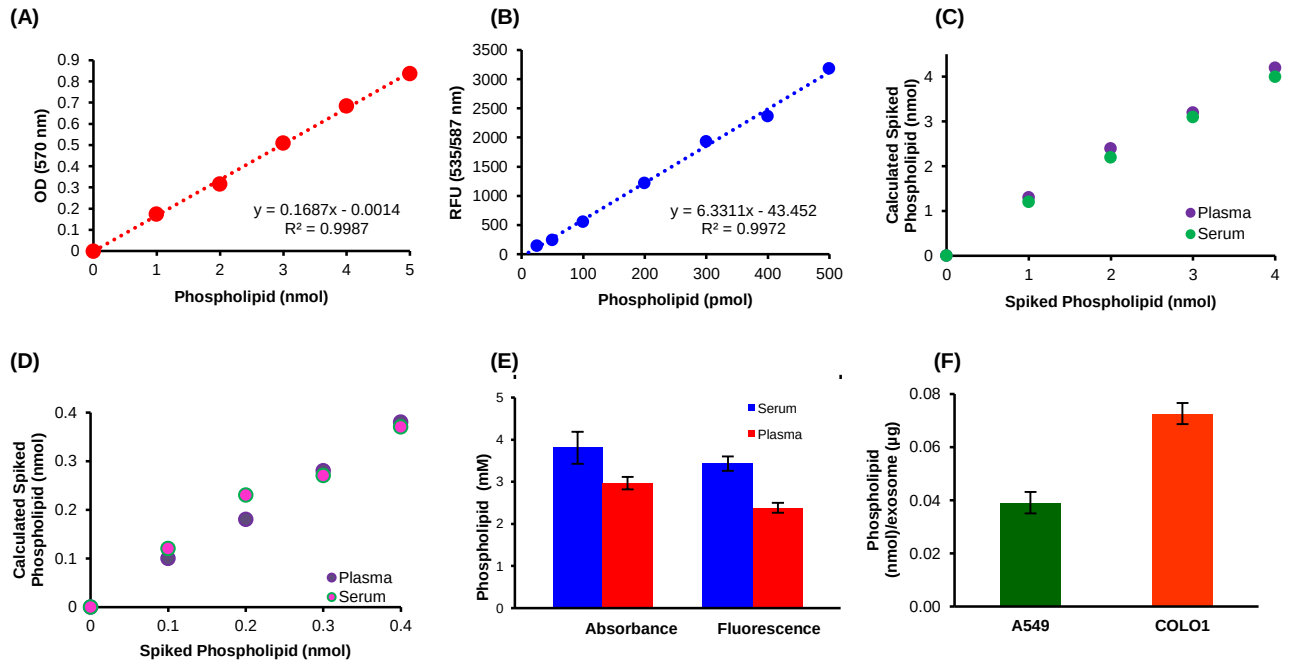

Figure: **A)** Phospholipid standard curve (colorimetric). **B)** Phospholipid standard curve (fluorometric). **C)** Determination of spiked phospholipid amount using human serum and plasma (Colorimetric) **D)** Determination of spiked phospholipid amount using human serum and plasma (Fluorometric). **E)** Determination of Phospholipid Concentration in Human Serum and plasma. Normal concentrations in human ranges between 1 and 4 mM. **F)** Determination of phospholipid in human lung (A549) and colon (M1049) carcinoma exosomes. Experiments were carried out in triplicate and followed kit protocols.

VIII. RELATED PRODUCTS:

Phosphatidylcholine Colorimetric/Fluorometric Assay Kit (K576)
Sphingomyelin Quantification Colorimetric Assay Kit (K600)
Lipase Activity Fluorometric Assay Kit (K724)
Free Fatty Acid Quantification Colorimetric/Fluorometric Kit (K612)
Adipogenesis Colorimetric/Fluorometric Assay Kit (K610)
Choline/Acetylcholine Quantification Kit (K615)
Triglyceride Quantification Colorimetric/Fluorometric Kit (K622)
HDL and LDL/VLDL Quantification Kit (K613)

FOR RESEARCH USE ONLY! Not to be used on humans.