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ab234050 Phospholipid Assay Kit (Colorimetric/Fluorometric)

For the measurement of choline-containing phospholipids in serum, plasma and exosomes.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Phospholipid Assay Kit (Colorimetric/Fluorometric) (ab234050) is a simple, accurate and reproducible way to measure total choline-containing phospholipids in biological samples such as serum, plasma and exosomes.

This kit provides a high-throughput assay of inhibitors/inducers affecting phospholipid metabolism. The signal is directly proportional to the phospholipid concentration of phospholipids in the sample and can detect phospholipids in the range of 0.1 to 10 nmol per sample.

Prepare all samples, reagents and standards as required.

Dilute standards for the standard curve.

Create 50 µL Reaction Mix for each well.

Incubate at 37°C for 45 minutes and measure fluorescence at Ex/Em=535/587 nm.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Reconstituted components are stable for 2 months.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer 1	25 mL	-20°C	-20°C
OxiRed™ Probe	0.2 mL	-20°C	-20°C
PC Hydrolysis Enzyme	1 vial	-20°C	-20°C
Enzyme Mix IV	1 vial	-20°C	-20°C
Phosphatidylcholine Standard	1 vial	-20°C	-20°C

PLEASE NOTE: Assay Buffer 1 was previously labelled as Assay Buffer I and Assay Buffer, OxiRed™ Probe was previously labelled as Probe solution, PC Hydrolysis Enzyme was previously labelled Hydrolysis Enzyme, Enzyme Mix IV was previously labelled Development Mix and Phosphatidylcholine Standard was previously labelled as Phospholipid Standard. The composition has not changed.

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at OD 570 nm or fluorescence at Ex/Em = 535/587 nm.
- 96 well plate with clear flat bottom (for colorimetric assay) / 96 well plate with clear flat bottom, preferably black (for fluorometric assay).

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening. Bring all reagents to room temperature before use.

5.1 Assay Buffer 1

1. Ready to use as supplied. Bring to room temperature before use. Assay Buffer 1 should be stored at -20°C, or 4°C for convenience.

5.2 OxiRed™ Probe

1. Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. Keep at room temperature during the assay. Store at -20°C and protect from light and moisture. Once the probe is opened and thawed, it is stable for at least 3 additional freeze/thaw cycles but should be used within two months. After use, promptly retighten the cap to minimize adsorption of airborne moisture.

5.3 PC Hydrolysis Enzyme

1. Reconstitute vial with 220 µL of Assay Buffer 1 when ready to use. Aliquot and store at -20°C..

5.4 Enzyme Mix IV

1. Reconstitute vial with 220 µL of Assay Buffer 1 when ready to use. Aliquot and store at -20°C.

5.5 Phosphatidylcholine Standard

1. Dissolve in 200 µL of distilled water to generate 50 mM phospholipid standard solution. The standard will go completely into solution after sonication and will look cloudy like an emulsion. Keep on ice while in use. Store at -20°C.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

For colorimetric detection:

1. Dilute 10 µL of the 50 mM Phosphatidylcholine Standard with 990 µL of distilled water to generate 0.5 mM Phosphatidylcholine Standard.
2. Add 0, 2, 4, 6, 8 and 10 µl of 0.5 mM Phosphatidylcholine Standard into series of wells in a 96-well plate to generate 0, 1, 2, 3, 4 & 5 nmol/well of Phospholipid. Adjust the volume to 50 µl/well with Assay Buffer 1 or prepare the standard curve dilution as described in the table below in a microplate or microcentrifuge tubes

Standard#	Phosphatidylcholine Standard (0.5 mM) (µL)	Assay Buffer 1 (µL)	Final volume standard in well (µL)	End amount of phospholipid in well (nmoles/well)
1	0	125	50	0
2	5	120	50	1
3	10	115	50	2
4	15	110	50	3
5	20	105	50	4
6	25	100	50	5

Each dilution has enough standard to set up duplicate readings (2 x 50 µL).

For fluorometric detection:

1. Dilute the 0.5 mM Phosphatidylcholine Standard to 0.05 mM with distilled water.
2. Add 0, 2, 4, 6, 8 and 10 µl of 0.05 mM Phosphatidylcholine Standard into series of wells in a 96-well plate to generate 0, 100, 200, 300, 400 & 500 pmol/well of phospholipid. Adjust the volume to 50 µl/well with Assay Buffer 1 or prepare the standard curve dilution as described in the table below in a microplate or microcentrifuge tubes.

Standard#	Phosphatidylcholine Standard (0.05 mM) (µL)	Assay Buffer 1 (µL)	Final volume standard in well (µL)	End amount of phospholipid in well (pmoles/well)
1	0	125	50	0
2	5	120	50	100
3	10	115	50	200
4	15	110	50	300
5	20	105	50	400
6	25	100	50	500

Each dilution has enough standard to set up duplicate readings (2 x 50 µL).

7. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples for the most reproducible assay.
- If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware, however, that this might affect the stability of your samples and the readings can be lower than expected. Avoid multiple freeze-thaws.
- Since endogenous compounds might interfere with the reaction, to ensure accurate measurement of PPi in the test wells, we recommend spiking the samples with known amount of PPi Standard within the standard curve range.

7.1 Serum, plasma or exosomes:

- Add samples directly into sample wells of the 96-well plate.
- Bring volume to 50 µL/well with Assay Buffer 1.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

Δ Note: If you suspect your samples contain substance that can generate background, set up Sample Background Controls to correct for background noise.

8.1 Reaction wells set up:

- Standard wells = 50 µL standard dilutions.
- Sample wells = 2-50 µL samples (adjust volume to 50 µL/well with Assay Buffer 1).
- Sample Background Control wells = 2-50 µL samples (adjust volume to 50 µL/well with Assay Buffer 1).

8.2 Reaction mix:

1. Prepare 50 µL of Reaction Mix and Background Mix for each reaction. Prepare a master mix to ensure consistency.

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer 1	44	46
PC Hydrolysis Enzyme	2	--
Enzyme Mix IV	2	2
OxiRed™ Probe	2	2

2. Add 50 µL of Reaction Mix into each standard and sample wells.
3. Add 50 µL of Background Reaction Mix into the background control sample wells.

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

4. **Measurement:** For colorimetric assays, incubate the plate protected from light for 45 minutes at 37°C and measure absorbance (OD) at 570 nm.
5. **Measurement:** For fluorometric assays, incubate the plate protected from light for 45 minutes at 37°C and measure fluorescence at Ex/Em = 535/587 nm.

9. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard, control and sample.
2. Subtract the mean value of the blank from all standards, controls and sample readings (including sample backgrounds). This is the corrected absorbance/fluorescence.
3. If significant, subtract the sample background control from sample readings.
4. Plot the corrected values for each standard as a function of the final amount of Phospholipid in nmol for colorimetric (or in pmol for fluorimetric).
5. Determine the linear equation for the standard curve.
6. Apply the corrected sample OD/RFU reading to the standard curve to get Phospholipid (B) amount in the sample wells.
7. Concentration of Phospholipid in B (nmol) / V (µL) in the test samples is calculated as:

$$\text{Phospholipid concentration} = \frac{B}{V} * D = \text{nmol}/\mu\text{L} = \text{mM}$$

Note: For fluorometric expiriments, B is in pmol, and the output of the equation above is pmol/µL or µM.

Where:

B = amount of Phospholipid in the sample well calculated from standard curve in nmol for colorimetric and pmol for fluorometric assays. .

V = sample volume added in the sample wells (µL).

D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

In case of spiked samples use the following equation, wherever required;

8. Using **spiked samples**, correct for any sample matrix interference by subtracting the sample reading from the spiked sample reading. This equation allows you to measure the Phospholipid concentration in your sample when matrix interference is significant.

$$B = \left(\frac{(ODsample\ corrected)}{(ODspiked\ corrected) - (ODsample\ corrected)}\right) * Phospholipid\ Spike\ (nm$$

Where:

B = Phospholipid amount in sample well (nmol).

OD sample corrected = OD/RFU of sample with blank and background readings subtracted.

OD spiked corrected = OD/RFU of spiked sample with blank and background readings subtracted.

Phospholipid Spike = amount of Phospholipid spiked (nmol) into the sample well.

Phospholipid MW = 770.123 g/mol

10. Typical Data

Data provided **for demonstration purposes only.**

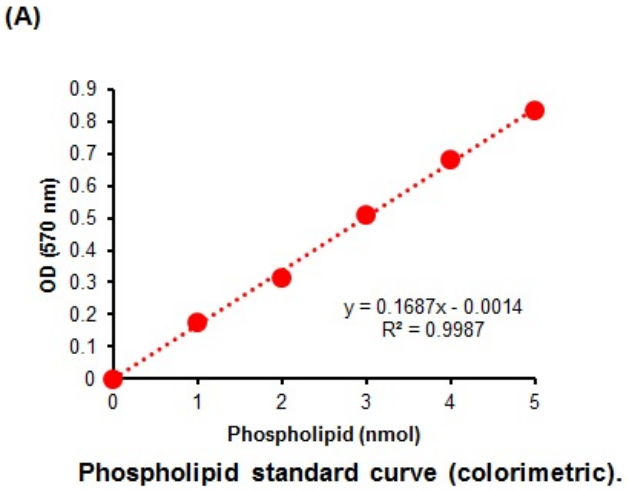

Figure 1: Phospholipid Standard curve (colorimetric).

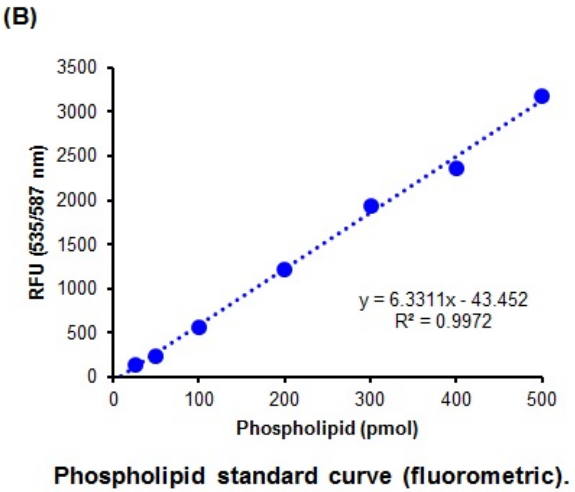

Figure 2: Phospholipid Standard curve (Fluorometric).

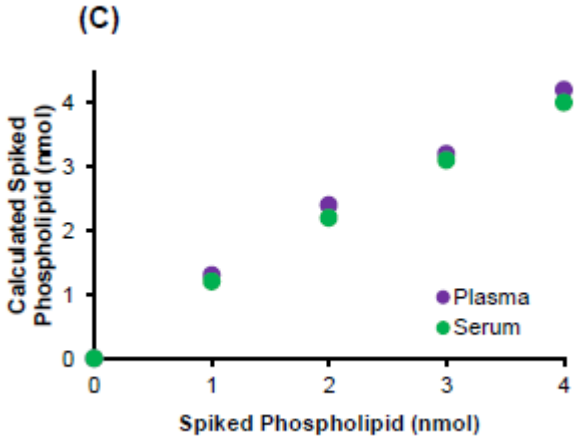

Figure 3: Determination of spiked phospholipid amount using human serum and plasma (colorimetric).

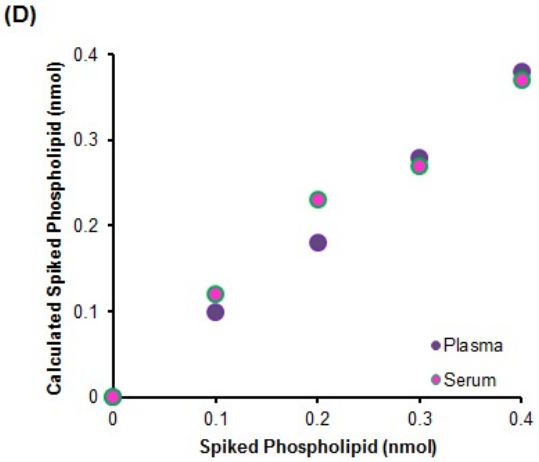

Figure 4: Determination of spiked phospholipid amount using human serum and plasma (Fluorometric).

Figure 5: Determination of Phospholipid Concentration in Human Serum and plasma. Normal concentrations in human ranges between 1 and 4 mM.

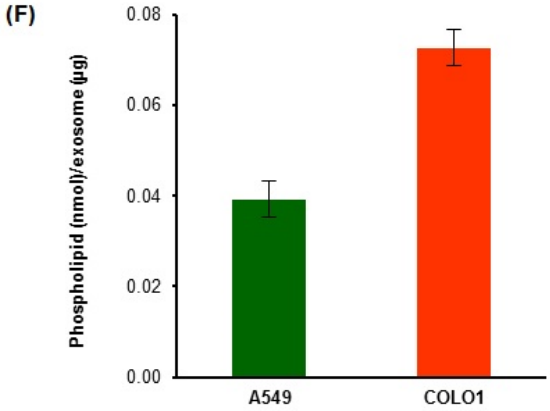

Figure 6: Determination of phospholipid in human lung (A549) and colon (M1049) carcinoma exosomes. Experiments were carried out in triplicate and followed kit protocols.

11. Notes

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)