

Version 3, Last updated 24 November 2023

# ab234050

## Phospholipid Assay Kit (Colorimetric/Fluorometric)

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

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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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 and plasma.

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  $\mu$ L Reaction Mix for each well.



Incubate at 25°C for 30 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 I/Assay Buffer	25 mL	-20°C	-20°C
OxiRed Probe/Probe solution	0.2 mL	-20°C	-20°C
PC Hydrolysis Enzyme/Hydrolysis Enzyme	1 vial	-20°C	-20°C
Enzyme Mix IV/Development Mix	1 vial	-20°C	-20°C
Phospholipid Standard	1 vial	-20°C	-20°C

### 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](http://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 I/Assay Buffer

1. Ready to use as supplied.

### 5.2 OxiRed Probe/Probe Solution

1. Ready to use as supplied.

### 5.3 PC Hydrolysis Enzyme/Hydrolysis Enzyme

1. Reconstitute each vial with 220  $\mu\text{L}$  of Assay Buffer I/Assay Buffer.

### 5.4 Enzyme Mix IV/Development Mix

1. Reconstitute each vial with 220  $\mu\text{L}$  of Assay Buffer I/Assay Buffer.

### 5.5 Phospholipid Standard

1. Dissolve in 200  $\mu\text{L}$  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.

## 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  $\mu\text{L}$  of the 50 mM phospholipid standard with 990  $\mu\text{L}$  of distilled water to generate 0.5 mM phospholipid standard.
2. Using the above prepared standard (0.5 mM) prepare standard curve dilution as described in the table below.

Standard #	Phospholipid standard (0.5 mM) ( $\mu\text{L}$ )	Assay Buffer l/Assay Buffer ( $\mu\text{L}$ )	Final volume standard in well ( $\mu\text{L}$ )	End amount of phospholipid standard in well (nmol/well)
1	0	100	50	0
2	4	96	50	1
3	8	92	50	2
4	12	88	50	3
5	16	84	50	4
6	20	80	50	5

Each dilution has enough standard to set up duplicate readings (2 x 50  $\mu\text{L}$ ).



**For fluorometric detection:**

1. Dilute the 0.5 mM phospholipid standard to 0.05 mM with distilled water.
2. Using the above prepared standard (0.05 mM) prepare standard curve dilution as described in the table below.

Standard #	Phospholipid standard (0.05 mM) (μL)	Assay Buffer I/Assay Buffer (μL)	Final volume standard in well (μL)	End amount of phospholipid standard in well (pmol/well)
1	0	100	50	0
2	4	96	50	100
3	8	92	50	200
4	12	88	50	300
5	16	84	50	400
6	20	80	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 and plasma:

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

## 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  $\mu$ L standard dilutions.
- Sample wells = XXX  $\mu$ L samples (adjust volume to 50  $\mu$ L/well with Assay Buffer I/Assay Buffer).
- Sample Background Control wells = XXX  $\mu$ L samples (adjust volume to 50  $\mu$ L/well with Assay Buffer I/Assay Buffer).

### 8.2 Reaction mix:

1. Prepare 50  $\mu$ L of Reaction Mix and Background Mix for each reaction. Prepare a master mix to ensure consistency.

Component	Reaction Mix ( $\mu$ L)	Background Reaction Mix ( $\mu$ L)
Assay Buffer I/Assay Buffer	44	46
PC Hydrolysis Enzyme/Hydrolysis Enzyme	2	--
Enzyme Mix IV/Development Mix	2	2
OxiRed Probe/Probe solution	2	2

2. Add 50  $\mu$ L of Reaction Mix into each standard and sample wells.
3. Add 50  $\mu$ L 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/Hydrolysis Enzyme and subtract this value from sample readings.

4. **Measurement:** For colorimetric assays, incubate the plate protected from light for 30 minutes at 25°C and measure absorbance (OD) at 570 nm.
5. **Measurement:** For fluorometric assays, incubate the plate protected from light for 30 minutes at 25°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. 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 concentration of Phospholipid.
5. Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).
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 units / V nmol in the test samples is calculated as:

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

Where:

B = amount of Phospholipid in the sample well calculated from standard curve in nmol.

V = sample volume added in the sample wells ( $\mu\text{L}$ ).

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.

$$B = \left( \frac{(OD_{\text{sample corrected}})}{(OD_{\text{spiked corrected}}) - (OD_{\text{sample corrected}})} \right) * \text{Phospholipid Spike (nmol)}$$

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.

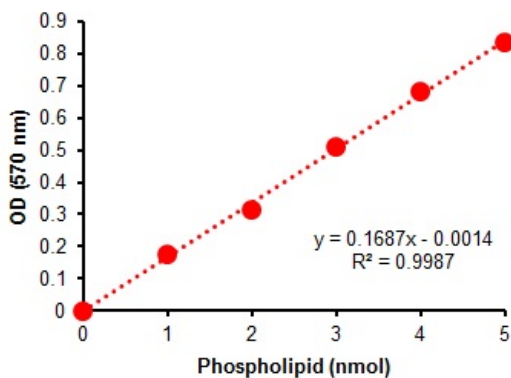
Phospholipid Spike = amount of Phospholipid spiked (nmol) into the sample well.

Phospholipid MW = 770.123 g/mol

## 10. Typical Data

Data provided for demonstration purposes only.

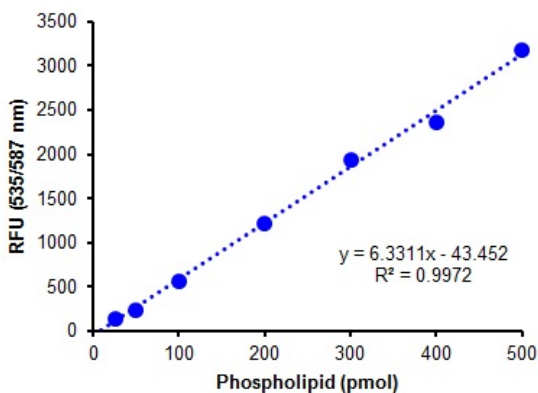
(A)



**Phospholipid standard curve (colorimetric).**

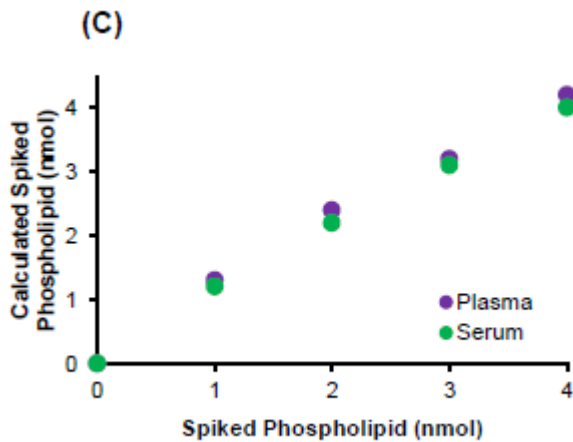
**Figure 1:** Phospholipid Standard curve (colorimetric).

(B)

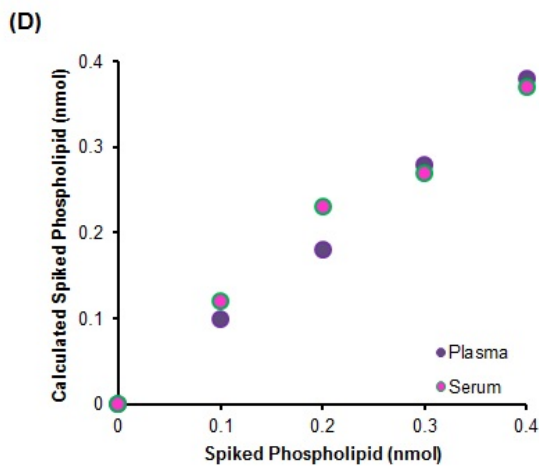


**Phospholipid standard curve (fluorometric).**

**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).

(E)



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

## Notes



## Technical Support

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