ab83377
Phosphatidylcholine Assay Kit
(Colorimetric/Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate measurement of phosphatidylcholine levels in various samples.

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

Phosphatidylcholine Assay Kit (colorimetric/fluorometric) (ab83377) is a simple convenient method for measuring phosphatidylcholine (PC) in a variety of biological samples. This assay uses an enzyme-coupled reaction to hydrolyze phosphatidylcholine to release choline, which subsequently oxidizes the OxiRed probe resulting in development of the Oxired Probe in order to generate fluorescence (Ex/Em 535 nm / 587 nm) or absorbance (OD=570 nm). This assay measures PC in the range of 0.1 to 10 nmol per sample.

Phosphatidylcholine (PC) is a phospholipid which incorporates choline as the headgroup of the lipid. PC is a major constituent of biological membranes and is involved in cell signalling through release of choline by phospholipase D leaving the second messenger phosphatidic acid. PC is present in serum at ~ 0.2-2.5 mM (~50-200 mg/dL).
2. **ASSAY SUMMARY**

- Standard curve preparation

  ↓

- Sample preparation

  ↓

- Add reaction mix and incubate RT for 30 min

  ↓

- Measure optical density (OD570 nm) or fluorescence (Ex/Em = 535/587 nm)
3. **PRECAUTIONS**

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. **STORAGE AND STABILITY**

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**
5. **MATERIALS SUPPLIED**

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Condition (Before Preparation)</th>
<th>Storage Condition (After Preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC Assay Buffer</td>
<td>25 mL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>OxiRed™ Probe</td>
<td>200 µL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>PC Hydrolysis Enzyme (lyophilized)</td>
<td>1 vial</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>PC Development Mix (lyophilized)</td>
<td>1 vial</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>PC Standard (10 µmol) (lyophilized)</td>
<td>1 vial</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
</tbody>
</table>

6. **MATERIALS REQUIRED, NOT SUPPLIED**

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

- MilliQ water or other type of double distilled water (ddH$_2$O)
- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric or fluorescent microplate reader – equipped with filter for OD 570 nm or Ex/Em = 535/587 nm (respectively)
- 96 well plate: clear plates for colorimetric assay; black plates (clear bottoms) for fluorometric assay
- Heat block or water bath
- Dounce homogenizer or pestle (if using tissue)
GENERAL INFORMATION

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.
8. **TECHNICAL HINTS**

- This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

- Keep enzymes, heat labile components and samples on ice during the assay.

- Make sure all buffers and solutions are at room temperature before starting the experiment.

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.

- Avoid foaming or bubbles when mixing or reconstituting components.

- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.

- Ensure plates are properly sealed or covered during incubation steps.

- Make sure you have the right type of plate for your detection method of choice.

- Make sure the heat block/water bath and microplate reader are switched on.
9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 PC Assay Buffer:
Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 OxiRed Probe – in DMSO:
Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. **NOTE:** DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for few minutes at 37°C. Aliquot probe so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light and moisture. Keep on ice while in use.

9.3 PC Hydrolysis Enzyme:
Reconstitute in 220 µL PC Assay Buffer. Pipette up and down to dissolve. Aliquot enzyme so that you have enough volume to perform the desired number of assays. Avoid repeated freeze/thaw cycles. Store at -20°C. Keep on ice while in use.

9.4 PC Development Mix:
Reconstitute in 220 µL PC Assay Buffer. Pipette up and down to dissolve. Aliquot development mix so that you have enough volume to perform the desired number of assays. Avoid repeated freeze/thaw cycles. Store at -20°C. Keep on ice while in use.

9.5 PC Standard:
Reconstitute the PC Standard (10 µmol) in 200 µL of ddH₂O to generate a 50 mM PC standard stock solution. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice while in use.
10. **STANDARD PREPARATION**

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used as soon as possible.

10.1 **For the colorimetric assay:**

10.1.1 Prepare 500 µL of 0.5 mM PC standard by diluting 5 µL of the reconstituted 50mM PC standard with 495 µL of ddH\textsubscript{2}O.

10.1.2 Using 0.5 mM PC standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

<table>
<thead>
<tr>
<th>Standard #</th>
<th>Volume of 0.5 mM Standard (µL)</th>
<th>ddH\textsubscript{2}O (µL)</th>
<th>Final volume standard in well (µL)</th>
<th>End [PC] in well</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>150</td>
<td>50</td>
<td>0 nmol/well</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>144</td>
<td>50</td>
<td>1 nmol/well</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>138</td>
<td>50</td>
<td>2 nmol/well</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>132</td>
<td>50</td>
<td>3 nmol/well</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>126</td>
<td>50</td>
<td>4 nmol/well</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>120</td>
<td>50</td>
<td>5 nmol/well</td>
</tr>
</tbody>
</table>

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).
10.2 For the flurometric assay:

10.2.1 Prepare 500 µL of 0.5 mM standard by diluting 5 µL of the reconstituted 50 mM PC standard with 495 µL of ddH₂O.

10.2.2 Prepare 500 µL of 0.05 mM standard by diluting 50 µL of the 0.5 mM PC standard with 450 µL of ddH₂O.

10.2.3 Using 0.05mM PC standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

<table>
<thead>
<tr>
<th>Standard #</th>
<th>Volume of 0.05 mM Standard (µL)</th>
<th>ddH₂O(µL)</th>
<th>Final volume standard in well (µL)</th>
<th>End [PC] in well</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>150</td>
<td>50</td>
<td>0 nmol/well</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>144</td>
<td>50</td>
<td>0.1 nmol/well</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>138</td>
<td>50</td>
<td>0.2 nmol/well</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>132</td>
<td>50</td>
<td>0.3 nmol/well</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>126</td>
<td>50</td>
<td>0.4 nmol/well</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>120</td>
<td>50</td>
<td>0.5 nmol/well</td>
</tr>
</tbody>
</table>

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

**NOTE:** If your sample readings fall out the range of your fluorometric standard curve, you might need to adjust the dilutions and create a new standard curve.
11. **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. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples 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.

11.1 **Cell (adherent or suspension) samples:**

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 2 x 10^6 cells).

11.1.2 Wash cells with cold PBS.

11.1.3 Resuspend cells in 100 µL of Assay Buffer.

11.1.4 Homogenize cells quickly by pipetting up and down a few times. Incubate on ice 10 minutes.

11.1.5 Centrifuge sample for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.

11.1.6 Collect supernatant and transfer to a clean tube.

11.1.7 Keep on ice.

11.2 **Tissue samples:**

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg).

11.2.2 Wash tissue in cold PBS.

11.2.3 Resuspend tissue in 100 µL of Assay Buffer.
11.2.4 Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes. Incubate on ice 10 minutes.

11.2.5 Centrifuge samples for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.

11.2.6 Collect supernatant and transfer to a clean tube.

11.2.7 Keep on ice.

11.3 **Plasma, Serum and Urine and other biological fluids:**

Samples can be assayed directly.

PC is present in serum at ~ 0.2-2.5 mM (~50-200 mg/dL).

**NOTE:** We suggest using different volumes of sample to ensure readings are within the Standard Curve range.
12. ASSAY PROCEDURE and DETECTION

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- It is recommended to assay all standards, controls and samples in duplicate.

12.1 Set up Reaction wells:
- Standard wells = 50 µL standard dilutions.
- Sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer).
- Background control sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer). **NOTE**: for samples containing choline as it can generate significant background.

12.2 PC Reaction Mix (COLORIMETRIC ASSAY):
Prepare 50 µL of Reaction Mix for each reaction

<table>
<thead>
<tr>
<th>Component</th>
<th>Colorimetric Reaction Mix (µL)</th>
<th>Background Control Reaction Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Buffer</td>
<td>44</td>
<td>46</td>
</tr>
<tr>
<td>PC Hydrolysis Enzyme</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>PC Development Mix</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>OxiRed Probe</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a Master Mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

\[ X \mu L \text{ component} \times (\text{Number samples + standards +1}) \]
12.3 **PC Reaction Mix (FLUOROMETRIC ASSAY):**
Prepare 50 µL of Reaction Mix for each reaction:

<table>
<thead>
<tr>
<th>Component</th>
<th>Fluorometric Reaction Mix (µL)</th>
<th>Background Control Reaction Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Buffer</td>
<td>45.8</td>
<td>47.8</td>
</tr>
<tr>
<td>PC Hydrolysis Enzyme</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>PC Development Mix</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>OxiRe Probe*</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

*For fluorometric readings, using 0.2 µL/well of the probe decreases the background readings, therefore increasing detection sensitivity.*

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

\[ X \ \mu L \ \text{component} \times (\text{Number samples} + \text{Background Control samples} + \text{standards} + 1) \]

12.4 Add 50 µL of appropriate Reaction Mix to each standard, sample and background control sample wells.

12.5 Incubate at room temperature for 30 min protected from light.

12.6 Measure output on a microplate reader.
- Colorimetric assay: measure OD570 nm.
- Fluorometric assay: measure Ex/Em = 535/587 nm.
13. **CALCULATIONS**

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 If the sample background control is significant, then subtract the sample background control from sample reading.

13.3 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.4 Plot the corrected absorbance values for each standard as a function of the final concentration of Phosphatidylcholine.

13.5 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).

13.6 Concentration of Phosphatidylcholine (as nmol/µL or mM) in the test samples is calculated as:

\[
\text{Phosphatidylcholine conc} = \left( \frac{A}{B} \right) \times D
\]

Where:

- A = Amount of Phosphatidylcholine in the sample well (nmol) from the standard curve.
- B = Sample volume added into the reaction well (µL).
- D = Sample dilution factor.

Phosphatidylcholine molecular weight: 768 g/mol.
14. **TYPICAL DATA**

**TYPICAL STANDARD CURVE** – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

![Figure 1](image1.png)

**Figure 1.** Typical Phosphatidylcholine standard calibration curve using colorimetric reading.

![Figure 2](image2.png)

**Figure 2.** Typical Phosphatidylcholine standard calibration curve using fluorometric reading.
Figure 3: Phosphatidycholine levels measured fluorometrically in mouse tissue lysates.

Figure 4: Phosphatidycholine levels colorimetrically measured in rat biological fluids.
Figure 5: Phosphatidycholine levels colorimetrically measured in cell lysates.
15. QUICK ASSAY PROCEDURE

**NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare standard, probe, enzyme and development mix (aliquot if necessary); get equipment ready.
- Prepare appropriate standard curve for your detection method of choice (colorimetric or fluorometric).
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (50 µL), samples (50 µL) and background wells (50 µL).
- Prepare PC Reaction Mix (Number samples + standards + 1).

<table>
<thead>
<tr>
<th>Component</th>
<th>Colorimetric Reaction Mix (µL)</th>
<th>Background Control Reaction Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Buffer</td>
<td>44</td>
<td>46</td>
</tr>
<tr>
<td>PC Hydrolysis Enzyme</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>PC Development Mix</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>OxiRed Probe</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Component</th>
<th>Fluorometric Reaction Mix (µL)</th>
<th>Background Control Reaction Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Buffer</td>
<td>45.8</td>
<td>47.8</td>
</tr>
<tr>
<td>PC Hydrolysis Enzyme</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>PC Development Mix</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>OxiRed Probe</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

- Add 50 µL of appropriate Reaction Mix to the standard, sample and background sample control wells.
- Incubate plate at RT 30 min protected from light.
- Measure plate at OD 570 nm for colorimetric assay or Ex/Em= 535/587 nm for fluorometric assay.
### 16. TROUBLESHOOTING

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Assay not working</strong></td>
<td>Use of ice-cold buffer</td>
<td>Buffers must be at room temperature</td>
</tr>
<tr>
<td></td>
<td>Plate read at incorrect wavelength</td>
<td>Check the wavelength and filter settings of instrument</td>
</tr>
<tr>
<td></td>
<td>Use of a different 96-well plate</td>
<td>Colorimetric: Clear plates, Fluorometric: black wells/clear bottom plate</td>
</tr>
<tr>
<td><strong>Sample with erratic readings</strong></td>
<td>Samples not deproteinized (if indicated on protocol)</td>
<td>Use PCA precipitation protocol for deproteinization</td>
</tr>
<tr>
<td></td>
<td>Cells/tissue samples not homogenized completely</td>
<td>Use Dounce homogenizer, increase number of strokes</td>
</tr>
<tr>
<td></td>
<td>Samples used after multiple free/thaw cycles</td>
<td>Aliquot and freeze samples if needed to use multiple times</td>
</tr>
<tr>
<td></td>
<td>Use of old or inappropriately stored samples</td>
<td>Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use</td>
</tr>
<tr>
<td></td>
<td>Presence of interfering substance in the sample</td>
<td>Check protocol for interfering substances; deproteinize samples</td>
</tr>
<tr>
<td><strong>Lower/Higher readings in samples and Standards</strong></td>
<td>Improperly thawed components</td>
<td>Thaw all components completely and mix gently before use</td>
</tr>
<tr>
<td></td>
<td>Allowing reagents to sit for extended times on ice</td>
<td>Always thaw and prepare fresh reaction mix before use</td>
</tr>
<tr>
<td></td>
<td>Incorrect incubation times or temperatures</td>
<td>Verify correct incubation times and temperatures in protocol</td>
</tr>
<tr>
<td>Problem</td>
<td>Cause</td>
<td>Solution</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>---------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Standard readings do not follow a linear pattern</td>
<td>Pipetting errors in standard or reaction mix</td>
<td>Avoid pipetting small volumes (&lt; 5 µL) and prepare a master mix whenever possible</td>
</tr>
<tr>
<td></td>
<td>Air bubbles formed in well</td>
<td>Pipette gently against the wall of the tubes</td>
</tr>
<tr>
<td></td>
<td>Standard stock is at incorrect concentration</td>
<td>Always refer to dilutions on protocol</td>
</tr>
<tr>
<td>Unanticipated results</td>
<td>Measured at incorrect wavelength</td>
<td>Check equipment and filter setting</td>
</tr>
<tr>
<td></td>
<td>Samples contain interfering substances</td>
<td>Troubleshoot if it interferes with the kit</td>
</tr>
<tr>
<td></td>
<td>Sample readings above/ below the linear range</td>
<td>Concentrate/ Dilute sample so it is within the linear range</td>
</tr>
</tbody>
</table>
17. **FAQ**
18. **INTERFERENCES**

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure:

- Choline.