PLAQPRO® Lp-PLA₂ Assay

Catalog #: ENZ-KIT179-0200
200 tests

For Roche cobas or other automated chemistry analyzers
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>2</td>
</tr>
<tr>
<td>Introduction</td>
<td>2</td>
</tr>
<tr>
<td>Principle of the Assay</td>
<td>3</td>
</tr>
<tr>
<td>Materials Supplied</td>
<td>4</td>
</tr>
<tr>
<td>Storage</td>
<td>5</td>
</tr>
<tr>
<td>Other Materials Needed</td>
<td>5</td>
</tr>
<tr>
<td>Precautions</td>
<td>5</td>
</tr>
<tr>
<td>Sample Storage and Handling</td>
<td>6</td>
</tr>
<tr>
<td>Reagent Preparation and Storage</td>
<td>6</td>
</tr>
<tr>
<td>Assay Procedure</td>
<td>7</td>
</tr>
<tr>
<td>Interpretation of Results</td>
<td>10</td>
</tr>
<tr>
<td>Performance Characteristics</td>
<td>10</td>
</tr>
<tr>
<td>Appendix</td>
<td>14</td>
</tr>
<tr>
<td>References</td>
<td>16</td>
</tr>
<tr>
<td>Contact Information</td>
<td>18</td>
</tr>
</tbody>
</table>

* Please read entire booklet before proceeding with the assay.

* Carefully note the handling and storage conditions of each kit component.

* Please contact Enzo Life Sciences Technical Support if necessary.
DESCRIPTION

The PLAQPRO® Lp-PLA₂ Assay measures the enzyme activity of Lp-PLA₂ (lipoprotein-associated phospholipase A2) in serum samples. The assay is designed to run on automated chemistry analyzers as well as injector equipped microtiter plate readers.

INTRODUCTION

Lipoprotein-associated phospholipase A2 (Lp-PLA₂), also known as platelet-activating factor acetylhydrolase (PAF-AH), is a member of the phospholipase A2 superfamily that recognizes the S₉² acyl bond of phospholipids and hydrolyzes the bond releasing products such as lysophosphatidic acid.¹⁻²

Lp-PLA₂ is produced by inflammatory cells and circulates primarily bound to low-density lipoprotein (LDL) and to a lesser extent associated with high-density lipoprotein (HDL) in human plasma.³ LDL oxidization is an early key event in the pathogenesis of atherosclerosis. The earliest event in LDL oxidization is the Lp-PLA₂ mediated hydrolysis of oxidatively modified phosphatidylcholines resulting in lysophosphatidylcholine and oxidized fatty acids.⁴ Elevated Lp-PLA₂ levels have been found in atherosclerotic plaques and rupture lesions.⁵ Unlike the systemic inflammation biomarker CRP or the major target of coronary artery disease (CAD), LDL-cholesterol, which are insufficient to identify CAD, Lp-PLA₂ is reported to be a specific biomarker for vascular inflammation and can serve as an independent marker for risk of cardiovascular disease (CVD).⁶⁻⁷
PRINCIPLE OF THE ASSAY

The PLAQPRO® assay for Lp-PLA₂ activity is a colorimetric readout enzyme assay. During the test, Lp-PLA₂ in serum samples hydrolyzes the S_n2 position of the substrate, 1-myristoyl-2-(4-nitrophenylsuccinyl) phosphatidylcholine. The resulting colored reaction product 4-nitrophenol is measured spectrophotometrically and the rate of formation of 4-nitrophenol is determined over successive measurements.

The assay kit includes five Lp-PLA₂ calibrators for use in generating a standard curve fit of change in absorbance versus Lp-PLA₂ activity level in nmol/min/mL. The activity of Lp-PLA₂ in serum samples is interpolated from the standard curve.
MATERIALS SUPPLIED

1. Lp-PLA\(_2\) Reagent 1
   Catalog No. 80-2778-0026
   One bottle containing 26 mL of Lp-PLA\(_2\) assay buffer.

2. Lp-PLA\(_2\) Reagent 2
   Catalog No. 80-2779-0008
   One bottle containing 8 mL of Lp-PLA\(_2\) substrate in a buffer.

3. Lp-PLA\(_2\)-Cal 1
   Catalog No. 80-2780
   One vial containing 2 mL of Lp-PLA\(_2\) calibrator with 0 nmol/min/mL.

4. Lp-PLA\(_2\)-Cal 2
   Catalog No. 80-2781
   One vial containing 2 mL of Lp-PLA\(_2\) calibrator with 50 nmol/min/mL.

5. Lp-PLA\(_2\)-Cal 3
   Catalog No. 80-2782
   One vial containing 2 mL of Lp-PLA\(_2\) calibrator with 100 nmol/min/mL.

6. Lp-PLA\(_2\)-Cal 4
   Catalog No. 80-2783
   One vial containing 2 mL of Lp-PLA\(_2\) calibrator with 250 nmol/min/mL.

7. Lp-PLA\(_2\)-Cal 5
   Catalog No. 80-2784
   One vial containing 2 mL of Lp-PLA\(_2\) calibrator with 400 nmol/min/mL.

8. Lp-PLA\(_2\)-QC-L
   Catalog No. 80-2785
   One vial containing 2 mL of QC Low control.
9. **Lp-PLA₂-QC-H**  
**Catalog No. 80-2786**  
One vial containing 2 mL of QC High control.

**STORAGE**  
Reagents can be stored at -20°C until expiration date. Avoid multiple freeze-thaw cycles. Once thawed, reagents can be stored at 2-8°C for up to 4 weeks. Calibrators and QC Controls can be stored at 2-8°C for up to 3 months.

**OTHER MATERIALS NEEDED**
- Automated chemistry analyzer and their system operation manuals
- Analyzer Application Sheet specific for the respective chemistry analyzer.
- PLAQPRO® Lp-PLA₂ Assay Application Sheet is available on request for the Roche cobas c501 and 8000 c502 analyzers. Please contact Technical Support for more information.
- Empty reagent cassette.

**PRECAUTIONS**
This assay is an enzyme kinetic assay which could be affected by temperature, pipetting, reagents storage condition, etc. To obtain reliable, accurate and reproducible results, users should fully understand the kit instructions and perform good laboratory practice.

- Do not use the reagents after the expiration date labeled on the outer box.
- Serum samples with any level of Lp-PLA₂ activity should not be diluted before use. Dilution of samples will cause erroneous results.
- Hemoglobin (>1.0 mg/mL) interferes with the assay and the hemolyzed sample may show a negative bias.
- Reversing the positions of the reagents on the analyzer will lead to the reversed loading order and erroneous results. Make sure to load the Reagent 1 and the Reagent 2 in the correct positions.

SAMPLE STORAGE AND HANDLING

- Serum samples can be kept 24 hours at 20-25°C, 7 days at 4°C or 30 days at -20°C with up to five freeze-thaw cycles.
- Handle all blood samples using Universal Biohazardous Precautions.

REAGENT PREPARATION

- **Reagents are provided frozen.** It is recommended to thaw all the reagents at room temperature, periodically inverting or gently mixing the bottles. Putting reagents in a room temperature water bath will help reduce the thawing time. Mix thoroughly before opening.
- Assign position A for Reagent 1 (which will be injected first), position B for Reagent 2 (which will be injected second). Remove the caps of position A and B sections on multi cassette cartridge.
- In position A, pipette 25.6 mL of Lp-PLA₂ Reagent 1.
- In position B, pipette 7.7 mL Lp-PLA₂ Reagent 2.
- Load Multi cassette on the instrument in an open channel.
- Make sure all the reagents are equilibrated to the set temperature prior to use.
- Cassette is sufficient to run 200 tests.
ASSAY PROCEDURE

Calibration

The analyzer is calibrated with a standard curve including 5 calibration points and should be recalibrated for each new lot. The absorbance at 405 nm is normalized by the background absorbance at 520 nm. The reaction rate (OD/min) is calculated following the instruction of each analyzer. The activity of Lp-PLA2 (nmol/min/mL) and the reaction rate (OD/min) will be plotted to generate the standard curve using appropriate curve fitting models. The sample activity is interpolated from the standard curve.

Quality Control

Under the conditions and temperature of calibration, the analyzer should be verified with two levels of QC controls each time when samples are analyzed, following the laboratory’s requirements. If QC controls do not match the expected range, recalibration should be performed to confirm the settings in the analyzer.

Procedural Notes

- Due to the nature of kinetic assays, the reaction temperature should be maintained the same in all tests applied with this kit. Small temperature variations (1°C warmer or cooler) can noticeably change the reaction rate range.
- It is recommended that the analyzer be calibrated with each new kit or new lot of reagents. Recalibration of the analyzer should also be performed if the QC Controls fall out of the expected range.
- Do not combine reagents from different vials or exchange the caps as this may cause contamination.
- Follow the instructions in STORAGE section and REAGENT PREPARATION section to store and thaw the reagents. All reagents and samples should be mixed well before loading onto the machine. Avoid
bubbles. Make sure reagents are balanced to the set temperature before running the tests.

**Example Setup of Assay**

The PLAQPRO® Lp-PLA₂ assay can be done in tubes or plates depending on the analyzer used. The test should be run using the appropriate machine settings for the analyzer used.

PLAQPRO® Lp-PLA₂ Assay Application Sheet is available on request for the Roche cobas 6000 c501 and 8000 c502 analyzers. Please contact Technical Support for more information.

A general assay procedure for use on other analyzer platform or injectors enabled plate readers is described below.

In each reaction, the reagents are loaded in the below order:

25 μL Calibrators/Samples
100 μL Reagent 1 (by injector)
25 μL Reagent 2 (by injector)
Assay Time: 3 minutes
Read cycle: 10 – 13
Wavelength: 405 nm and 520 nm
Assay Range: 0 – 400 nmol/min/mL
Calibration Method: appropriate curve fit of 5 points
An Example Calibration Curve from cobas 8000 c502

<table>
<thead>
<tr>
<th>Calibrators (nmol/min/mL)</th>
<th>Absorbance (OD x 10⁴/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>141</td>
</tr>
<tr>
<td>50</td>
<td>1177</td>
</tr>
<tr>
<td>100</td>
<td>2174</td>
</tr>
<tr>
<td>250</td>
<td>4793</td>
</tr>
<tr>
<td>400</td>
<td>6908</td>
</tr>
</tbody>
</table>

The example calibration curve provided is not to be used to interpolate sample activity. The user must generate their own calibration curve using the reagents provided in the specific analyzer of their choice.
INTERPRETATION OF RESULTS

Lp-PLA₂ has been extensively studied to define the relationship with heart disease and vascular inflammation in general⁵-⁷. This assay is intended to provide a reproducible method for measuring a key biomarker for coronary heart disease (CHD).

The analytical measurement range for the PLAQPRO® Lp-PLA₂ Assay is 0.0 to 400.0 nmol/min/mL. Any sample has activity beyond the measurement range should be reported as ≥ 400.0 nmol/min/mL.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

The assay sensitivity was determined by calculating the Limit of blank at 2 standard deviations above zero (n=20). The sensitivity of the assay was determined to be 0.2355 nmol/min/mL.

Intra-assay and Inter-assay Precision

The intra-assay reproducibility of the PLAQPRO® Lp-PLA₂ Assay was evaluated by running three replicates of five serum specimens in the measuring range of the assay. The specimens were run on a single day in one run testing a range from 84.7 to 376.3 nmol/min/mL. The intra-assay CV was observed to be 0.24-0.83% and is shown in the table below.

The inter-assay reproducibility of the PLAQPRO® Lp-PLA₂ Assay was demonstrated by testing three replicates of five serum specimens in the measuring range of the assay. The samples were run on three separate days in triplicate on each day. The inter-assay CV was observed to be 0.52-0.65% and is shown in the Table below.
### Intra-assay and inter-assay precision.

#### Linearity

Three lots of calibrators were tested for linearity. The linearity of the calibrators show a slope range of 0.986 to 1.020.

**Example of Calibrator Linearity Plot**

The linearity data was analyzed using EP Evaluator. The assay demonstrated a linear response from 0.0 to 400.0 nmol/min/mL.
Interfering Substances

Endogenous Substances

Endogenous substances were spiked into samples at the indicated concentrations. For all endogenous substances tested the interference was less than 5% at the levels indicated.

<table>
<thead>
<tr>
<th>Potential Interferent</th>
<th>Tested Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>60 g/L</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>20 mg/dL</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>300 mg/dL</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>400 mg/dL</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>1 mg/mL</td>
</tr>
</tbody>
</table>
Exogenous Substances

Exogenous substances were spiked into samples at the indicated concentrations. For all endogenous substances tested the interference was less than 10% at the levels indicated.

<table>
<thead>
<tr>
<th>Potential Interferent</th>
<th>Tested Concentration (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>1511.6</td>
</tr>
<tr>
<td>Aspirin</td>
<td>1801.6</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>558.6</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>510.7</td>
</tr>
<tr>
<td>Fenofibrate</td>
<td>721.7</td>
</tr>
<tr>
<td>Lisinopril</td>
<td>405.5</td>
</tr>
<tr>
<td>Niacin</td>
<td>2462.2</td>
</tr>
<tr>
<td>Tolbutamide</td>
<td>2703.5</td>
</tr>
<tr>
<td>Warfarin</td>
<td>3083.3</td>
</tr>
<tr>
<td>Metformin</td>
<td>2583.2</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>2099.5</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>4403.0</td>
</tr>
</tbody>
</table>
APPENDIX

An example procedure used in Perkin Elmer Victor 2 Multilabel Counter is described below.

Temperature in Victor 2: 25°C
The Lp-PLA₂ reaction is done in a clear, half area, 96 well plate.
In each reaction, the reagents are loaded in the order below:
25 μL Calibrators/Samples
100 μL Reagent 1 (by injector)
25 μL Reagent 2 (by injector)

Assay Time: 3 minutes
Read cycle: 11
Analytical cycles: 3rd to 7th
Wavelength: 405 nm and 520 nm
Assay Range: 0 – 400 nmol/min/mL

Calibration Method: The absorbance at 405nm was normalized by the background absorbance at 520nm. The reaction rate (OD/min) was calculated using the change of absorbance between 3rd and 7th cycles. The activity of Lp-PLA₂ (nmol/min/mL) and the reaction rate (OD/min) was plotted to generate the standard curve with point-to-point curve fit model.
Example of a Calibration Curve

<table>
<thead>
<tr>
<th>Calibrators (nmol/min/mL)</th>
<th>Absorbance (OD x 10^4/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>50</td>
<td>550</td>
</tr>
<tr>
<td>100</td>
<td>1139</td>
</tr>
<tr>
<td>250</td>
<td>2579</td>
</tr>
<tr>
<td>400</td>
<td>4034</td>
</tr>
</tbody>
</table>

*Note: The difference of absorbance between cobas curve and Victor 2 curve may be caused by the different reaction temperature. However, the sample returned values obtained in different analyzers should be similar.

The example calibration curve provided is not to be used to interpolate sample activity. The user must generate their own calibration curve using the reagents provided in the specific analyzer of their choice.
REFERENCES


