Lp-PLA$_2$ FS*

Diagnostic reagent for quantitative in vitro determination of Lp-PLA$_2$ (Lipoprotein-associated phospholipase A$_2$) in serum and plasma on photometric systems

Order Information

Cat. No.	Kit size
171819910936	R1 1 x 20 mL + R2 1 x 4.75 mL + R3 1 x 0.25 mL
171819910937	R1 1 x 10 mL + R2 1 x 3.8 mL + R3 1 x 0.2 mL

Summary [1-4]

Lipoprotein-associated phospholipase A$_2$ (Lp-PLA$_2$), also known as platelet-activating factor acetylhydrolase (PAF-AH), is a calcium-independent phospholipase released by inflammatory cells in atherosclerotic plaques. In circulation, Lp-PLA$_2$ is predominantly associated with LDL particles whereas only a small portion of enzyme is associated with HDL. Lp-PLA$_2$ hydrolyzes oxidized LDL to generate two pro-atherogenic and pro-inflammatory compounds: Lysophosphatidylcholine (lyso-PC) and oxidized free fatty acids (oxFFA). Both substances play a major role in the development of vulnerable atherosclerotic plaques. Concentration of Lp-PLA$_2$ is independent of the presence of other cardiovascular risk factors, shows minimal biovariability and is not elevated in systemic inflammatory reactions. Lp-PLA$_2$ is a beneficial indicator for cardiovascular disease (CVD) risks, and may represent a potential therapeutic target for the reduction of such risks.

Method

UV test using 1-myristoyl-2-(4-nitrophenylsuccinyl)-sn-glycero-3-phosphocholine.

Principle

Lp-PLA$_2$ hydrolyzes the sn-position of the substrate 1-myristoyl-2-(4-nitrophenylsuccinyl)-sn-glycero-3-phosphocholine producing 4-nitrophenylsuccinate. After degradation in aqueous solution, 4-nitrophenol develops which can be detected photometrically; Lp-PLA$_2$ activity is determined by a change in absorbance at the defined wavelengths.

Reagents

Components and Concentrations

R1:	Buffer	pH 7.6	< 500 mmol/L
	EDTA		< 50 mmol/L
R2:	Buffer	pH 2.7	< 200 mmol/L
R3:	Alcohol		99%
	1-myristoyl-2-(4-nitrophenylsuccinyl)-sn-glycero-3-phosphocholine		< 200 mmol/L

Storage Instructions and Reagent Stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8℃ and contamination is a voided. Do not freeze the reagents!

Warning and Precautions

1. Reagent 3: Danger. H225 Highly flammable liquid and vapor. H318 Causes serious eye damage. H336 May cause drowsiness or dizziness. P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P240 Ground/bond container and receiving equipment. P261 Avoid breathing vapors. P280 Wear protective gloves/protective clothing/eye protection/face protection. P303+P361+P353 If on skin (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. P305+P351+P338 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P370+P378 In case of fire: Use dry powder, foam or water spray for extinction. P403+P233 Store in a well-ventilated place. Keep container tightly closed.
2. In very rare cases, samples of patients with gammopathies might give falsified results [6].
3. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
4. For professional use only!

Waste Management

Please refer to local legal requirements.

Reagent Preparation

Reagent 2 and reagent 3 must be premixed before use. Due to hygroscopic components, reagent 3 shall be stored tightly closed, and should not stand open for longer than 5 min. Bring reagents to room temperature before mixing.

Pipette below-mentioned reagent volume R3 into reagent bottle R2 of the same kit:

Cat. No	Reagent volume R3
1 7181 99 10 936	0.25 mL
1 7181 99 10 937	0.20 mL

Mix very gently to avoid foaming. In case of precipitation, leave premixed reagent until it is completely homogenized.
Stability of premixed R2/R3: 8 weeks if stored at 2 – 8℃.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen

Serum, heparin plasma or EDTA plasma
Stability [5]:

4 weeks at	2 – 8℃
2 days at	20 – 25℃
3 months	–20℃

Freeze only once! Discard contaminated specimens!

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength	405 nm/505 nm (bichromatic)
Optical path	1 cm
Temperature	37℃
Measurement	Against reagent blank

	Reagent blank	Sample/ calibrator
Sample or calibrator	-	10 µL
Dist. water	10 µL	-
Reagent 1	1000 µL	1000 µL
Mix, incubate approx. 5 min, then add:		
Reagent 2	250 µL	250 µL
Mix, read absorbance after 2 min and start stop watch. Read absorbance again exactly after 1, 2 and 3 minutes.		

Calculation

With calibrator

$$\text{Lp-PLA2 [U/L]} = \frac{\Delta A/\text{min. Sample}}{\Delta A/\text{min. Cal.}} \times \text{Conc. Cal. [U/L]}$$

Calibrators and Controls

For the calibration of automated photometric systems, DiaSys TruCal Lipid calibrator is recommended. The method is traceable to the molar extinction coefficient of 4-nitrophenol. For internal quality control, DiaSys TruLab L Level 1 and Level 2 should be assayed. Each laboratory should establish corrective action in case of deviations in control recovery.

Note: For reconstitution of TruLab L Level 2 add exactly 1 mL of distilled water. Reconstitution of TruLab L Level 1 should be done according to the instruction supplied with the product. **Replacement labels are attached to the reagent kit to identify TruLab L Level 2 with reduced reconstitution volume.**

	Cat. No.	Kit size
TruCal Lipid	1 3570 99 10 045	3 x 2 mL
TruLab L Level 1	5 9020 99 10 065	3 x 3 mL
TruLab L Level 2	5 9030 99 10 065	3 x 1 mL

Performance Characteristics

Measuring range

The test has been developed to determine Lp-PLA_2 activities from 50 U/L up to 2000 U/L.

Specificity/Interferences

No interference was observed by ascorbic acid up to 50 mg/dL, bilirubin up to 50 mg/dL, hemoglobin up to 1000 mg/dL, and lipemia up to 2000 mg/dL triglycerides.

Sensitivity/Limit of Detection

The lower limit of detection is 10 U/L.

Precision

Intra-assay n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	319	2.02	0.63
Sample 2	633	4.40	0.69
Sample 3	1113	7.98	0.72

Total precision CLSI n = 80	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	314	4.80	1.53
Sample 2	625	10.0	1.61
Sample 3	1105	13.3	1.20

Method Comparison

A comparison of DiaSys Lp-PLA_2 FS (y) with an activity test (x) using 97 samples gave following results:
y = 0.909 x – 4.28 U/L; r = 0.999

Reference Range [5]

Adults	Men	< 639 U/L
	Women	< 507 U/L

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

1. Ridker, P.M.; MacFadyen, J.G.; Wolfert R.L.; Koenig W. Relationship of lipoprotein-associated phospho-lipase A_2 mass and activity with incident vascular events among primary prevention patients allocated to placebo or to statin therapy: An analysis from the JUPITER trial. Clin Chem 2012; 58(5):877–886.
2. Münzel, T.; Gori, T. Lipoprotein-associated phospholipase A_2, a marker of vascular inflammation and systemic vulnerability. Eur Hear J 2009; 30:2829–2831.
3. Madjid, M.; Ali, M.; Willerson, J.T. Lipoprotein-associated phospholipase A_2 as a novel risk marker for cardiovascular disease. Tex Heart Inst J 2010; 37(1): 25–39.
4. Mannheim, D; Herrmann, J et al. Enhanced expression of Lp-PLA_2 and Lysophosphatidylcholine in Symptomatic Carotid Atherosclerotic Plaques. Stroke 2008;39:1448–1455.
5. Personal communication from Prof. Dr. med. Karl Winkler, Universitaetsklinikum Freiburg, Germany
6. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: Mechanism, detection and prevention. Clin Chem Lab Med 2007; 45(9): 1240–1243.

Manufacturer

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