Lp-PLA$_2$ FS*

Order Information

Cat. No.	Kit size
1 7181 99 10 936	R1 1 x 20 mL + R2 1 x 4.75 mL + R3 1 x 0.25 mL
1 7181 99 10 937	R1 1 x 10 mL + R2 1 x 3.8 mL + R3 1 x 0.2 mL

Intended Use

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

Summary

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 atherogenic and 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. [1-4]

Method

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

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 and Stability

The reagents are stable up to the date of expiry indicated on the kit, if stored at 2 – 8°C and contamination is avoided. Do not freeze reagent R3, protect from light and moisture.

Warnings and Precautions

1. ⚠ Reagent 3: Warning. Contains: Diethylene glycol. H302 Harmful if swallowed. H373 May cause damage to organs through prolonged or repeated exposure. P260 Do not breathe mist/vapours/spray. P264 Wash hands and face thoroughly after handling. P314 Get medical advice/attention if you feel unwell.
2. In very rare cases, samples of patients with gammopathy might give falsified results [5].
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

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. Make sure that there is no air bubble on the bottom of the reagent vial R3 by tapping the vial two to three times on the table.

Aspirate slowly with a pipet below-mentioned volume of highly viscous reagent R3 and transfer 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 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°C.

Materials Required

General laboratory equipment

Specimen

Human serum or heparin plasma

Stability [6]:		
2 days	at	20 – 25°C
4 weeks	at	2 – 8°C
3 months	at	–20°C

Only freeze once. Discard contaminated specimens.

Assay Procedure

Basic settings for BioMajesty® JCA-BM6010/C

Wavelength	410/505 nm
Temperature	37°C
Measurement	Kinetic
Sample/calibrator	1.0 µL
Reagent 1	100 µL
Reagent 2	25 µL
Addition Reagent 2	Cycle 19 (286 s)
Absorbance 1	Cycle 25 (367 s)
Absorbance 2	Cycle 32 (464 s)
Calibration	Linear

Calculation

With calibrator

$$\text{Lp-PLA}_2\ [\text{U/L}] = \frac{\Delta A/\text{min Sample}}{\Delta A/\text{min Cal}} \times \text{Conc. Cal [U/L]}$$

Calibrators and Controls

DiaSys TruCal Lipid calibrator is recommended for calibration. TruCal Lipid calibrator values have been made traceable to the molar extinction coefficient of 4-nitrophenol. Use DiaSys TruLab L Level 1 and Level 2 for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

	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 3 mL**

****Note:** For reconstitution of TruLab L Level 2, add exactly 1 mL of distilled water. For analyzers having problems in processing highly viscous solutions, reconstitution may alternatively be performed with exactly 1.5 mL distilled water. **Carefully choose the appropriate target values.** Replacement labels for TruLab L Level 2 are attached to the reagent kit to identify vials with reduced reconstitution volume.

TruLab L Level 1 should be reconstituted according to the instructions provided with the product.

Performance Characteristics

Data evaluated on BioMajesty® JCA-BM6010/C

Exemplary data mentioned below may slightly differ in case of deviating measurement conditions.

Measuring range up to to 2000 U/L. When values exceed this range, samples should be diluted 1 + 4 with NaCl solution (9 g/L) and the result multiplied by 5.

Limit of detection***	50.0 U/L

Interfering substance	**Interferences ≤ 10% up to**
Ascorbic acid	60 mg/dL
Bilirubin (conjugated)	50 mg/dL
Bilirubin (unconjugated)	50 mg/dL
Hemoglobin	1000 mg/dL
Lipemia (triglycerides)	1800 mg/dL
N-acetylcysteine (NAC)	1500 mg/L

Precision			
Within run (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [U/L]	293	585	848
CV [%]	0.790	0.666	0.774
Total Precision CLSI (n=80)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [U/L]	288	572	834
CV [%]	2.48	2.15	2.46

Method comparison (n=100)	
Test x	DiaSys Lp-PLA_2 FS
Test y	DiaSys Lp-PLA_2 FS (improved)
Slope	1.03
Intercept	–7.02
Coefficient of correlation	0.992

*** according to CLSI document EP17-A2, Vol. 32, No. 8

Reference Range [6]

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 A2 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 A2, 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 A2 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 PLA2 and Lysophosphatidylcholine in Symptomatic Carotid Atherosclerotic Plaques. Stroke 2008;39:1448-1455.
5. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: Mechanism, detection and prevention. Clin Chem Lab Med 2007; 45(9): 1240-1243.
6. Personal communication from Prof. Dr. med. Karl Winkler, Universitaetsklinikum Freiburg, Germany.

DiaSys Diagnostic Systems GmbH
Alte Strasse 9 65558 Holzheim Germany
www.diasys-diagnostics.com

* Fluid Stable