

Lp-PLA₂ FS*

Order Information

Cat. No.	Kit size
1 7181 99 10 922	 50 (1 x 50)

Intended Use

Diagnostic reagent for quantitative in vitro determination of Lp-PLA₂ (lipoprotein-associated phospholipase A₂) in human serum or heparin plasma on automated DiaSys respons[®]910.

Summary

Lipoprotein-associated phospholipase A₂ (Lp-PLA₂), also known as platelet-activating factor acetylhydrolase (PAF AH), is a calcium-independent phospholipase released by inflammatory cells in atherosclerotic plaques. In circulation, Lp-PLA₂ is predominantly associated with LDL particles whereas only a small portion of enzyme is associated with HDL. Lp-PLA₂ 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-PLA₂ is independent of the presence of other cardiovascular risk factors, shows minimal biovariability and is not elevated in systemic inflammatory reactions. Lp-PLA₂ 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-PLA₂ 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-PLA₂ 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

-  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.
- In very rare cases, samples of patients with gammopathy might give falsified results [5].
- 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.
- 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.

Transfer 250 µL R3 into the R2 compartment of the twin container by pipetting.

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°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.

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 1 mL

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.

Performance Characteristics

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

Measuring range up to 2000 U/L. In case of higher activities re-measure samples after manual dilution with NaCl solution (9 g/L) or use rerun function.		
Limit of detection**	50 U/L	
Onboard stability	10 days	
Calibration stability	10 days	
Interfering substance	Interferences ≤ 10% up to	Analyte concentration [U/L]
Ascorbic acid	60 mg/dL	455
	60 mg/dL	907
Bilirubin (conjugated)	50 mg/dL	446
	50 mg/dL	884
Bilirubin (unconjugated)	50 mg/dL	414
	50 mg/dL	860
Hemoglobin	1000 mg/dL	425
	1000 mg/dL	904
N-acetylcysteine (NAC)	1500 mg/L	445
	1500 mg/L	898
Lipemia (triglycerides)	1800 mg/dL	415
	1800 mg/dL	932

Precision			
Within run (n=20)	Sample 1	Sample 2	Sample 3
Mean [U/L]	287	577	842
CV [%]	2.29	1.41	1.80
Total Precision CLSI (n=80)	Sample 1	Sample 2	Sample 3
Mean [U/L]	273	539	778
CV [%]	3.70	3.86	3.56
Method comparison (n=100)			
Test x	DiaSys Lp-PLA ₂ FS		
Test y	DiaSys Lp-PLA ₂ FS (improved)		
Slope	0.958		
Intercept	4.21		
Coefficient of correlation	0.995		

** according to CLSI document EP17-A, Vol. 24, No. 34

Conversion Factor

Lp-PLA₂ [U/L] x 0.0167 = Lp-PLA₂ [µkat/L]

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.

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* Fluid Stable

Lp-PLA2 FS

Application for serum and plasma samples

This application was set up and evaluated by DiaSys. It is based on the standard equipment at that time and does not apply to any equipment modifications undertaken by unqualified personnel.

Identification	
This method is usable for analysis:	Yes
Twin reaction:	No
Name:	LpPLA2
Shortcut:	
Reagent barcode reference:	065
Host reference:	

Technic	
Type:	Linear kinetic
First reagent:[μ L]	200
Blank reagent	Yes
Sensitive to light	
Second reagent:[μ L]	50
Blank reagent	Yes
Sensitive to light	
Main wavelength:[nm]	405
Secondary wavelength:[nm]	508
Polychromatic factor:	1.000
1 st reading time [min:sec]	6:00
Last reading time [min:sec]	8:12
Reaction way:	Increasing
Linear Kinetics	
Substrate depletion: Absorbance limit	1.50
Linearity: Maximum deviation [%]	
Fixed Time Kinetics	
Substrate depletion: Absorbance limit	
Endpoint	
Stability: Largest remaining slope	
Prozone Limit [%]	

Reagents	
Decimals	
Units	

Sample	
Diluent	System water
Hemolysis:	
Agent [μ L]	0 (no hemolysis)
Cleaner	
Sample [μ L]	0
Technical limits	
Concentration technical limits-Lower	50
Concentration technical limits-Upper	2000
SERUM	
Normal volume [μ L]	2
Normal dilution (factor)	1
Below normal volume [μ L]	10
Below normal dilution (factor)	1
Above normal volume [μ L]	2
Above normal dilution (factor)	6
URIN	
Normal volume [μ L]	2
Normal dilution (factor)	1
Below normal volume [μ L]	10
Below normal dilution (factor)	1
Above normal volume [μ L]	2
Above normal dilution (factor)	6
PLASMA	
Normal volume [μ L]	2
Normal dilution (factor)	1
Below normal volume [μ L]	10
Below normal dilution (factor)	1
Above normal volume [μ L]	2
Above normal dilution (factor)	6
CSF	
Normal volume [μ L]	2
Normal dilution (factor)	1
Below normal volume [μ L]	10
Below normal dilution (factor)	1
Above normal volume [μ L]	2
Above normal dilution (factor)	6
Whole blood	
Normal volume [μ L]	2
Normal dilution (factor)	1
Below normal volume [μ L]	10
Below normal dilution (factor)	1
Above normal volume [μ L]	2
Above normal dilution (factor)	6

Results	
Decimals	2
Units	U/L
Correlation factor-Offset	0.000
Correlation factor-Slope	1.000

Range	
Gender	Male
Age	
SERUM	<=639
URINE	
PLASMA	<=639
CSF	
Whole blood	
Gender	Female
Age	
SERUM	<=507
URINE	
PLASMA	<=507
CSF	
Whole blood	

Contaminants	
Please refer to r910 Carryover Pair Table	

Calibrators details	
Calibrator list	Concentration
Cal. 1/Blank	0
Cal. 2	*
Cal. 3	
Cal. 4	
Cal. 5	
Cal. 6	
	Max delta abs.
Cal. 1	0.010
Cal. 2	0.005
Cal. 3	
Cal. 4	
Cal. 5	
Cal. 6	
Drift limit [%]	0.8

Calculations	
Model	X
Degree	1

* Enter calibrator value