respons®910

# Lp-PLA$_2$ FS*

## Order Information

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

## Intended Use

Diagnostic reagent for quantitative in vitro determination of Lp-PLA$_2$ (lipoprotein-associated phospholipase A2) in human serum or heparin plasma on automated DiaSys respons®910.

## 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.

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 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

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

| | |
|---|---|
| Measuring range up to 2000 U/L.<br>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-$PLA_2$ [U/L] x 0.0167 = Lp-$PLA_2$ [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

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# 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: | 065 |

| Technic | |
|---|---|
| Type: | Linear kinetic |
| First reagent:[µL] | 200 |
| Blank reagent | Yes |
| Sensitive to light | |
| Second reagent:[µL] | 50 |
| Blank reagent | No |
| Sensitive to light | |
| Main wavelength:[nm] | 405 |
| Secondary wavelength:[nm] | 508 |
| Polychromatic factor: | 1.0000 |
| 1 st reading time [min:sec] | 6:00 |
| Last reading time [min:sec] | 8:12 |
| Reaction way: | Increasing |
| Linear Kinetics<br>Substrate depletion: Absorbance limit | 1.5000 |
| Linearity: Maximum deviation [%] | |
| Fixed Time Kinetics<br>Substrate depletion: Absorbance limit | |
| Endpoint<br>Stability: Largest remaining slope | |
| Prozone Limit [%] | |

| Reagents | |
|---|---|
| Decimals | |
| Units | |

| Sample | |
|---|---|
| Diluent | DIL A (NaCl) |
| Hemolysis: | |
| Agent [µL] | 0 (no hemolysis) |
| Cleaner | |
| Sample [µL] | 0 |
| Technical limits | |
| Concentration technical limits-Lower | 50.0000 |
| Concentration technical limits-Upper | 2000.0000 |
| SERUM | |
| Normal volume [µL] | 2.0 |
| Normal dilution (factor) | 1 |
| Below normal volume [µL] | |
| Below normal dilution (factor) | |
| Above normal volume [µL] | 2.0 |
| Above normal dilution (factor) | 6 |
| URINE | |
| Normal volume [µL] | 2.0 |
| Normal dilution (factor) | 1 |
| Below normal volume [µL] | |
| Below normal dilution (factor) | |
| Above normal volume [µL] | 2.0 |
| Above normal dilution (factor) | 6 |
| PLASMA | |
| Normal volume [µL] | 2.0 |
| Normal dilution (factor) | 1 |
| Below normal volume [µL] | |
| Below normal dilution (factor) | |
| Above normal volume [µL] | 2.0 |
| Above normal dilution (factor) | 6 |
| CSF | |
| Normal volume [µL] | 2.0 |
| Normal dilution (factor) | 1 |
| Below normal volume[ µL] | |
| Below normal dilution (factor) | |
| Above normal volume [µL] | 2.0 |
| Above normal dilution (factor) | 6 |
| Whole blood | |
| Normal volume [µL] | 2.0 |
| Normal dilution (factor) | 1 |
| Below normal volume[ µL] | |
| Below normal dilution (factor) | |
| Above normal volume [µL] | 2.0 |
| Above normal dilution (factor) | 6 |

| Results | |
|---|---|
| Decimals | 2 |
| Units | U/L |
| Correlation factor-Offset | 0.0000 |
| Correlation factor-Slope | 1.0000 |

| Range | |
|---|---|
| Gender | Male |
| Age | |
| SERUM | <=639.00 |
| URINE | |
| PLASMA | <=639.00 |
| CSF | |
| Whole blood | |
| Gender | Female |
| Age | |
| SERUM | <=507.00 |
| URINE | |
| PLASMA | <=507.00 |
| 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 [%] | 0.80 |

| Calculations | |
|---|---|
| Model | X |
| Degree | 1 |

* Enter calibrator value