

Lp-PLA₂ Activity Assay Kit

Research Use Only (RUO)

【Product Name】

Lp-PLA₂ Activity Assay Kit

【Package Size】

Lp-PLA₂ Activity Assay Kit (35 mL)

Reagent R1	28 mL
Reagent R2a	7 mL
Reagent R2b	370 μL
Calibrators	2 × 1 mL
Quality Controls	2 × 3 mL

【Intended Use】

Lp-PLA₂ Activity Assay Kit is used for the quantitative determination of Lp-PLA₂ enzyme activity in human serum and plasma samples.

【Background】

Lp-PLA₂, also known as platelet-activating factor acetylhydrolase (PAF-AH), is a phospholipase A2 enzyme produced by inflammatory cells. Lp-PLA₂ is involved in the development of atherosclerosis and serves a specific marker of cardiac disease^{1,2}. Studies found that Lp-PLA₂ levels are positively correlated with increased risk of coronary disease, stroke, and mortality³.

【Assay Principle】

Lp-PLA₂ Activity Assay is an enzymatic assay. In the assay reaction, sample Lp-PLA₂ hydrolyses acetyl group at the sn-2 position of the phospholipid substrate, 1- myristoyl-2-(4-nitrophenylsuccinyl)-sn-glycerol-3-phosphocholine (MNP), resulting in a release of a colorful 4-nitrophenyl group, which can be monitored spectrophotometrically at 405-410 nm. Absorbance increase is proportional to the activity of Lp-PLA₂, allowing the instrument to calculate the activity of a sample using the Lp-PLA₂ Activity Calibrator generated curve.

【Reagent Composition】

Material		Amount
R1	CHAPS hydrate	< 1.0 %
	EDTA disodium salt	< 0.5 %
	HEPES hemisodium salt	< 2.0 %
	1-Nonanesulfonic acid sodium salt	< 0.5 %
	Sodium chloride	< 5.0 %
R2	Citric acid monohydrate	< 1.0 %
	Dimethylformamide	< 5.0 %
	1-tetradecanoyl-2-(4-nitrophenyl succinyl)-sn-glycerol-3-phospho choline	< 1.0 %
Calibrators & Controls	Purified human serum	< 60 %
	Potassium phosphate monobasic	< 0.1 %
	Sodium azide	< 0.1 %
	Sodium chloride	< 5.0 %
	Sodium phosphate dibasic	< 0.1 %

【Reagent Preparation And Handling】

- R1: Supplied as ready to use, stable liquid Reagent 1 (R1). Store at 2-8°C.
- R2: Prepare Reagent 2 (R2) by mixing R2a and R2b solutions in a ratio of 19:1 right before use (example: 19 mL of R2a + 1 mL of R2b). Prepared R2 is stable for two weeks when stored at 2-8°C. Discard unused R2 after **one month**.
- Calibrators: Supplied as ready to use, store at 2-8°C.

【Reagent Stability and Storage】

All reagents are stable when stored at 2-8°C until the expiration date on the label. Do not mix reagents of different lots. DO NOT FREEZE.

【Specimen Collection and Handling】

The Lp-PLA₂ Activity Assay is formulated for use with serum and plasma. Samples can be tested immediately or stored prior to testing under the following conditions:

- 8 hours at room temperature
- Up to 2 weeks at 2-8 °C
- At least 2 months at -20 °C
- When transporting samples, ship samples on cold packs at 2-8 °C

【Analyzers Application】

This product can be applied to run on chemistry analyzers including: Hitachi (7180, 7600); Beckman (AU640, AU5400, AU5800; Mindray (BS-200, BS-480); Roche (P800, Cobas701); Bayer-Siemens (ADVIA); Siemens Dimension RXL; and MEDICA EasyRA.

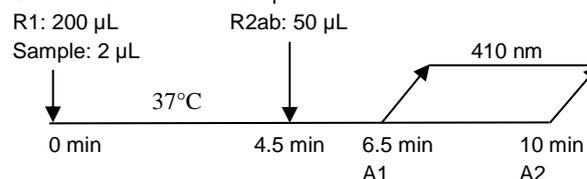
【Assay Requirements】

Lp-PLA₂ Activity should be measured according to specific application parameters for specific chemistry analyzers. SJK will provide application parameters for specific chemistry analyzer per customer request.

Essential parameters (Beckman Coulter AU)

Method:	Rate	Sample Vol.:	2.0 μL
Measuring Point	12-20	Pre-Dilution Rate	1
Reagent 1 (R1):	200 μL	Reagent 2 (R2):	50 μL
Reaction Time:	10 Min	Main Wavelength:	410 nm
Temperature:	37°C	Sub Wavelength:	520 nm
Reaction Direction/ Indication:		Positive/Increasing	

Generalized reaction description:



【Calibration】

Lp-PLA₂ activity Assay should be calibrated using Lp-PLA₂ Activity Calibrator Set.

$$\text{Activity (U/L)} = \frac{\Delta\text{OD}/\text{min} \times V_t \times 10^6}{\epsilon \times V_s \times d}$$

Activity (U/L): μmol/min/L

ΔOD/min: optical density change per min

V_t: total reaction volume

V_s: sample volume

10⁶: factor converting moles to micromoles

ε: molar extinction coefficient of p-nitrophenol at 410 nm

d: cuvette light path length (1 cm)

Lp-PLA₂ Activity Calibrator Set is a two-level set. Two-point calibration curve obtained from the Lp-PLA₂ Activity Calibrators is used to determine Lp-PLA₂ activity (U/L) in the sample.

Calibration Frequency: daily calibration is recommended.

【Quality Control】

Users should follow the appropriate federal, state and local guideline concerning the running of external quality control.

To ensure adequate quality control, low and high controls with known values should be run as unknown samples.

【Results】

Assay results are reported in U/L.

Unit Definition: 1 U/L of Lp-PLA₂ activity is defined as 1 μmol/L or 1 nmol/mL of substrate converted to 4-nitrophenol product per minute.



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【Limitations】

Samples with Lp-PLA₂ Activity level exceeding the linearity limit of 2000 U/L should be diluted with saline (0.9% NaCl) and retested. Retest results are to be multiplied by the dilution factor.

【Analytical Characteristics】

Precision

Within-run precision of the Lp-PLA₂ Activity Assay was evaluated. In the study, two levels of Lp-PLA₂ Activity samples containing 232 U/L and 658 U/L Lp-PLA₂ Activity respectively were tested with 20 replicates in a single run.

	Level 1	Level 2
N	20	20
Mean (U/L)	232	658
SD	2.069	4.922
CV%	0.89	0.76

Accuracy

The performance of this assay was compared with the performance of a commercial Lp-PLA₂ Activity Assay using serum samples. For the 60 serum samples with Lp-PLA₂ Activity ranging from 84 U/L to 1778 U/L, the correlation coefficient between the two methods was 0.995, slope was 0.978, and y intercept was 4.5.

Limit of Quantitation

The Limit of Quantitation (LOQ) of the Lp-PLA₂ Activity Assay was determined to be 25 U/L.

Linearity

The linearity of the assay is from 25-2000 U/L. Results that exceed 2000 U/L should be diluted with saline and retested.

Interference

The substances normally present in the serum were tested. Less than 10% deviation was produced when tested up to the concentrations shown below.

Name	Concentration
Ascorbic Acid	10 mM
Bilirubin	40 mg/dL
Hemoglobin	400 mg/dL
Triglyceride	1000 mg/dL

【Caution】

1. Reagents are "Research Use Only". Not for use in diagnostic procedures.
2. Do not use the reagents after the expiration date labeled on the outer box.
4. Reagent 2b highly flammable and evaporative. Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. Avoid breathing vapors.
5. Assay calibration frequency is dependent on instrument used. Additionally, the assay should be recalibrated and controls run with each new lot of reagents.
6. Avoid ingestion and contact with skin and eyes.
7. Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures, such as those outlined.

【References】

- [1] Zalewski A, Macphee C (May 2005). "Role of lipoprotein-associated phospholipase A2 in atherosclerosis: biology, epidemiology, and possible therapeutic target". *Arteriosclerosis, Thrombosis, and Vascular Biology* **25** (5): 923–31
- [2] Meher ER, Ballantyne CM, Davidson MH, Hanefeld M, Ruilope LM, Johnsonn JL, Zalewski A (April 2008). "The effect of darapladib on plasma lipoprotein-associated phospholipase A2 activity and cardiovascular biomarkers in patients with stable coronary heart disease or coronary heart disease risk equivalent: the results of a multicenter, randomized, double-blind, placebo-controlled study". *J. Am. Coll. Cardiol.* **51**(17):1632-41
- [3] The Lp-PLA₂ Studies Collaboration (2010). "Lipoprotein-associated phospholipase A2 and risk of coronary disease, stroke, and mortality: collaborative analysis of 32 prospective studies". *The Lancet* **375** (9725): 1536–1544

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