Instruction For Use

【Product Name】

Lipoprotein-Associated Phospholipase A2 (Electrochemiluminescence Immunoassay)

【Order Information】

REF NO.	Package Size
691051	50T
691052	2×50T
691053	100T
691054	2×100T

【Intended Use】

Immunoassay for in vitro quantitative determination of Lp-PLA2 in human serum and plasma.

Lipoprotein-Associated Phospholipase A2 (Lp-PLA2) is a subtype in the phospholipase superfamily, also known as platelet-activating factor acetylhydrolase, secreted by macrophages, T cells, and mast cells in the intima. The expression of Lp-PLA2 in atherosclerotic plaques was up-regulated and strongly expressed in macrophages in the fibrous cap of vulnerable plaques.[1] The study suggests that with increased Lp-PLA2 levels, the risk of coronary heart disease and post-stroke increases, especially in the elderly and asymptomatic atherosclerotic disease populations.[2-3]

2011 AHA/American stroke association stroke in primary prevention guide[4] suggested detecting inflammation indexes such as the hs CRP or Lp-PLA2 can identify high-risk patients with stroke (IIb/b). 2012 European society of cardiology cardiovascular disease prevention and clinical practice guidelines[5] is pointed out that the Lp-PLA2 to reflect the latest atherosclerotic plaque rupture and thrombosis events of a highly consistency, accuracy of the independent risk factors, and can be used as a kind of acute thrombosis of atherosclerosis accurate risk assessment markers of recurrent events in high-risk patients.

【Test Principle】

Sandwich principle. Total duration of assay: 18 minutes.

1st incubation: 15 μL sample, biotinylated monoclonal Lp-PLA2 specific antibody and monoclonal Lp-PLA2 specific antibody labeled by ruthenium trisbipyidyl NHS ester form a sandwich compound by immunoreaction.

2nd incubation: After addition of streptavidin-coated micro magnetic beads, the compounds bind to the micro beads via interaction of biotin and streptavidin.

Measurement: The reaction mixture is aspirated into the measuring cell where the micro beads and immuno-compounds are magnetically captured onto the surface of the electrode.

(Unbound substances are then removed by Buffer. Application of a voltage to the electrode then induces emission of luminescence which is measured by a photomultiplier.

Results are determined via calibration and a master curve provided via the reagent barcode.

【Main Components】

The reagent pack consists of MB, RA, RB, calibrators and control materials(Optional). Different lots cannot be used at the same time or mixed up together.

Components	Ingredients	Volume (50T)	Volume (2×50T)	Volume (100T)	Volume (2×100T)
(MB)	Streptavidin-coated micro magnetic beads: preservative	1×1.8 mL	2×1.8 mL	1×3.5 mL	2×3.5 mL
(RB)	Anti-Lp-PLA2-Ab~biotin: 0.1M PBS; preservative	1×3.8 mL	2×3.8 mL	1×7.5 mL	2×7.5 mL
(RA)	Anti-Lp-PLA2-Ab~Ru(bpy)$^{2+}_3$: 0.1M PBS; preservative	1×3.8 mL	2×3.8 mL	1×7.5 mL	2×7.5 mL
Calibrators	Bovine serum, preservative, lyophilized	High: 1×1.0 mL; Low: 1×1.0 mL			
Control Materials (Optional)	Bovine serum, preservative, lyophilized	High: 1×1.0 mL; Low: 1×1.0 mL			

Traceability: This method can be traced to manufacturer's selected measurement procedures.

See the control material value sheet for the target values and ranges of control materials.

Supporting instruments and materials required but not provided in this pack (provided by Lifotronic).

Additional materials for Automated ECL Immunoassay Analyzer eCL8000, eCL8000i, eCL8000p, eCL8000x:

- REF 679005, Auffer, 480 mL, or REF 679004, Auffer, 6×480 mL
- REF 679007, Buffer, 480 mL, or REF 679006, Buffer, 6×480 mL
- REF 679009, Concentrated Washing Buffer, 1 L, or REF 679008, Concentrated Washing Buffer, 6×1 L
- REF 679011, Assay Cup, 300 T, or REF 679012, Assay Cup, 3000 T

Additional materials for Automated ECL Immunoassay Analyzer eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p:

- REF 0320501804, Auffer, 2×1 L, or REF 0320501805, Auffer, 4×1 L
- REF 0320507502, PreClean, 2×800 mL, or REF 0320507503, PreClean, 4×800 mL
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, or REF 032120111, Assay Cup/Assay Tip, 12×2×105

Additional materials for Automated ECL Immunoassay Analyzer eCL9000, eCL9600, eCL9900, eCL9900i:

- REF 0320501801, Auffer, 2×2 L, or REF 0320501806, Auffer, 4×2 L
- REF 0320501901, Buffer, 2×2 L, or REF 0320501906, Buffer, 4×2 L
- REF 0320507501, PreClean, 2×2 L, or REF 0320507504, PreClean, 4×2 L
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, or REF 0320510002, Assay Cup/Assay Tip, 1×6×105.

【Storage and Shelf Life】

Store at 2℃-8℃.

Do not freeze.

Store the reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Damaged, expired or contaminated reagents should be discarded.

Stability of the reagents	
Unopened at 2℃~8℃	18 months
Store at 2℃~8℃ after opening	8 weeks
Stored on the analyzers at 2℃~15℃	8 weeks

The calibrators and control materials are stable up to the stated expiration date.

Stability of the calibrators and control materials	
Unopened store at 2℃~8℃	18 months
Store at 2℃~8℃ after reconstituted	7 days
Store at -25℃~-15℃ after reconstituted	3 months (freeze only once)

【Applicable Instrument】

Automated ECL Immunoassay Analyzers: eCL8000, eCL8000i, eCL8000p, eCL8000x, eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p, eCL9000, eCL9600, eCL9900, eCL9900i.

【Specimen collection, handling and storage】

It is recommended to use serum samples or plasma samples collected from EDTA-2K, EDTA-3K, heparin lithium, heparin sodium blood collection tubes.

Blood samples should be collected by standard operation of venous puncture; after complete coagulation, the tangible component should be removed by centrifugation. The sample should avoid bubbling during testing. Lipid layer floating on the upper of the sample should be removed.

Samples would better to be tested in 4 hours after collection, otherwise, should be stored 2~8℃ for no more than 24 hours or -25~-15℃ for 30 days. Freezing and thawing cycle is permitted only once.

【Assay Procedure】

Testing procedures and precautions

Previous to operation, users should read the operational manual carefully to obtain system operation procedure, sample processing, security precaution, maintenance and other related information.

Set up Lp-PLA2 test according to the operational manual.

Put the Lp-PLA2 rackpack into the correct analyzer slot to automatically suspend magnetic beads at least 30 min before testing.

Recommended ambient temperature:10~30℃; Relative humidity: ≤ 80%.

Handing of lyophilized calibrators and control materials

Carefully dissolve the contents of calibrator by adding exactly 1.0 mL of distilled or deionized water and allow to stand closed at room temperature for at least 15 minutes to reconstitute. Mix carefully, avoiding foam formation. Transfer aliquots of the reconstituted calibrators to the additional plastic bottles. After bottles is marked and dispensed. If you do not test immediately, you need to store the aliquots immediately at -15℃ or lower than -15℃.

Carefully dissolve the contents of control materials by adding exactly 1.0 mL of distilled or deionized water and allow to stand closed at room temperature for at least 15 minutes to reconstitute. Mix carefully, avoiding foam formation. Transfer aliquots of the reconstituted control materials to the additional plastic bottles. After bottles is marked and dispensed. If you do not test immediately, you need to store the aliquots immediately at -15℃ or lower than -15℃.

Calibration

Calibration should be performed using lot-matching reagent and calibrators.

Before calibration, the reagent and main curve information should be imported into the analyzer via radio frequency identification (RFID) reagent card(refer to instrument user manual). The analyzer adjusts the main curve to produce working curve according to the calibrator results, and identifies validity of the working curve automatically.

Recalibration is recommended when:

(1) The reagents of different lot are used;

(2) The same lot of reagents were used on the analyzer beyond 4 weeks;

(3) As required: if the quality control results exceed the specified limit.

(4)The Buffer of different lots are used.

Quality control

For quality control, use Lp-PLA2 control materials.

In addition, other suitable control material can be used.

Control materials for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.
The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.
If necessary, repeat the measurement of the samples concerned.
Follow the applicable government regulations and local guidelines for quality control.
Please make sure that the correct values are used.

Calculation

The system software automatically calculates the analyte concentration using particular algorithm, and the result unit is ng/mL.

Specimen dilution

Samples with Lp-PLA2 concentrations above the measuring range would be manually diluted with Lp-PLA2-negative serum.
The recommended dilution ratio is 1:10. After manual dilution, multiply the result by the dilution factor.

【Biological reference interval】

According to serum test results of aged from 25 to 93 years 244 healthy human (males 122, females 122) in hospitals. The percentile 95 gives the reference range of < 197.41 ng/mL.
Due to differences in the region, race, gender and age, laboratories are recommended to set up their own reference ranges.

【Result Interpretation】

For explaining the result, the patient's overall clinical manifestation should be referred to, including symptoms, medical history and other relevant data and information.

【Limitations】

Test results are used only for clinical reference and cannot be used as the basis for diagnosis or rule-out of diseases alone.
If the Lp-PLA2 contents is above the upper detection limit 1000 ng/mL, then the sample is tested after dilution with a recommended dilution ratio of 1:10.The results are reported after multiplying with the dilution factor.
There is no high-dose hook effect at Lp-PLA2 concentrations 4000 ng/mL.
When the sample containing bilirubin ≤ 80 mg/dL, triglyceride ≤ 2500 mg/dL, hemoglobin ≤ 200 mg/dL, biotin ≤ 30 ng/mL, HAMA ≤120 ng/mL, interference deviation to the test result lies within ±10%. When the RF concentration in the sample < 1500 IU/mL, the relative recovery of the test results is between 90% and 110%.

【Analytical Performance】

Lower Limits of measurement

Lower detection limit is 0.3 ng/mL.

Accuracy

When measuring the appropriate concentration of the added sample, the recovery ratio should be in the range of 90% to 110%. Or measuring the metrologically traceable accuracy control materials, the ratio between resulting value and target value lies between 0.900 and 1.100.

Linearity

The correlation coefficient (Pearson's r) is not less than 0.9900 in the interval of 30~1000 ng/mL.

Within-run imprecision (repeatability)

The coefficient of variation (CV) ≤ 8%.

Between-lot imprecision

The coefficient of variation (CV) ≤ 10%.

Homogeneity of calibrators and control materials

Within-bottle homogeneity: coefficient of variation (CV) ≤ 10%.
Between-bottle homogeneity: coefficient of variation (CV) ≤ 10%.

Method comparison

A comparison of the Lifotronic Lp-PLA2 assay (y) with the commercial Lp-PLA2 method (x) using clinical samples gave the following correlations:
Number of serum samples measured: 216
Linear regression:
Y=1.0082 x-4.8338
r = 0.9912
The sample concentrations were between 0.546 and 977.8 ng/mL.

【Precaution and Warning】

The kit is only used for in vitro diagnostics.
When using the kit, it's necessary to comply with regulations in the laboratory.
All reagents and samples including specimen, calibrators and control materials, should avoid foaming before and during test.
Test results of the kit are for clinical reference only, and clinical evaluation of patients should be combined with their symptoms and signs, medical history, other laboratory examination results and treatment responses.
Due to factors such as methodology or antibody specificity, testing identical samples with reagents from different manufacturers may get different results, and the results from different kits should not compare directly, lest cause the wrong medical explanation. It's suggested that laboratorians should point out the characteristics of used reagent in the test report to the clinician. In serial monitoring, if the reagent type is changed, a continuous parallel test should be performed and comparison of the results to the former to determine a new baseline value.
This product contains animal-sourced materials and may have potential biological risk. All samples and test wastes should be treated as the source of infection, and all wastes must be disposed according to local regulations.
The preservative ProClin™300 which contain Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1): May cause an allergic skin reaction. thus, proper personal protection should be adopt while handling the reagent: avoid breathing dust/fume/gas/mist/vapours/spray, wear protective gloves and contaminated work clothing should not be allowed out of the workplace. If skin irritation or rash occurs: get medical advice/attention, take off contaminated clothing and wash it before reuse, and dispose of contents/container to an approved waste disposal plant

【Symbols Explanation】

Symbol	Title of Symbol	Symbol	Title of Symbol
	Manufacturer		Consult instructions for use
	Use-by date		In vitro diagnostic medical device
	Batch code		Sufficient for <n> tests
	Serial number		Biological risks
	Temperature limit		This way up
	Authorized representative in the European Community		Indicates this device is in compliance with Europe Directive.
	Catalogue number	P/N	Material code
	Warning		Date of manufacture
	Unique device identifier		

【Literature References】

1. O. Vittos, et al. Lipoprotein-associated phospholipase A2 (Lp-PLA2): a review of its role and significance as a cardiovascular biomarker. Biomarkers. 2012; 17(4):289-302.
2. Persson, et al. Elevated Lp-PLA2 Levels Add Prognostic Information to the Metabolic Syndrome on Incidence of Cardiovascular Events Among Middle-Aged Nondiabetic Subjects. Arterioscler Thromb Vasc Biol. 2007; 27:1411-1416.
3. N. Khuseyinova et al. Association between Lp-PLA2 and coronary artery disease: Focus on its relationship with lipoproteins and markers of inflammation and hemostasis. Atherosclerosis. 2005; 182:181–188.
4. Goldstein LB, Bushnell CD, Adams RJ, et al. Guidelines for the Primary Prevention of Stroke. Stroke 2011; 42: 517-584.
5. Perk J, De Backer G, Gohlke H, et al. European Guidelines on Cardiovascular Disease Prevention in Clinical Practice (Version 2012). International Journal of Behavioral Medicine 2012; 19: 403-488.

【Manufacturer】

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