



PLAC[®] Test Reagent Kit

Turbidimetric Immunoassay for the Quantitative Determination of Lp-PLA₂ in Human Plasma or Serum



www.plactest.com



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REF

90107

90112

90115

90116

10-0112

10-0113

10-0115

10-0126

Symbol Key

	Catalog Number		Reagent 1
	<i>In vitro</i> diagnostic medical device		Reagent 2
	Batch		Consult Instructions for Use
	Expiration Date		Manufacturer
	Store at 2 to 8 °C		Authorized Representative in the European Community
	Irritant		European Conformity

Read this package insert completely before using the product. Follow instructions carefully when performing tests. Failure to follow the instructions may result in inaccurate results.

Additional information is available in Application Sheets for specific automated clinical chemistry analyzers by contacting Customer Service at 1-877-752-2837 or 1-650-246-6400 or www.plactest.com.

This product is covered by U.S. Patent Nos. 5532152, 5641669, 5698403, 5847088, 5968818, 5981252, 6177257, 7045329, 7416853 and European Patent Nos. 658205 and 673426. Additional patents pending.

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INTENDED USE

The PLAC[®] Test Reagent Kit is a turbidimetric immunoassay for the quantitative determination of Lp-PLA₂ (lipoprotein-associated phospholipase A₂) in human plasma or serum on automated clinical chemistry analyzers, to be used in conjunction with clinical evaluation and patient risk assessment as an aid in predicting risk for coronary heart disease, and ischemic stroke associated with atherosclerosis.

SUMMARY AND EXPLANATION

Lp-PLA₂ is a calcium-independent serine lipase that is associated with both low-density lipoprotein (LDL) and, to a lesser extent, high-density lipoprotein (HDL) in human plasma and serum [1] and is distinct from other phospholipases such as cPLA₂ and sPLA₂ [2]. Lp-PLA₂ is produced by macrophages and other inflammatory cells and is expressed in greater concentrations in advanced atherosclerotic lesions than early-stage lesions [3,4]. Several lines of evidence suggest that oxidation of LDL plays a critical step in the development and progression of atherosclerosis [5,6]. Lp-PLA₂ participates in the oxidative modification of LDL by hydrolyzing oxidized phosphatidylcholine, generating lysophosphatidylcholine and oxidized free fatty acids, both of which are potent proinflammatory products that contribute to the formation of atherosclerotic plaques [7,8,9]. Lp-PLA₂ has demonstrated modest intra- and inter-individual variation, commensurate with other cardiovascular lipid markers and substantially less than C-reactive protein (CRP). In addition, Lp-PLA₂ is not elevated in systemic inflammatory conditions, and may be a more specific marker of vascular inflammation. The relatively small biological variation of Lp-PLA₂ and its specificity are of value in the detection and monitoring of cardiovascular risk [10,11].

Elevated levels of Lp-PLA₂, as measured by immunoassay, were found in patients with angiographically proven coronary heart disease (CHD), when compared to age matched controls [1]. In a retrospective case-control study, using samples from hypercholesterolemic men (n=1740) in the West of Scotland Coronary Prevention Study (WOSCOPS), a 2-fold greater risk of coronary heart disease was observed for subjects in the highest quintile of Lp-PLA₂ levels, compared to the lowest quintile [12]. Furthermore, the CHD risk association of Lp-PLA₂ was shown to be independent of LDL and other markers of inflammation: C-reactive protein, white cell count and fibrinogen. The authors of the study stated in their conclusions, "Elevated levels of lipoprotein-associated phospholipase A₂ appear to be a strong risk factor for coronary heart disease, a finding that has implications for atherogenesis and the assessment of risk." In another report, using samples from the Atherosclerosis Risk in Communities (ARIC) study, which followed 12,819 apparently healthy middle-aged (45 to 64 years) men and women for six to eight years, Lp-PLA₂ was found to be an important predictor of CHD risk. For individuals with LDL less than 130 mg/dL, Lp-PLA₂ was significantly and independently associated with a 2-fold higher risk for CHD events including the need for revascularization, myocardial infarction and death from cardiac disease [13].

The ARIC study was re-analyzed to determine the risk of stroke associated with increased levels of Lp-PLA₂. A total of 223 stroke events were identified from the study group; of this, 194 (87%) were ischemic stroke associated with atherosclerosis, as classified by the ARIC investigators. This proportion of ischemic stroke to the total is consistent with the percentage found in the general population [14]. The results of this study indicated that Lp-PLA₂ was a strong predictor of ischemic strokes, with an increased risk of nearly 2-fold, even after adjustment for blood pressure, lipids, diabetes, body mass index and other inflammatory markers [15].

PRINCIPLE OF THE TEST

The PLAC Test is a turbidimetric immunoassay using two highly specific monoclonal antibodies (2C10 and 4B4) for the direct measurement of Lp-PLA₂ concentration in human plasma or serum. Lp-PLA₂ in patient samples binds specifically to the monoclonal antibodies that are linked to polymeric microparticles in suspension. As Lp-PLA₂ binds, a change in suspension turbidity occurs, resulting in a measurable absorbance change that is read at 570 nm on a clinical chemistry analyzer. This change in absorbance is proportional to the concentration of Lp-PLA₂ in the patient sample. A set of Lp-PLA₂ calibrators is used to plot a standard curve of absorbance versus Lp-PLA₂ concentration from which the Lp-PLA₂ concentration in the test sample can be determined by interpolation.

REAGENTS AND MATERIALS

PLAC Test Reagent Kit is supplied as a liquid, ready-to-use, two-reagent kit. Materials supplied with the PLAC Test Reagent Kit:

R1 Reagent 1 Borate-based buffer solution with protein stabilizers and 0.03% methylisothiazolinone preservative

R2 Reagent 2 Mouse monoclonal anti-Lp-PLA₂ antibody-coated microparticles with 0.05% methylisothiazolinone preservative

Product catalog numbers (**REF**) are instrument specific.

PLAC Test Reagent Kit (sufficient for 100 tests)	REF 90107
R1 Reagent 1	60012
R2 Reagent 2	60013

PLAC Test Reagent Kit (sufficient for 50 tests)	REF 10-0112
R1 Reagent 1	60022
R2 Reagent 2	60023

PLAC Test Reagent Kit (sufficient for 50 tests)	REF 90112
R1 Reagent 1	60022
R2 Reagent 2	60023

PLAC Test Reagent Kit (sufficient for 100 tests)	REF 10-0113
R1 Reagent 1	60022
R2 Reagent 2	60023

PLAC Test Reagent Kit (sufficient for 50 tests)	REF 90115
R1 Reagent 1	60040
R2 Reagent 2	

PLAC Test Reagent Kit (sufficient for 50 tests)	REF 10-0115
R1 Reagent 1	60040
R2 Reagent 2	

PLAC Test Reagent Kit (sufficient for 250 tests)	REF 90116
R1 Reagent 1	60041
R2 Reagent 2	60042

PLAC Test Reagent Kit (sufficient for 100 tests)	REF 10-0126
R1 Reagent 1	60055
R2 Reagent 2	

Materials required but not provided:

- Automated clinical chemistry analyzer and system operation manual
- Analyzer Application Sheet specific to the clinical chemistry analyzer used (available separately, contact diaDexus Customer Service)
- PLAC Test Calibrator Kit, **REF** 90108 or 10-0108 (available separately, contact diaDexus Customer Service)
- Lp-PLA₂ Control Kit, **REF** 90109 or 10-0109 (available separately, contact diaDexus Customer Service)

WARNINGS AND PRECAUTIONS

- For *In Vitro* Diagnostic Use.
- Treat all blood samples as potentially biohazardous material.
- Exposure of samples to room temperature should be minimized to less than 6 hours (including blood draw, processing, transport and laboratory sample analysis time).
- Storage of samples at -20 °C for longer than 24 hours is not recommended.
- Do not report samples values >360 ng/mL.
- Do not dilute samples in order to report sample value >360 ng/mL.
- Dispose of reagents in a manner consistent with relevant regulations.
- Do not use reagents past their expiration dates.
- Do not mix reagents from different kit lot numbers.

REAGENT PREPARATION AND STORAGE

Reagents are provided ready to use. Store reagents at 2 to 8 °C upon receipt. Unopened reagents are stable until the expiration date shown, provided they are stored at 2 to 8 °C. Testing on specific clinical chemistry analyzers indicate opened bottles of reagents stored in the refrigerated compartment of most analyzers should be stable for up to 2 weeks. Please refer to the specific clinical chemistry analyzer Application Sheet for information specific to your analyzer. Laboratories should verify on board reagent stability on their own analyzers under typical laboratory conditions.

SPECIMEN COLLECTION AND STORAGE

- Fasting is not required
- Collect blood samples in
 - serum or plasma gel separation tubes
 - EDTA or heparin plasma collection tubes
 - any serum collection tubes
- Process samples using standard separation procedures
 - Samples should be centrifuged and separated within four hours of venipuncture as per good laboratory practices, but no longer than 36 hours after blood draw. Samples must be stored refrigerated (2 to 8 °C).
- Unprocessed blood samples:
 - Store and transport on cold packs (at 2 to 8 °C) and process within 36 hours of collection.
- Processed samples:

There are several options for the handling and storage of processed samples. Adopt the option that best suits your laboratory.

 - **Option #1: Transport and store samples at 2 to 8 °C.**
 - Samples must be stored refrigerated at 2 to 8 °C for a minimum of 3 days after sample is drawn before testing can occur.
 - Samples should be tested between the 3rd and 10th day after the sample is drawn when stored at 2 to 8 °C.
Example - a sample drawn on Monday may be tested any time from Thursday through the next Thursday, when stored at 2 to 8 °C.

Day 0	Day 1–2	Day 3–10	Day 11
DRAW DAY Store @ 2-8°C	NO TESTING	TESTING OK	NO TESTING

- **Option #2: Transport samples at 2 to 8 °C. Freeze samples at or below -20 °C for a minimum of 16 hours up to 24 hours. Thaw the following day and store samples at 2 to 8 °C.**
 - Samples must be frozen at or below -20 °C for a minimum of 16 hours up to 24 hours anytime up to 3 days (≤ 72 hours) after the sample is drawn.
 - Do not freeze at -20 °C for more than 24 hours.
 - Samples may be tested as soon as they are thawed but no longer than 10 days after the sample is drawn when stored at 2 to 8 °C.

Day 0	Day 0–3	Day 1–10	Day 11
DRAW DAY NO TESTING	Freeze for 16-24 hours; then THAW and Store @ 2-8°C	TESTING OK after Freeze/Thaw	NO TESTING

- **Option #3: Transport samples frozen (on dry ice).**
 - Samples can be frozen on dry ice for 16 hours up to 24 hours.
 - Samples can be thawed and tested up to 10 days after the sample is drawn when stored at 2 to 8 °C.

Day 0	Day 1	Day 2–10	Day 11
DRAW DAY Freeze During Transport (16-24 hrs)	THAW Store @ 2-8°C TESTING OK	TESTING OK	NO TESTING

- **Option #4: For longer term storage, freeze samples at or below -70 °C.**
 - Samples must be frozen overnight for a minimum of 16 hours.
 - Samples can be tested as soon as they are thawed after a minimum of 16 hours at or below -70 °C.
 - Once thawed, the sample can be tested up to 7 days when stored at 2 to 8 °C.
 - Samples may be frozen at or below -70 °C and thawed twice without affecting the Lp-PLA₂ quantitation.

ASSAY PROCEDURE

Calibration

The assay must be calibrated using a full calibration (5-point) curve (REF 90108 or 10-0108). A standard curve is generated using the appropriate curve fit model indicated in the instrument Application Sheet. Verify the calibration curve with at least two levels of Controls according to the laboratory's requirements. Recalibrate and run Controls for each new lot of reagents. If Controls fall outside of laboratory's acceptable range, recalibrate as necessary.

Quality Control

Test at least two levels of an appropriate Quality Control material (REF 90109 or 10-0109) a minimum of once per day for each day of use. In addition, run Controls after each new calibration run. It is recommended that Low and High Controls be included in each run. If control values are not within acceptance limits, repeat the assay. Additional quality control testing may be necessary according to local, state and/or federal regulations or accreditation requirements.

Example Assay Steps

The PLAC Test should be assayed using the appropriate settings for the analyzer to be used. For detailed instructions and settings for the analyzer, please refer to the Analyzer Application Sheet for the specific automated clinical chemistry analyzer used. A general explanation of the assay procedure for Hitachi 917 analyzer is described.

1. 6 µL of sample is added into a reaction cell and diluted with 185 µL of buffer reagent **R1**.
2. Sample is incubated with reagent **R1** for 5 minutes at 37 °C.
3. 60 µL of reagent **R2** is added to the reaction cell.
4. The absorbance at 570 nm is read continuously over a 5 minute period.
5. The absorbance differential, starting from the addition of reagent **R2** to the final read, is used to calculate Lp-PLA₂ concentration by interpolation from the standard curve.

Settings for the Hitachi 917 Clinical Analyzer

Assay Code	2 point end
Assay Time	10 minutes
Assay Point	18 to 34
Sample Volume	6 µL
Reagent T1	185 µL R1 reagent (R1 position)
Reagent T3	60 µL R2 reagent (R2 position)
Wavelength	1° 570 nm, 2° 800 nm

Calibration Method	Spline 5 point
Assay Range	0 to 500 ng/mL
Patient Specimen Reportable Range	7 to 360 ng/mL

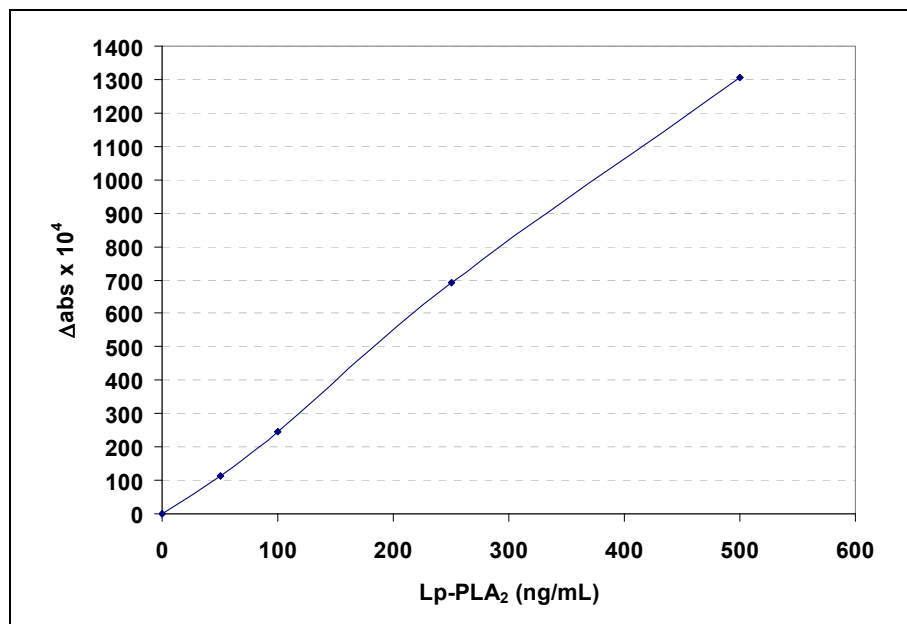
PROCEDURAL NOTES

- It is recommended that each lab determine a suitable calibration frequency. A new calibration curve should be generated whenever there is a reagent lot change or if Controls fall out of range.
- If samples have been frozen, thaw at 2 to 8 °C or on ice.
- If the Lp-PLA₂ concentration in a sample is above 360 ng/mL, do not report the patient result and do not dilute sample in order to report patient result.
- Do not switch caps on reagent solutions as this may lead to contamination.

EXAMPLE OF CALIBRATOR CURVE

Results of a typical standard calibration curve with absorbance readings at 570 nm on the Hitachi 917 are shown on the y-axis plotted against Lp-PLA₂ concentrations (ng/mL) shown on the x-axis. This calibration curve is for the purpose of illustration only. A standard calibration curve should be generated by the laboratory.

Lp-PLA ₂ (ng/mL)	Absorbance ($\Delta\text{abs} \times 10^4$)
0	1
50	113
100	246
250	692
500	1306



LIMITATIONS

Procedure

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
- As with any immunoassay system, particularly those employing mouse monoclonal antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) or other heterophilic interferences present in the sample that could cause falsely elevated or depressed values.
- As with any analytical method, the possibility exists that substances and/or factors not tested (e.g. technical or procedural) may interfere with the test and cause false results. Results should be considered in conjunction with other clinical and analytical methods.

Clinical Interpretation

- Lp-PLA₂ levels should be interpreted in conjunction with clinical findings and other diagnostic tests.
- This test does not replace blood cholesterol tests or other traditional risk factors identified for coronary heart disease or ischemic stroke.

EXPECTED VALUES

Samples from apparently healthy males (n=251) and apparently healthy females (n=174), in the clinically relevant age range of 40 to 70 years, were evaluated with the diaDexus PLAC Test. The reference population was represented by the following ethnic backgrounds: African-American n=26, Caucasian n=390, Hispanic n=8 and not specified n=1. The distributions of Lp-PLA₂ values across the entire population and divided by gender appear in the following table:

Percentile	Lp-PLA ₂ ng/mL		
	All (n=425)	Females (n=174)	Males (n=251)
5	126	120	131
20	174	169	179
33	201	188	205
50	235	228	244
67	262	252	268
80	289	285	293
95	369	342	376

These reference ranges are provided as guidelines only and are not intended to address “critical values” or medical decision limits. Each laboratory should establish its own reference intervals. Guidance for establishing reference intervals can be found in CLSI Standard C28-A2 (*How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline - Second Edition*). Based on the median population Lp-PLA₂ concentration of 235 ng/mL, it has been suggested to use that value as a clinical decision threshold [16]. More recently, an expert consensus panel has suggested applying a decision threshold of 200 ng/mL, based on a significant body of published literature for Lp-PLA₂ risk assessment [17].

PERFORMANCE CHARACTERISTICS

Performance characteristics were established using the Hitachi 917, a representative automated clinical chemistry analyzer. The assay range studied was 7 to 500 ng/mL. The current patient reportable range is 7 to 360 ng/mL. Refer to the specific clinical chemistry analyzer Application Sheet for performance characteristics, including reportable range.

Sensitivity

The clinical sensitivity of the assay is 7 ng/mL as determined by the limit of quantitation (the lowest concentration with acceptable precision).

The analytical sensitivity of the assay is 4 ng/mL, as calculated by interpolation of the mean plus two standard deviations of 20 replicates of the 0 ng/mL Lp-PLA₂ calibrator from the standard curve.

Assay Precision

Intra-assay and inter-assay variability were determined by testing one human serum sample and two buffer controls with Lp-PLA₂ concentrations distributed throughout the calibration range of the assay. The samples were assayed in duplicate using a single lot of reagents, twice per day, for 20 days. The data from the testing are summarized below and serve as representative performance for the assay:

Sample	Mean Concentration Lp-PLA ₂ (ng/mL)	Intra-assay % CV n=80	Inter-assay % CV n=20	Total % CV n=80
Serum	68.5	2.4	2.1	3.2
Buffer Control 1	143.9	2.0	1.6	2.5
Buffer Control 2	449.8	1.6	0.8	1.8

Linearity

From six pairs of serum samples with known high or low Lp-PLA₂ levels, a dilution series was prepared for each pair combination to assess linearity. Percent recoveries of the combined samples were determined as the measured value divided by the expected value, multiplied by 100. The average recovery was 97%, demonstrating linearity of the diluted samples over a range of 0 to 472 ng/mL Lp-PLA₂. The current patient reportable range is 7 to 360 ng/mL.

Interfering Substances

Endogenous substances found in blood and exogenous substances (common and prescription drugs) were evaluated for interference in the assay. Three individual serum samples with Lp-PLA₂ values ranging from 160 to 470 ng/mL were spiked with potential interferents. No appreciable interference was observed for the following substances at the spiked levels tested.

Endogenous		Exogenous (OTC Drugs, etc.)	
Potential Interferent	Test Concentration	Potential Interferent	Test Concentration
Bilirubin	20 mg/dL	Acetaminophen	1.66 µmol/L
Cholesterol [†]	500 mg/dL	Aspirin	3330 µmol/L
Hemoglobin	500 mg/dL	Atorvastatin	20 µmol/L
Triglycerides	3000 mg/dL	Clopidogrel bisulfate	100 µmol/L
Total Albumin*	~9000 mg/dL	Diphenhydramine	19.6 µmol/L
		Fenofibrate	125 µmol/L
		Lisinopril	0.74 µmol/L
		Metformin	310 µmol/L
		Niacin	4800 µmol/L
		Pravastatin	100 µmol/L
		Tolbutamide	2400 µmol/L
		Vitamin C	227 µmol/L
		Warfarin	64.9 µmol/L

[†] Human based lipids

* 5 g/dL albumin added to plasma pool of presumptively 4 g/dL albumin

No hook effect was observed with samples up to 1500 ng/mL.

Method Comparison

Correlation studies were performed comparing the PLAC Test turbidimetric immunoassay to the PLAC Test ELISA microplate method. Human serum samples with Lp-PLA₂ concentrations ranging from 0 to 499 ng/mL were tested. Results from linear regression analysis are shown below.

Linear Regression

Slope = 1.02

y-intercept = -24.5

Correlation coefficient r = 0.93

Number of samples = 794

The current patient reportable range is 7 to 360 ng/mL.

CLINICAL STUDIES

Coronary Heart Disease

To determine the efficacy of the diaDexus PLAC Test as a predictor of risk for coronary heart disease (CHD), Lp-PLA₂ levels were measured in 1348 banked EDTA-plasma samples from a large, multi-center epidemiology study, the Atherosclerosis Risk In Communities (ARIC) study, sponsored by the National Institutes of Health's National Heart, Lung, and Blood Institute. Participants were followed for the development of CHD for six to eight years. Samples used for the PLAC Test were from participants, age 47 to 69, who were free of CHD at the time of blood drawn. This was a case-cohort study where samples from all the CHD cases (607) were tested together with 741 appropriately matched participants without CHD at the time of censor (controls).*

Cox regression models were used to evaluate the association of Lp-PLA₂ and CHD in a univariate analysis (Model 1), a univariate analysis adjusted for demographics (Model 2), and a multivariate model adjusted for demographics and other prognostics factors (Model 3). Using high and low tertile cutpoints of Lp-PLA₂, generated from the ARIC data set (420 and 310 ng/mL, the 67th and 33rd percentiles, respectively), the hazard ratios of the Cox regression analyses demonstrated that Lp-PLA₂ was a significant predictor of risk for CHD, for the highest and intermediate levels when compared to the lowest level of Lp-PLA₂, for all participants (see Table 1). It should be noted that different cutpoints may be appropriate for different clinical populations.

* NOTE: 86 results (5.5%) were outside the assay acceptance criteria and were excluded from data analyses.

Table 1. Risk Ratios of CHD for Subjects Across All LDL Levels

Lp-PLA ₂	Lp-PLA ₂ Risk Ratio (95% CI, p value)*		
	Tertile 1	Tertile 2	Tertile 3
#CHD cases/total subjects in category	127/366 (34.7%)	192/444 (43.2%)	288/538 (53.5%)
Model 1	1.0	1.49 (1.11 to 1.99, p=0.008)	2.50 (1.89 to 3.31, p<0.001)
Model 2	1.0	1.24 (0.92 to 1.66, p=0.154)	1.76 (1.32 to 2.36, p<0.001)
Model 3	1.0	1.71 (1.06 to 2.75, p=0.029)	2.12 (1.29 to 3.48, p=0.003)

*The lowest tertile with Lp-PLA₂ values <310 ng/mL was used as the reference group.

Model 1: univariate analysis

Model 2: adjusted for age, race and gender

Model 3: Model 2, plus adjustment for current smoking status, blood pressure, diabetes, HDL, LDL, CRP and Lp-PLA₂ - LDL interaction

A statistical interaction was found between Lp-PLA₂ and LDL. Therefore, it was appropriate to evaluate Lp-PLA₂ risk ratios in the high and low LDL subgroups. The median value of LDL for the cohort population was 130 mg/dL. This defined the high and low LDL subgroups. Tables 2a and 2b represent the univariate analysis of the risk ratios in the high and low LDL subgroups. The risk ratios were calculated from Cox regression employing the weighted case-cohort method with Barlow adjustment, n=1348.

Table 2a. Risk Ratios of CHD for Subjects with LDL <130 mg/dL

	Lp-PLA ₂ Risk Ratio (95% CI)*		
Lp-PLA ₂ [†]	Tertile 1	Tertile 2	Tertile 3
Risk Ratio	1.0	2.17 (1.41 to 3.36)	3.52 (2.25 to 5.49)
#CHD cases/total subjects in category	51/215 (23.7%)	75/195 (38.5%)	77/163 (47.2%)

*The lowest tertile with Lp-PLA₂ values <310 ng/mL was used as the reference group.

[†]Lp-PLA₂ cutpoints based on the ARIC study population across all LDL levels.

Table 2b. Risk Ratios of CHD for Subjects with LDL ≥130 mg/dL

	Lp-PLA ₂ Risk Ratio (95% CI)*		
Lp-PLA ₂ [†]	Tertile 1	Tertile 2	Tertile 3
Risk Ratio	3.15 (2.08 to 4.77)	3.66 (2.43 to 5.51)	5.10 (3.43 to 7.57)
#CHD cases/total subjects in category	110/234 (47.0%)	126/247 (51.0%)	169/294 (57.5%)

*The lowest tertile for LDL <130 subgroup, with Lp-PLA₂ values <310 ng/mL, was used as the reference group.

[†]Lp-PLA₂ cutpoints based on the ARIC study population with LDL ≥130 mg/dL.

In the high LDL subgroup, the subgroup specific tertile groups yielded cutpoints of 350 and 460 ng/mL; the risk ratio increased with higher Lp-PLA₂ values. Therefore, for individuals with high LDL, a higher Lp-PLA₂ cutpoint should be considered. Further research is warranted to evaluate the Lp-PLA₂ - LDL interaction in the subgroup with high LDL. For the total population, Lp-PLA₂ was a significant predictor of risk for CHD for the high and intermediate groups versus the low Lp-PLA₂ (reference) group.

Ischemic Stroke

The levels of Lp-PLA₂ were evaluated in the ARIC study to determine its efficacy as a predictor of risk for stroke. A total of 223 stroke events were identified from the study group; of this, 194 (87%) were ischemic stroke associated with atherosclerosis, as classified by the ARIC investigators. A similar case-cohort study was designed, where samples from all the available ischemic stroke cases (194) were tested together with 762 appropriately matched participants without CHD or stroke at the time of censor (controls).

As with the study for CHD risk, Cox regression models were used to evaluate the association of Lp-PLA₂ and stroke in a univariate analysis (Model 1), a univariate analysis adjusted for demographics (Model 2), a multivariate model adjusted for demographics and other prognostic factors (Model 3), and all factors including CHD status (Model 4). The same tertile cutpoints (420 and 310 ng/mL, the 67th and 33rd percentiles, respectively) were applied to this study as for the earlier analyses. CHD status itself was found to be a predictor of risk, with a hazard ratio of 2.26 in a fully adjusted model. The hazard ratios of the Cox regression analyses demonstrated that Lp-PLA₂ was a significant and independent predictor of risk for ischemic stroke for the highest tertile, when compared to the lowest tertile of Lp-PLA₂, for all participants, with an increase of up to nearly 2-fold, even after adjustment for diabetes, lipids, blood pressure, smoking status, body mass index (BMI), other inflammatory markers and CHD status (see Table 3).

Table 3. Risk Ratios of Ischemic Stroke for All Subjects

Lp-PLA ₂	Lp-PLA ₂ Risk Ratio (95% CI, p-value)*		
	Tertile 1	Tertile 2	Tertile 3
# ischemic stroke cases/total subjects	47/283 (16.6%)	44/305 (14.4%)	103/368 (28.0%)
Model 1	1.0	0.85 (0.57 to 1.29, p=0.45)	1.79 (1.27 to 2.52, p=0.0010)
Model 2	1.0	0.89 (0.59 to 1.35, p=0.58)	2.09 (1.46 to 3.01, p=0.0001)
Model 3	1.0	0.89 (0.58 to 1.36, p=0.59)	1.81 (1.22 to 2.69, p=0.0034)
Model 4	1.0	0.86 (0.56 to 1.31, p=0.48)	1.75 (1.18 to 2.60, p=0.0057)

* The lowest tertile with Lp-PLA₂ values <310 ng/mL was used as the reference group.

Model 1: univariate analysis

Model 2: adjusted for age, race and gender

Model 3: Model 2, plus adjustment for diabetes, LDL, HDL, blood pressure, smoking, BMI and CRP

Model 4: Model 3, plus adjustment for CHD

Further analyses were performed to determine if Lp-PLA₂ was predictive of ischemic stroke across the complete range of systolic blood pressure (SBP) in the population, and to determine whether blood pressure and Lp-PLA₂ were additive in assessing risk for ischemic stroke. Systolic blood pressure tertile cutpoints were assigned by the 33rd and 67th percentiles of the population (113 and 130 mm Hg, respectively). The study population was divided into the low, mid and high range (1st, 2nd and 3rd tertile) of SBP and the low and high range of Lp-PLA₂ (below and above the median, 377 ng/mL in the ARIC study). The relative risk of each group was compared to the risk of events associated with the group in the 1st tertile of SBP and the group below the median of Lp-PLA₂ (Table 4).

Table 4. Risk Ratios of Ischemic Stroke: Additive Effects of Lp-PLA₂ and Systolic Blood Pressure

		Lp-PLA ₂	
		Below Median	Above Median
SBP (mm Hg)	# ischemic stroke cases/total subjects in category	68/478 (14.2%)	126/478 (26.4%)
<113	29/270 (10.7%)	1.00	2.29 (p=0.03)
113 to 130	60/337 (17.8%)	2.05 (p=0.06)	3.53 (p=0.0004)
>130	105/349 (30.1%)	3.52 (p=0.0005)	6.75 (p<0.0001)

The individuals above the median of Lp-PLA₂ concentration in the ARIC study and in the top tertile of systolic blood pressure (>130 mm Hg) had a risk ratio of 6.75 (p<0.0001), compared to those individuals below the median of Lp-PLA₂ and in the lowest tertile of blood pressure. These results indicated that Lp-PLA₂ and blood pressure were additive in their ability to predict risk, and that individuals in the highest groups of both variables were at the greatest risk of suffering an ischemic stroke associated with atherosclerosis.

PRODUCT SAFETY INFORMATION



Xi R36
S24/25-26-46

R36	Irritating to eyes
S24	Avoid contact with skin
S25	Avoid contact with eyes
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S46	If swallowed, seek medical advice immediately and show this container or label

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