PLAC®
Test for Lp PLA$_2$

REF 10-0148

PLAC® Test for Lp-PLA$_2$ Activity

Enzyme Assay for the Quantitative Determination of Lp-PLA$_2$ Activity in Human Plasma or Serum

Diazyme Laboratories, Inc.
12889 Gregg Court,
Poway, CA 92064 USA
Tel: 1-858-455-4768
www.diazyme.com

Symbol Key

REF	Catalog Number	R1	Buffer
IVD	In Vitro Diagnostic Medical Device	R2	Substrate
LOT	Batch	CAL	Calibrator
	Expiration Date	CONTROL LOW	Control Low
	Store at 2-8 deg C	CONTROL HIGH	Control High
	Irritant		Consult Instructions for Use
Rx only	CAUTION: Federal law restricts the device to sale or on the order of a physician		Manufacturer
			Read

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.

For specific automated clinical chemistry Analyzer Application Sheets, contact Diazyme Customer Service at 1-858-455-4768.

DIAZYME LABORATORIES

INTENDED USE

The **PLAC® Test for Lp-PLA$_2$ Activity** is an enzyme assay for the *in vitro* quantitative determination of Lp-PLA$_2$ (lipoprotein-associated phospholipase A$_2$) activity in EDTA plasma and serum on automated clinical chemistry analyzers. Lp-PLA$_2$ activity is to be used in conjunction with clinical evaluation and patient risk assessment as an aid in predicting risk of coronary heart disease (CHD) in patients with no prior history of cardiovascular events.

SUMMARY AND EXPLANATION

Lp-PLA$_2$ is a calcium-independent phospholipase A2 enzyme that is associated with both low-density lipoprotein (LDL) and, to a lesser extent, high-density lipoprotein (HDL) in human plasma and serum (Zalewski and MacPhee, 2005) and is distinct from other such phospholipases such as cPLA$_2$ and sPLA$_2$ (Kudo and Murakami 2002, Burke and Dennis 2009). Lp-PLA$_2$ is produced by macrophages and other inflammatory cells and is expressed in greater concentrations in advanced atherosclerotic lesions than early-stage lesions (Hakkinen, Luoma et al. 1999, Kolodgie, Burke et al. 2006). Several lines of evidence suggest that oxidation of LDL plays a critical step in the development and progression of atherosclerosis (Witztum 1994, Chisolm and Steinberg 2000). Lp-PLA$_2$ participates in the breakdown of oxidized LDL in the vascular wall by hydrolyzing the oxidized phospholipid, producing lysophosphatidylcholine and oxidized free fatty acids, both of which are potent pro-inflammatory products that contribute to the formation of atherosclerotic plaques (Macphee, Moores et al. 1999, Macphee 2001, Suckling and Macphee 2002). Lp-PLA$_2$ has demonstrated modest intra- and inter-individual variation, commensurate with other cardiovascular lipid markers and substantially less variability than high sensitivity C-reactive protein (hs-CRP). In addition, Lp-PLA$_2$ is not elevated in systemic inflammatory conditions, and may be a more specific marker of vascular inflammation. The relatively small biological variation of Lp-PLA$_2$ and its vascular specificity are of value in the detection and monitoring of cardiovascular risk (Wolfert, Kim et al. 2004, Lerman and McConnell 2008, Thompson, Gao et al. 2010).

PRINCIPLE OF THE TEST

The PLAC Test for Lp-PLA$_2$ Activity is an enzyme assay. Lp-PLA$_2$, in plasma or serum, hydrolyzes the sn-2 position of the substrate, 1-myristoyl-2-(4-nitrophenylsuccinyl) phosphatidylcholine, producing a colored reaction product, 4-nitrophenol. The rate of formation of 4-nitrophenol is measured spectrophotometrically and the Lp-PLA$_2$ activity is calculated from the rate of change in absorbance. A set of five Lp-PLA$_2$ calibrators is used to generate a standard curve fit of change in absorbance versus Lp-PLA$_2$ activity level in nmol/min/mL from which the sample Lp-PLA$_2$ activity is derived.

REAGENTS AND MATERIALS

The PLAC Test for Lp-PLA$_2$ Activity is supplied with:

- Lp-PLA$_2$ Substrate, 1-myristoyl-2-(4-nitrophenylsuccinyl) phosphatidylcholine
- Calibrators
- Control Low
- Control High
- Buffer
- Certificate of Analysis – Control Range

Other materials required but not provided:

- Automated Clinical Chemistry Analyzer and System Operation Manual.
- Analyzer Application Sheet specific to the clinical chemistry analyzer used is available separately. Contact Diazyme Customer Service.

WARNINGS AND PRECAUTIONS

Dilution of samples will create erroneous results. Samples cannot be diluted at any Lp-PLA$_2$ activity level.
Samples with Lp-PLA$_2$ values above the measuring range should be reported as >382 nmol/min/mL. In the Clinical Validation Study, > 99% of subjects reported values ≤382 nmol/min/mL.

Hemolyzed samples interfere with the assay and should not be tested. Samples that are visibly hemolyzed should be redrawn. Testing hemolyzed samples with >1.0 mg/mL hemoglobin may cause erroneous results.

Reversing the positions of the reagents on the analyzer will lead to erroneous results. Make sure to load the R1 reagent in the right location on the analyzer, R1 position, and the R2 reagent in the instrument's R2 position.

- For *In Vitro* Diagnostic Use.
- CAUTION: Federal law restricts the device to sale by or on the order of a physician.
- Treat all blood samples, calibrators and controls as potentially biohazardous material.
- Visually observe samples for excessive turbidity and clots prior to analysis. Samples with excessive turbidity and clots may impact results and should not be used.
- Dispose of reagents in a manner consistent with relevant regulations.
- Do not use reagents, calibrators or controls past their expiration dates.
- Do not mix reagents, calibrators or controls from different kit lots.

REAGENT PREPARATION AND STORAGE

Reagents are provided ready to use. Remove the caps of reagent R1 and R2 and place on the instrument. Reagents are stable for up to 4 weeks on board the analyzer.

Once opened:

- Reagents can be stored at 2-8°C for up to 4 weeks
- Calibrators can be stored at 2-8°C for up to 3 months
- Controls can be stored at 2-8°C for up to 3 months

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 whole blood by venipuncture in:
  - K_2 EDTA plasma collection tubes with or without gel
  - K_3 EDTA plasma collection tubes without gel
  - Serum collection tubes, with or without gel.
- Process blood using standard separation procedures.
  - Whole blood can be kept up to 4 hours at 20-22°C or up to 30 hours at 2-8°C prior to separation.
- Following centrifugation:
  - Examine for hemolysis. If present, discard sample and re-draw.
    - Sample can be tested immediately or stored prior to testing under the following conditions:
      - 24 hours at 20-26°C
      - Up to 2 weeks at 2-8°C
      - Up to 18 months at -20°C
      - Up to 2 years at -70°C
  - Plasma and serum samples can be freeze-thawed up to 5 times after freezing at either -70°C or -20°C.
  - When transporting samples, ship samples on cold packs at 2-8°C.
- Handle and dispose of all samples using Universal Biohazardous Precautions.

ASSAY PROCEDURE

Calibration

The assay is calibrated using a 5-point calibration curve. A standard curve is generated using the appropriate curve fit model indicated in the Analyzer Application Sheet. Verify the calibration with at least two levels of controls according to the laboratory's requirements. Recalibrate and run controls for each kit from a new lot and thereafter, every 4 weeks for kits from the same lot. If controls fall outside of laboratory's acceptable range, recalibrate as necessary **up to the expiration date of the opened reagents**.

Quality Control

Test at least two levels of an appropriate quality control material 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 Procedure

The PLAC Test for Lp-PLA$_2$ Activity should be run using the appropriate settings for the analyzer to be used. For detailed instructions and settings for each analyzer, please refer to the Analyzer Application Sheet for the specific automated clinical chemistry analyzer used. A general explanation of the assay procedure for the Beckman Coulter (Olympus) AU400® Analyzer is described.

Settings for the Beckman Coulter (Olympus) AU400 Clinical Chemistry Analyzer

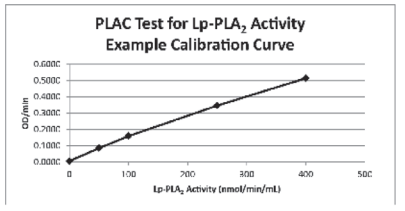
Assay Code	Rate
Assay Time	8.5 minutes
Read Cycle	12 to 14
Sample Volume	25 µL
Reagent R1 vol.	100 µL R1 reagent (R1 position)
Reagent R2 vol.	25 µL R2 reagent (R2 position)
Wavelength	1° 410 nm, 2° 520 nm
Calibration Method	Spline 5 point
Assay Range	10 to 382 nmol/min/mL

Procedural Notes

- It is recommended that each lab determine a suitable calibration frequency. At a minimum, a new calibration curve should be generated with a kit from a new lot, and thereafter, every 4 weeks for kits from the same lot. Run calibration when and if controls fall outside acceptable range.
- Upon storage, do not switch caps on reagent solutions as this may lead to contamination.
- All samples should be well mixed before testing, and especially after thawing stored samples. A vortex mixer may be used; however, any air bubbles or foaming of the samples should be avoided.

Example of Calibration Curve

Lp-PLA$_2$ Activity nmol/min/mL	Absorbance OD/min
0	0.0067
50	0.0857
100	0.1601
250	0.3469
400	0.5142



LIMITATIONS

- Reliable, accurate and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
- 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.

Interpretation of Results

The PLAC Test for Lp-PLA$_2$ Activity provides information on the risk of coronary heart disease (CHD) events. In the Clinical Validation Study (see below), the overall population rate of CHD events at 5-year follow-up was 4.1% (95% CI 3.8 - 4.38%), prior to consideration of subject PLAC Activity values. Using a PLAC Activity cut-point value of 225 nmol/min/mL, subjects were then classified into a high PLAC Activity group (Lp-PLA$_2$ activity ≥ 225 nmol/min/mL) or a low PLAC Activity group (Lp-PLA$_2$ activity < 225 nmol/min/mL). The following absolute rates of CHD events at 5-year follow-up were observed (including 95% CI):

- High PLAC Activity group CHD event rate 7.0% [6.2% - 7.8%]
- Low PLAC Activity group CHD event rate 3.3% [3.0% - 3.6%]

The high PLAC Activity group was found to have a 5-year absolute rate for CHD events that was over twice that of the low PLAC Activity group. The difference in absolute rates between the two PLAC Activity groups is both statistically significant (p <0.001) and clinically important given the continuing lifetime risk of CHD events. The finding that an individual with no prior history of cardiovascular events has high PLAC Activity would suggest that they are at higher than average risk. Similarly, the finding that such an individual has low PLAC Activity would suggest that they are at lower than average risk.

Additional analyses showed that high PLAC Activity was a significant predictor of CHD events relative to low PLAC Activity, even after adjustment for other demographic and cardiovascular risk factors including age, race, gender, smoking, hypertension, diabetes, LDL-cholesterol and HDL-cholesterol.

Use of PLAC Activity results in conjunction with the other clinical parameters (e.g., age, life expectancy, etc.) and traditional risk factors (e.g., LDL-cholesterol, total cholesterol, blood pressure, etc.) may be used to refine risk assessment in primary prevention.

Cut-Point

Analyte	Units	Reduced Risk	Increased Risk
Lp-PLA$_2$ Activity	nmol/min/mL	< 225	≥ 225

PERFORMANCE CHARACTERISTICS

Performance characteristics were established using one Beckman Coulter (Olympus) AU400 Analyzer. Refer to the specific clinical chemistry Analyzer Application Sheet for performance characteristics.

Analytical Sensitivity

The analytical sensitivity (limit of quantitation) of the assay is 10 nmol/min/mL.

Assay Precision

Within-run and within-lab variability were determined by testing four human plasma samples and two controls with Lp-PLA$_2$ activities ranging from 113 to 315 nmol/min/mL. Samples were assayed in duplicate, twice a day, over 20 days and with 3 kit lots using one instrument. Total precision CV's for each reagent lot and sample were <3%. The assay results for within-run repeatability and total lab variability which includes within-run, between-run, between-day and between-lot variance components are summarized below for each sample tested:

Sample	Mean Value (nmol/min/mL) n=240	Repeatability (within-run)		Within-Lab Precision	
		SD	%CV	SD	%CV
Kit Control Low	120.0	1.81	1.5%	3.40	2.8%
Kit Control High	302.8	4.31	1.4%	8.24	2.7%
Plasma 1	113.0	1.41	1.2%	4.24	3.8%
Plasma 2	208.1	2.97	1.4%	6.94	3.3%
Plasma 3	244.4	3.67	1.5%	8.61	3.5%
Plasma 4	314.6	3.86	1.2%	9.79	3.1%

Linearity

Several dilution series were prepared from plasma samples with known high and low Lp-PLA$_2$ activity levels and were tested with 3 kit lots. In the dynamic range of 6 to 382 nmol/min/mL, linear regression of Lp-PLA$_2$ activity levels resulted in slopes ranging from 0.98 to 1.04, with intercepts ranging from -0.40 to -0.03 nmol/min/mL and R2 values ranging from 0.995 to 0.999. Linearity was demonstrated from 10 to 382 nmol/min/mL with a deviation from linearity of ≤ 10%. The measuring range of the assay is determined to be 10 to 382 nmol/min/mL.

Interfering Substances

Endogenous substances were titrated into samples (above and below the Lp-PLA$_2$ cut-point) with known levels of each endogenous substance and were tested. No appreciable interference was observed for the following substances:

Interference Testing: Endogenous Substances

Potential Interferent	High Concentration
Albumin, g/L	60
Unconjugated Bilirubin, mg/dL	20
Conjugated Bilirubin, mg/dL	12
Cholesterol, mg/dL	300
Triglycerides, mg/dL	400
Hemoglobin, mg/mL	1

Exogenous substances (common and prescription drugs) were evaluated for interference in the assay. Samples were spiked with two levels of the potential interferent and tested. No appreciable interference was observed for the following substances at the spiked levels tested.

Interference Testing: Exogenous Substances

Potential Interferent	Test Concentration Low	Test Concentration High
Acetaminophen, µmol/L	33	1324
Aspirin, µmol/L	720	3600
Atorvastatin, µmol/L	2	20
Diphenhydramine, µmol/L	2	20
Fenofibrate, µmol/L	42	125
Lisinopril, µmol/L	0.25	0.74
Niacin, µmol/L	480	4800
Tolbutamide, µmol/L	400	2300
Warfarin, µmol/L	10	33
Metformin, µmol/L	31	310
Clopidogrel bisulfate, µmol/L	10	100
Vitamin C, µmol/L	14	342

Recovery

Various amounts of a high Lp-PLA_2 activity level solution were added to an enzyme-free diluent to create seven activity levels. These spiked solutions were assayed with 3 lots of reagents and the Lp-PLA_2 activity levels were then compared to expected values resulting in slopes ranging from 0.99 to 1.10, with intercepts ranging from -2.9 to 4.2 nmol/min/mL and R2 ranging from 0.997 to 1.000.

Clinical Studies

The clinical effectiveness of the PLAC Test for Lp-PLA_2 Activity in its intended use population was validated in a Clinical Validation Study utilizing participants in the REasons for Geographic And Racial Differences in Stroke (REGARDS) study. Begun in 2003, REGARDS is an active observational population study which continues to follow its participants longitudinally for the development of cardiovascular events (CHD and Stroke). Designed to elucidate racial and geographical differences in cardiovascular disease within the general US population, the study targeted a balanced enrollment of gender and race, and enrolled Black and White participants only. The REGARDS study design, inclusion and exclusion criteria, and coronary heart disease outcomes have been previously published (Howard, Cushman et al. 2005, Safford, Brown et al. 2012). With its nationwide scope and 30,183 total enrolled participants, REGARDS is one of the largest single such study ever undertaken in the United States by the National Institutes of Health.

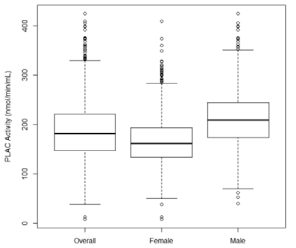
The primary endpoint used in the Clinical Validation Study for the PLAC Test for Lp-PLA_2 Activity was a composite of total CHD events, comprising 1) acute myocardial infarction, 2) coronary revascularization, and 3) CHD-related death.

The Clinical Validation Study used a case-cohort study design (Prentice 1986). Consistent with the intended use, subjects were randomly sampled from all REGARDS participants with no prior history of cardiovascular events at baseline enrollment (23,019 participants). This sampled population was then enriched with all remaining REGARDS CHD cases that occurred after the same baseline exclusion.

The resulting Clinical Validation Study is a case-cohort of 4,598 subjects, including a total of 933 cases and 3,665 controls. In the sampled population, subject ages ranged from 45 to 92 years (median 63 years), with gender distributed 41.7% male and 58.3% female, and race distributed 41.5% black and 58.5% white. Statistical tests confirmed that the case-cohort was representative of the full REGARDS population for the intended use. Efficacy analyses, Kaplan-Meier and Cox proportional hazards models were all weighted to adjust the Clinical Validation Study to the underlying prevalence of cases in the parent study population (Barlow 1994).

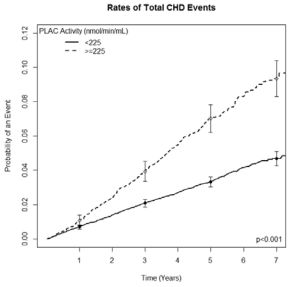
Frozen EDTA plasma specimens were tested with the PLAC Test for Lp-PLA_2 Activity following the recommended sample handling protocol and the observed values were analyzed. The median population follow-up was 5.3 years from the draw date of the specimens tested.

PLAC Activity Distribution: PLAC Test for Lp-PLA_2 Activity values in the sampled population ranged from a minimum of 8 to a maximum of >382 nmol/min/mL, with a median value of 178 nmol/min/mL (interquartile range (IQR) 145 – 216 nmol/min/mL).



Analysis Cut-point: For the Clinical Validation Study efficacy analyses, a PLAC Test for Lp-PLA_2 Activity cut-point of 225 nmol/min/mL was pre-specified based on prior studies and publications using PLAC activity in other independent cohorts. This analysis cut-point was used as a binary classifier in the study, dividing the study population into the low (below the cut-point) and high (at or above the cut-point) PLAC Activity groups.

Kaplan-Meier Analyses: In the Kaplan-Meier analysis of the Clinical Validation Study, absolute risk of CHD events was higher in the high PLAC Activity group (log-rank p-value < 0.001), as seen in the figure below. The absolute risk of CHD events of the two groups separated early and was consistently different after one year of follow-up and at all subsequent timepoints pre-specified for the analysis. The same analysis was also statistically significant within each gender and race analyzed independently (log-rank p-value < 0.001 for each analysis).



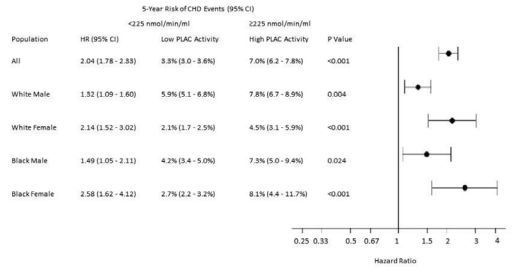
Absolute Rates of CHD by PLAC Activity Group: The absolute risk of CHD events at 5-year follow-up is presented for each PLAC Activity group of the Clinical Validation Study in the table below. The study indicated that the absolute risk of CHD events in the high PLAC Activity group is 2.1 times the absolute risk of CHD events in the low PLAC Activity group.

5-Year Risk of CHD Events (Kaplan-Meier Absolute Risk/Rate Analysis)

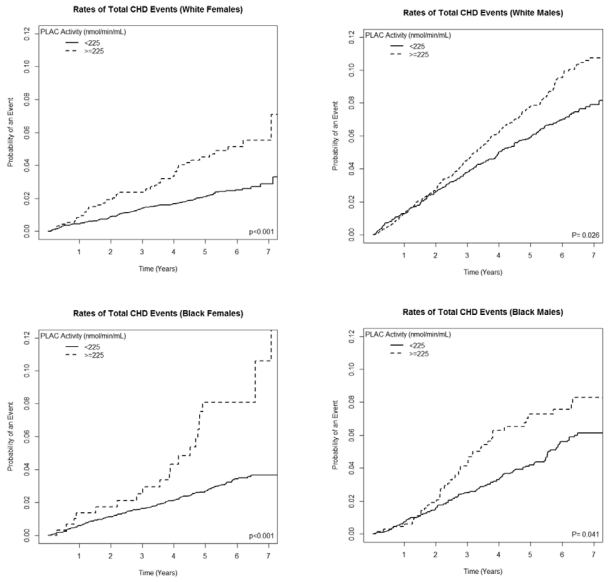
Population	Absolute Risk Pre-Test	Absolute Risk Post-Test Low Group (<225 nmol/min/mL)	Absolute Risk Post-Test High Group (≥225 nmol/min/mL)	Relative Risk (High/Low)
All	4.1%	3.3%	7.0%	2.1
Females	2.7%	2.4%	5.3%	2.2
Males	6.1%	5.2%	7.7%	1.5
White	4.4%	3.5%	6.9%	2.0
Black	3.6%	3.2%	7.6%	2.4

Race by Gender Subgroup Analyses: The predictive power of the PLAC Test for Lp-PLA_2 Activity was also assessed for each race by gender subgroup of the Clinical Validation Study. The figure below shows the predictive power of PLAC Activity within each race by gender subgroup using univariate Cox proportional hazards model, [high versus low PLAC activity group] with 5-year Kaplan-Meier rate estimates provided for each PLAC Activity group. The PLAC Test for Lp-PLA_2 Activity was shown to be a statistically significant predictor of total CHD events in all subgroups, with a consistently higher hazard ratio (HR) estimate observed for women compared to that for men, regardless of race.

5-Year Risk of CHD Events and Cox Proportional Hazards Models by Race by Gender Subgroup



Kaplan-Meier Analyses in Each Race by Gender Subgroup



Risk Adjusted Cox Proportional Hazards Models: Cox proportional hazards models showed the PLAC Test for Lp-PLA2 Activity to be a statistically significant predictor of total CHD events when comparing the high versus the low PLAC Activity groups of the Clinical Validation Study, with a fully-adjusted hazard ratio of 1.54 (1.31 – 1.82, p < 0.001) after adjustment for age, gender, race, diabetes, hypertension, smoking, LDL and HDL.

Secondary Component Endpoint Analysis: The predictive power of the PLAC Test for Lp-PLA_2 Activity was also assessed using Cox proportional hazards models for each of the individual component endpoints of the Clinical Validation Study. The PLAC Test for Lp-PLA_2 Activity was shown to be a consistent and statistically significant predictor for each of acute myocardial infarction, coronary revascularization and CHD-related death (p < 0.001 for each analysis).

Expected Values

EDTA-plasma samples were obtained from an all-comers population of 300 subjects of a clinically relevant age of 35 to 75 years old (median age 57) from two different geographical locations. Information regarding age, gender and race was collected from each subject. No health information was obtained. The testing population included 154 males and 146 females with a racial distribution of 38% White, 32% Black, 21% Hispanic and 9% Asian. Samples were tested with the PLAC Test for Lp-PLA_2 Activity following the recommended sample handling protocol and the expected values were computed.

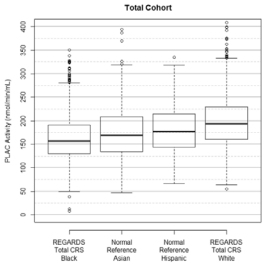
The distribution of baseline PLAC activity values for this population are shown in the following table:

Percentile	All (N=300) Lp-PLA_2 (nmol/min/mL)	Female (N=146) Lp-PLA_2 (nmol/min/mL)	Male (N=154) Lp-PLA_2 (nmol/min/mL)
Min	50	50	70
2.5	84	74	92
5	94	86	102
25	137	130	149
33	148	139	155
50	**167**	**154**	**176**
67	196	179	204
75	211	200	219
95	276	264	295
97.5	303	300	329
99	369	339	>382
Max	>382	370	>382
Mean	**176**	**166**	**186**

A low-frequency homozygous null mutation (V279F) has been reported in individuals with two Asian parents that results in a low level of circulating Lp-PLA_2 (Jang, Waterworth et al. 2011). Such individuals generally present with Lp-PLA_2 activity values that are either unmeasurable or below the minimum population values shown in the previous table.

These reference ranges are provided as guidelines only and are not intended to address "critical values" or medical decision limits. The results indicate that each clinical laboratory may observe a median and range of Lp-PLA_2 activity values that depend on the patient population tested (e.g. patients that are generally at greater risk for cardiovascular disease versus those at a generally lower risk). Each laboratory should establish its own reference intervals.

The distribution of PLAC Activity values observed in the Clinical Validation Study, which enrolled Blacks and Whites, encompasses those seen in other races (tested in a separate study) as shown in the figure below for Asians (n = 285) and Hispanics (n = 199).



PRODUCT SAFETY INFORMATION

Calibrator Set (1-5), Control Low and High R36 S25/26/36/37/39	R1 Buffer R36 S25/26/36/37/39

R36	Irritating to eyes
S25	Avoid contact with eyes
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S36/37/39	Wear suitable protective clothing, gloves, eye/face protection

REFERENCES

Barlow, W. E. (1994). "Robust variance estimation for the case-cohort design." Biometrics **50**(4): 1064-1072.

Burke, J.E. and Dennis, E.A. (2009). "Phospholipase A2 structure/function, mechanism, and signaling." J. Lipid Res **50**: s237-42.

Chisolm, G. M. and D. Steinberg (2000). "The oxidative modification hypothesis of atherogenesis: an overview." Free Radic Biol Med **28**(12): 1815-1826.

CLSI (2008). "Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory: Approved Guideline--Third Edition." **CLSI Document EP28-A3C**.

Hakkinen, T., J. S. Luoma, M. O. Hiltunen, C. H. Macphee, K. J. Milliner, L. Patel, S. Q. Rice, D. G. Tew, K. Karkola and S. Yla-Herttuala (1999). "Lipoprotein-associated phospholipase A(2), platelet-activating factor acetylhydrolase, is expressed by macrophages in human and rabbit atherosclerotic lesions." Arterioscler Thromb Vasc Biol **19**(12): 2909-2917.

Howard, V. J., M. Cushman, L. Pulley, C. R. Gomez, R. C. Go, R. J. Prineas, A. Graham, C. S. Moy and G. Howard (2005). "The reasons for geographic and racial differences in stroke study: objectives and design." Neuroepidemiology **25**(3): 135-143.

Jang, Y., D. Waterworth, J. E. Lee, K. Song, S. Kim, H. S. Kim, K. W. Park, H. J. Cho, I. Y. Oh, J. E. Park, B. S. Lee, H. J. Ku, D. J. Shin, J. H. Lee, S. H. Jee, B. G. Han, H. Y. Jang, E. Y. Cho, P. Vallance, J. Whittaker, L. Cardon and V. Mooser (2011). "Carriage of the V279F null allele within the gene encoding Lp-PLA(2) is protective from coronary artery disease in South Korean males." PLoS One **6**(4): e18208.

Kolodgie, F. D., A. P. Burke, K. S. Skorija, E. Ladich, R. Kutys, A. T. Makuria and R. Virmani (2006). "Lipoprotein-associated phospholipase A2 protein expression in the natural progression of human coronary atherosclerosis." Arterioscler Thromb Vasc Biol **26**(11): 2523-2529.

Kudo, I. and M. Murakami (2002). "Phospholipase A2 enzymes." Prostaglandins Other Lipid Mediat **68-69:** 3-58.

Lerman, A. and J. P. McConnell (2008). "Lipoprotein-associated phospholipase A2: a risk marker or a risk factor?" Am J Cardiol **101**(12A): 11F-22F.

Macphee, C. H. (2001). "Lipoprotein-associated phospholipase A2: a potential new risk factor for coronary artery disease and a therapeutic target." Curr Opin Pharmacol **1**(2): 121-125.

Macphee, C. H., K. E. Moores, H. F. Boyd, D. Dhanak, R. J. Ife, C. A. Leach, D. S. Leake, K. J. Milliner, R. A. Patterson, K. E. Suckling, D. G. Tew and D. M. Hickey (1999). "Lipoprotein-associated phospholipase A2, platelet-activating factor acetylhydrolase, generates two bioactive products during the oxidation of low-density lipoprotein: use of a novel inhibitor." Biochem J **338 (Pt 2)**: 479-487.

Prentice, R. L. (1986). "A Case-Cohort Design for Epidemiologic Cohort Studies and Disease Prevention Trials." Biometrika **73**(1): 1-11.

Safford, M. M., T. M. Brown, P. M. Muntner, R. W. Durant, S. Glasser, J. H. Halanych, J. M. Shikany, R.J. Prineas, T. Samdarshi, V. A. Bittner, C. E. Lewis, C. Gamboa, M. Cushman, V. Howard and G. Howard (2012). "Association of race and sex with risk of incident acute coronary heart disease events." JAMA **308**(17): 1768-1774.

Suckling, K. E. and C. H. Macphee (2002). "Lipoprotein-associated phospholipase A2: a target directed at the atherosclerotic plaque." Expert Opin Ther Targets **6**(3): 309-314.

Thompson, A., P. Gao, L. Orfei, S. Watson, E. Di Angelantonio, S. Kaptoge, C. Ballantyne, C. P. Cannon, M. Criqui, M. Cushman, A. Hofman, C. Packard, S. G. Thompson, R. Collins and J. Danesh (2010). "Lipoprotein-associated phospholipase A(2) and risk of coronary disease, stroke, and mortality: collaborative analysis of 32 prospective studies." Lancet **375**(9725): 1536-1544.

Witztum, J. L. (1994). "The oxidation hypothesis of atherosclerosis." Lancet **344**(8925): 793-795.

Wolfert, R. L., N. W. Kim, R. G. Selby, M. J. Sarno, G. R. Warnick and K. Sudhir (2004). "Biological variability and specificity of lipoprotein-associated phospholipase A2 (Lp-PLA_2), a novel marker of cardiovascular risk (abstract)." Circulation **110**(Supplement 3): 309.

Zalewski A, Macphee C. (2005) "Role of lipoprotein-associated phospholipase A2 in atherosclerosis." Arterioscler Thromb Vasc Biol **25**:923-931.

P/N 30050F-01
Rev. A