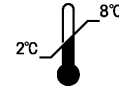








**Human Lp-PLA<sub>2</sub> ELISA Kit**  
**Enzyme Immunoassay to Quantitatively Measure**  
**Lp-PLA<sub>2</sub> in Human Plasma and Serum**

**REF**  
**90023**

**For Research Use Only**  
**Not for use in diagnostic procedures**



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Symbol Key			
<b>REF</b>	Catalog Number	<b>STOP</b>	Stop Solution
<b>PLATE</b>	Antibody Coated Plate	<b>LOT</b>	Batch
<b>CAL</b>	Calibrator		Expiration Date
<b>CONTROL LOW</b>	Control Low		Store at 2 to 8 °C
<b>CONTROL HIGH</b>	Control High		Irritant
<b>WASH</b>	Wash Buffer		Consult Instructions for Use
<b>CONJ</b>	Conjugate		Manufacturer
<b>TMB</b>	TMB Substrate		

*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.*

## INTRODUCTION

Lp-PLA<sub>2</sub> 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 cPLA<sub>2</sub> and sPLA<sub>2</sub> [2]. Lp-PLA<sub>2</sub> is produced by macrophages and other inflammatory cells.

## PRINCIPLE OF THE TEST

The diaDexus Human Lp-PLA<sub>2</sub> ELISA Kit is a sandwich enzyme immunoassay that uses two highly specific monoclonal antibodies. The assay system utilizes monoclonal anti-Lp-PLA<sub>2</sub> antibody (2C10) directed against Lp-PLA<sub>2</sub> for solid phase immobilization on the microwell plate. Sample is added to the plate and incubated for 10 minutes at 20 to 26 °C. A second monoclonal anti-Lp-PLA<sub>2</sub> antibody (4B4) labeled with the enzyme horseradish peroxidase (HRP) is then added and reacted with the immobilized antigen at 20 to 26 °C for 180 minutes, resulting in the Lp-PLA<sub>2</sub> molecules being captured between the solid phase and the enzyme-labeled antibodies. The wells are washed with a supplied buffer to remove any unbound antigen. The substrate, tetramethylbenzidine (TMB), is then added and incubated at 20 to 26 °C for 20 minutes, resulting in the development of a blue color. Color development is stopped with the addition of Stop Solution, changing the color to yellow. The absorbance of the enzymatic turnover of the substrate is determined spectrophotometrically at 450 nm and is directly proportional to the concentration of Lp-PLA<sub>2</sub> present. A set of Lp-PLA<sub>2</sub> Calibrators is used to plot a standard curve of absorbance versus Lp-PLA<sub>2</sub> concentration from which the Lp-PLA<sub>2</sub> concentration in the test sample can be determined. Two levels of Controls are provided.

## REAGENTS AND MATERIALS

Materials supplied with the kit: (Sufficient for 96 wells)

PART NUMBER	SYMBOL	COMPONENT DESCRIPTION	QUANTITY
60001	<b>PLATE</b>	<b>Antibody Coated Plate</b> Mouse monoclonal anti-Lp-PLA <sub>2</sub> (2C10) antibody coated microwell plate	1
60006	<b>CAL</b>	<b>Calibrators</b> (0, 50, 100, 250, 500 and 1000 ng/mL)	1 set, 0.25 mL each
60007			
60008			
60009			
60010			
60011			
60002	<b>WASH</b>	<b>20X Wash Buffer</b> Non-ionic detergent in a buffered solution	50 mL
60003	<b>CONJ</b>	<b>Conjugate</b> Mouse monoclonal anti-Lp-PLA <sub>2</sub> (4B4) antibody conjugated to horseradish peroxidase in a buffered reagent with bovine and murine carrier proteins	23 mL
60004	<b>TMB</b>	<b>TMB Reagent</b> 3,3',5,5'-tetramethylbenzidine in a mildly acidic buffer	11 mL
60005	<b>STOP</b>	<b>Stop Solution</b> 1N HCl	11 mL
65009	<b>CONTROL LOW</b>	<b>Control Low</b> (~150 ng/mL) diaDexus recombinant Lp-PLA <sub>2</sub> antigen (DDX-RA) in a liquid, protein (BSA) buffered matrix	1 bottle, 0.5 mL
65010	<b>CONTROL HIGH</b>	<b>Control High</b> (~350 ng/mL) diaDexus recombinant Lp-PLA <sub>2</sub> antigen (DDX-RA) in a liquid, protein (BSA) buffered matrix	1 bottle, 0.5 mL
FMD-01-027		<b>Certificate of Analysis – Control Range</b> The control ranges for the lot are indicated on the Certificate of Analysis	1 each

### Materials required but not provided:

- Precision single and multi-channel pipettors: 0.02, 0.10, 0.20 mL
- Disposable pipette tips (a new pipette tip must be used for each addition of different samples or reagents during the assay procedure)
- Vortex mixer or equivalent
- Deionized water
- A microwell plate reader with a bandwidth of 10 nm or less and an optical density (O.D.) range of 3 or greater at 450 nm
- Computer software capable of point-to-point curve fit for calculating concentration of analyte from optical density (optional)

### WARNINGS AND PRECAUTIONS

- For Research Use Only. Not for use in diagnostic procedures.
- 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 time and laboratory sample analysis). This does not include incubation on the ELISA plate.
- Storage of samples at -20 °C for longer than 24 hours is not recommended.
- Certain components are labeled with safety precautions. See the Product Safety Information section.
- 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.
- If there is evidence of contamination, do not use.
- Hemolysis may affect results. Do not test hemolyzed samples.
- It is recommended that both Low and High Controls be included in each run. If control values are not within acceptance limits, repeat the assay.
- To avoid erroneous results, store the material as indicated.
- The control ranges provided were derived from replicate testing of the specific control lots using Human Lp-PLA<sub>2</sub> ELISA Kits. Values listed are specific for each lot and should be used as guides. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences or reagent variability. It is recommended that each laboratory establish its own acceptable ranges.

### REAGENT PREPARATION AND STORAGE

Store unopened test kits at 2 to 8 °C upon receipt. In addition, keep the microwell plate sealed in the foil pouch with desiccant to minimize exposure to moisture. Opened test kits will remain stable until the expiration date shown, provided they are stored as described above.

Prepare 1X Wash Buffer by diluting 20X Wash Buffer 1:20 with deionized water. Mix 1 part of Wash Buffer to 19 parts of deionized water. Store at room temperature (20 to 26 °C). Use 1X Wash Buffer within four weeks of preparation. If microbial growth is seen, discard.

### 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:
  - Samples must be stored refrigerated at 2 to 8 °C for a minimum of 24 hours after the sample is drawn before testing can occur.
  - Samples can be tested up to 7 days after the sample is drawn when stored at 2 to 8 °C.
  - For longer term storage, serum/plasma samples must be stored 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 and thawed up to six times without affecting the Lp-PLA<sub>2</sub> quantitation.

## ASSAY PROCEDURE

### Calibration

Each plate or strip run must be calibrated using a full (6-point) calibration curve. A standard curve is generated using a point-to-point curve fit model. Verify the calibration curve with at least two levels of Controls according to the laboratory's requirement. Calibrate and run Controls for each plate run.

### Quality Control

Test the High and Low Controls with each run. If control values are not within acceptance limits, repeat the assay.

### Preparatory Steps

1. Bring the microwell plate, Conjugate, Wash Buffer and TMB to room temperature (20 to 26 °C) before use.
2. Remove the plate frame and the required number of coated microwell strips from the foil pouch. Completely reseal the foil pouch containing any unused strips with the desiccant that came in the pouch and store at 2 to 8 °C.
3. Prepare 1X Wash Buffer by diluting 20X Wash Buffer 1:20 with deionized water (1 part Wash Buffer and 19 parts of deionized water). Store at room temperature (20 to 26 °C). Use 1X Wash Buffer within four weeks of preparation.
4. Allow samples to thaw at 2 to 8 °C, if needed, and place on ice or at 2 to 8 °C as soon as thawed.
5. Store the Controls at 2 to 8 °C or on ice until used.
6. Vortex the samples and Controls to mix thoroughly. Avoid foaming.

### Sample Incubation

1. Using a pipettor and tip with appropriate low volume precision, dispense 20 µL of Calibrators, samples and Controls into the appropriate wells after vortexing. Use a calibrated pipette and new pipette tip for each Calibrator, Control or sample.
2. Allow the samples to incubate on the plate for 10 ± 2 minutes before adding the Conjugate.
3. Pipette 200 µL of room temperature Conjugate into the appropriate wells of the coated microwell plate. Avoid contamination by adding the Conjugate without touching the samples with the pipette tips. If there is cross over, change tips and continue adding Conjugate to the wells.
4. Gently swirl the plate on a flat surface for 10 to 15 seconds to ensure mixing.
5. Incubate for 3 hours at room temperature.
6. At the end of the incubation period, wash the microwells four (4) times with at least 300 µL of the supplied room temperature 1X Wash Buffer. (DO NOT USE TAP or DISTILLED WATER.)
7. Blot the plate on absorbent paper after the final wash. Immediately (in less than 2 minutes) proceed to the next step. Do not allow the microwell plate to dry.

### Substrate Incubation

1. Pipette 100 µL of room temperature TMB Reagent into each well.
2. Gently swirl the plate on a flat surface for 10 to 15 seconds to ensure mixing.
3. Incubate the plate at room temperature for 20 minutes in the dark.
4. Stop the reaction by adding 100 µL of room temperature Stop Solution to each well.
5. Gently swirl the plate on a flat surface for 20 to 30 seconds to ensure mixing. It is important to make sure that the blue color completely changes to yellow color.
6. Wipe moisture from the bottom of the plate using a paper towel.
7. Within 15 minutes of adding the Stop Solution, read the optical density (O.D.) at 450 nm using a microwell plate reader.

## PROCEDURAL NOTES

- Store all test reagents at 2 to 8 °C. Except for the Calibrators and Controls, allow the reagents to equilibrate to room temperature prior to use. A 23 mL bottle of reagent may require an hour or more to reach room temperature. Keep Calibrators and Controls at 2 to 8 °C or on ice until used.
- Bring the microwell plate to room temperature before opening the bag. Store the plate in the foil pouch with desiccant to minimize exposure to moisture. Always keep the unused microwell plate in the foil pouch with desiccant.
- Always have the next step reagent ready 2 to 3 minutes before the washing step.
- For accurate measurement of samples, the addition of samples, Calibrators and Controls must be precise. Pipette carefully using only calibrated equipment.
- This assay may be performed using any validated washing method.
- Do not use plate sealers during incubations.
- Do not use a plate shaker for incubation steps.

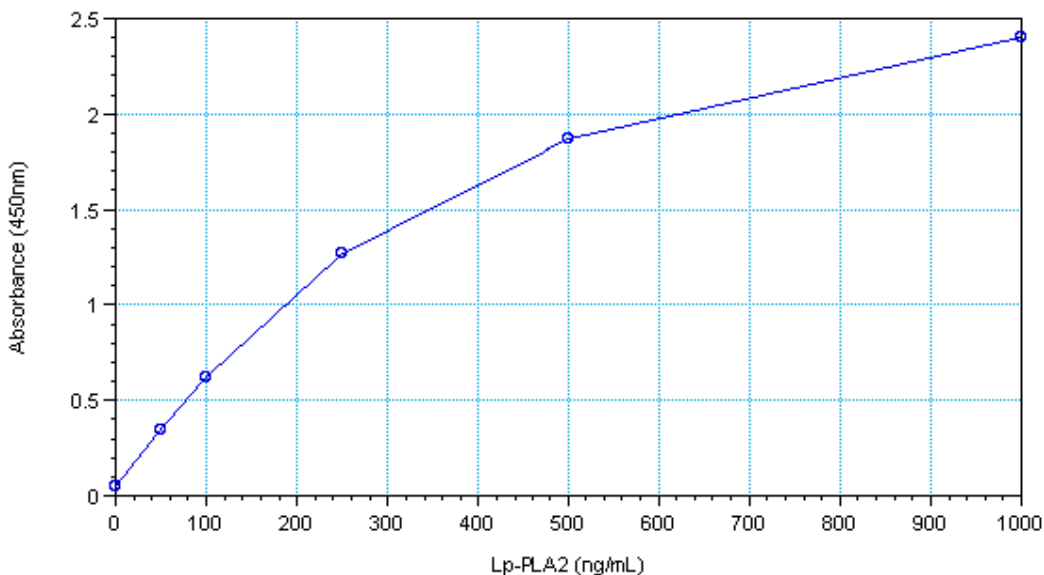
## CALCULATION OF RESULTS

1. Construct a standard calibration curve by plotting the absorbance obtained for each Calibrator on the y-axis versus the Lp-PLA<sub>2</sub> concentration in ng/mL on the x-axis. Use a point-to-point curve fit with appropriate computer software to construct the standard calibration curve.
2. Using the absorbance value for each sample and Control, determine the corresponding concentration of Lp-PLA<sub>2</sub> in ng/mL from the calibration curve.

## EXAMPLE OF CALIBRATOR CURVE

Results of a typical standard calibration curve with O.D. readings at 450 nm are shown on the y-axis against Lp-PLA<sub>2</sub> 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 user for each assay performed.

Lp-PLA <sub>2</sub> (ng/mL)	Absorbance (O.D. at 450 nm)
0	0.048
50	0.348
100	0.623
250	1.269
500	1.868
1000	2.402



## LIMITATIONS

### Procedure

- The wash procedures are critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
- 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 analytical methods.

## ANALYTICAL CHARACTERISTICS

### Low End Detection

The minimum detection limit is 0.34 ng/mL, as calculated by interpolation of the mean plus two standard deviations of 20 replicates of the 0 ng/mL Lp-PLA<sub>2</sub> calibrator from the standard curve.

### Assay Precision

Intra-assay and inter-assay variability were determined by testing four human serum pools with Lp-PLA<sub>2</sub> concentrations distributed throughout the calibration range of the assay. The serum pools were assayed using a single lot of reagents, in duplicate, on two separate stripwells per day, for 5 days, four plates per day. The data from the testing are summarized below:

Serum Pool	Mean Concentration Lp-PLA <sub>2</sub> (ng/mL)	Intra-assay % CV n=80	Inter-assay % CV n=20	Total % CV n=80
1	143	6.2	4.6	7.7
2	211	4.1	5.1	6.6
3	368	5.1	8.5	9.9
4	830	9.5	8.7	12.8

In a repeatability study conducted with a set of 108 serum samples, assay results determined from the first wells (single point assay) were compared to the mean results from two successive measurements (duplicate wells). The Lp-PLA<sub>2</sub> levels of the samples ranged from 87 to 575 ng/mL, and the mean %CV between replicates was 2.3%. In linear regression analysis, single point results were highly correlated to duplicate mean results: correlation coefficient  $r=0.997$  (slope 1.0 and intercept -1.3 ng/mL).

### Linearity

Six serum samples with known high Lp-PLA<sub>2</sub> levels were intermixed with six serum samples with known low Lp-PLA<sub>2</sub> levels. Percent recovery was determined as the measured value divided by the expected value, multiplied by 100. The average recovery was 93%, demonstrating linearity of the diluted samples over a range of 151 to 810 ng/mL Lp-PLA<sub>2</sub>.

## PRODUCT SAFETY INFORMATION

Calibrator Set (1-6) Control Low and High Xi R36 S24/25-26-46 	20X Wash Buffer Xi R36-37-38 S24/25-26-46 	Stop Solution Xi S24/25 	Conjugate, TMB Reagent Xi 
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R36	Irritating to eyes
R37	Irritating to respiratory system
R38	Irritating to skin
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

## REFERENCES

- [1] Caslake M.J., Packard C.J., et al. (2000). Atherosclerosis 150: 413-9.
- [2] Kudo I. and Murakami M. (2002). Prostaglandins Other Lipid Mediat 68-69: 3-58.