

PLAC FIA Rapid Test Device (Whole Blood/Serum/Plasma) FI2-PLA2-001

A Fluorescence Immunoassay for the quantitative detection of Lipoprotein-associated phospholipase A2 (Lp-PLA2) in human whole blood, serum, or plasma with the use of the Biopanda Fluorescence Immunoassay Device (BR-FIA-2000). For professional *in vitro* diagnostic use only.

INTENDED USE

The PLAC FIA Rapid Test Device is intended for *in vitro* quantitative determination of human Lipoprotein-associated phospholipase A2 (Lp-PLA2) in whole blood, serum, or plasma as an aid in the diagnosis of Coronary disease.

SUMMARY

Lipoprotein-associated phospholipase A2 (Lp-PLA2), also known as platelet-activating factor acetyl hydrolase, is an enzyme that plays a significant role in vascular inflammation and atherosclerosis. It is primarily associated with low-density lipoprotein (LDL) particles in the bloodstream and is involved in the hydrolysis of oxidized phospholipids, generating pro-inflammatory mediators such as lysophosphatidylcholine and oxidized free fatty acids.¹ These bioactive compounds contribute to endothelial dysfunction, plaque formation, and instability, making Lp-PLA2 a key player in the pathogenesis of cardiovascular diseases.²

Elevated levels of Lp-PLA2 have been identified as an independent risk factor for coronary heart disease and stroke, as it reflects vascular inflammation and plaque vulnerability. Due to its specific association with atherosclerotic processes, Lp-PLA2 has emerged as a promising biomarker for assessing cardiovascular risk and guiding therapeutic strategies. Inhibitors of Lp-PLA2 have been developed and studied for their potential to reduce inflammation and stabilize atherosclerotic plaques, offering a targeted approach to managing cardiovascular disease. Understanding the role of Lp-PLA2 in vascular biology continues to provide insights into the mechanisms of atherosclerosis and the development of novel therapeutic interventions.^{3,4}

PRINCIPLE

The PLAC FIA Rapid Test Device is a membrane-based fluorescence immunoassay for the quantitative detection of Lp-PLA2 in human whole blood, serum or plasma specimen. During testing, the sample moves through the strip from sample pad to absorbent pad. Lp-PLA2 in the sample will combine with the Lp-PLA2 antigen coated on the membrane. The fluorescent microspheres coupled to the Lp-PLA2 antibody show a positive correlation with the Lp-PLA2 concentration in the sample, which can be captured by the Lp-PLA2 antigen coated on the membrane (Test line). According to the fluorescence intensity of the test and the standard curve, the concentration of Lp-PLA2 in the sample can be calculated by the Biopanda Fluorescence Immunoassay Device. The testing result of Lp-PLA2 will display on the analyzer screen.

REAGENTS

The test contains Lp-PLA2 coated with cellulose nitrate membrane and Lp-PLA2 antibody which conjugated with fluorescence particles.

PRECAUTIONS

- For professional *in vitro* diagnostic use only.
- Do not use after the expiration date indicated on the package. Do not use the test if the foil pouch is damaged. Do not reuse.
- Avoid cross-contamination of specimens by using a new specimen collection container for each specimen obtained.
- Do not eat, drink or smoke in the area where the specimens and tests are handled. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow standard procedures for proper disposal of specimens. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- Do not interchange or mix reagents from different lots.
- Humidity and temperature can adversely affect results.
- Used testing materials should be discarded in accordance with local regulations.
- Read the entire procedure carefully prior to any testing.
- The Biopanda PLAC FIA Rapid Test should only be used with the Biopanda Fluorescence Immunoassay Device by approved medical professionals.

STORAGE AND STABILITY

- The kit should be stored at 4-30°C until the expiry date printed on the sealed pouch.
- The test must remain in the sealed pouch until use.
- Do not freeze.
- Care should be taken to protect the components of the kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

KIT COMPONENTS

- 25 x foil wrapped PLAC FIA test devices
- 25 x Specimen collection tubes with dilution buffer
- 1 x ID card (PLAC)
- Package Insert

MATERIALS REQUIRED BUT NOT PROVIDED

- Timer
- Centrifuge
- Pipette
- Biopanda Fluorescence Immunoassay Device (BR-FIA-2000)

SPECIMEN COLLECTION AND PREPARATION

For Venipuncture Specimens

- Collect the specimen according to standard procedures.
- Separate the serum or plasma from blood as soon as possible to avoid hemolysis. Only clear, non-hemolyzed specimens can be used.
- Do not leave specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8 °C for up to 3 days, for long term storage, specimens should be kept below -20 °C. Whole blood collected by venipuncture should be stored at 2-8 °C if the test is to be run within 2 days of collection. Do not freeze whole blood specimens. Whole blood collected by fingerstick should be tested immediately.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Avoid repeated freezing and thawing of specimens.
- EDTA K2, Heparin sodium can be used as the anticoagulant for collecting the specimen. A clean tube without anticoagulants can be used to collect serum specimens.

For Finger-pricked Whole Blood Specimens, please refer to the DIRECTIONS FOR USE for further information.

DIRECTIONS FOR USE

Refer to the Biopanda Fluorescence Immunoassay Analyser Operation Manual for the complete instructions on use of the test. The test should be conducted at room temperature. Cold buffer solution or moisture condensation on the membrane can lead to invalid test results.

Note: There are two test modes for the Biopanda Fluorescence Immunoassay Device: Standard Test mode and Quick Test mode. *Standard Test* mode is a 'set and forget' method where the test will automatically be read after 15 minutes. *Quick Test* mode provides an instant result but the user must monitor the 15 minute test time themselves. It is suitable when running multiple tests concurrently.

Refer to the Biopanda Fluorescence Immunoassay Device Operation Manual for further details.

Allow the test cassette, specimen, buffer and/or controls to reach room temperature (15-30°C) prior to testing.

- Turn on the Analyser. Then according to users' requirements, select "Standard test" or "Quick test" mode.
- Take out the ID card and insert it into the Analyser port.
- Remove the test cassette from the sealed foil pouch and start testing as soon as possible.
- Follow the appropriate steps below for the chosen specimen type:
 - For venipuncture whole blood/serum/plasma specimens:**
 - Pipette **20 µl of whole blood/serum/plasma** into the buffer tube.
 - Close the tube cap and shake the tube for approximately **10 seconds** to mix the specimen and dilution buffer well.
 - Let the diluted specimen homogenise for approximately 1 minute. Diluted specimens should be used as soon as possible.
 - Pipette 75 µl of diluted specimen** into the specimen well (S) of the test cassette. Start the timer at the same time.
 - For finger-prick whole blood specimens:**
 - Wash hands with soap and warm water or clean finger with an

alcohol pad. Allow to dry.

- ii. Massage the hand without touching the puncture site by applying pressure down the hand towards the finger to be pricked. The middle or ring finger is recommended.
- iii. Use a sterile lancet to puncture the skin. Wipe away the first sign of blood.
- iv. Gently apply pressure from palm to the pricked finger so a rounded drop of blood forms over the puncture site.
- v. Using the provided capillary dropper and ensuring the dropper is level, touch the open end to the rounded drop of blood without squeezing the dropper bulb. The dropper will automatically collect the correct volume of blood (approx. 20 µl), see Figure 1 below.
- vi. Dispense the whole blood specimen into the buffer tube by squeezing the dropper bulb.
- vii. Close the tube cap and shake the tube for approximately **10 seconds** to mix the specimen and dilution buffer well.
- viii. Using a disposable dropper, **draw the diluted specimen to the fill line** marked on the dropper (approx. 75 µl), then add to the specimen well (S) of the test cassette. Start the timer.

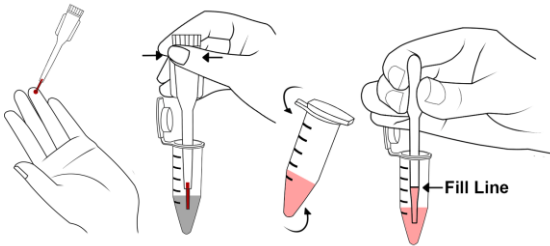


Figure 1

5. Test results should be read at **15 minutes** with the use of the Biopanda Fluorescence Immunoassay Analyser.

INTERPRETATION OF RESULTS

The result of test for the PLAC FIA Rapid Test is calculated by the Biopanda Fluorescence Immunoassay Device and is displayed on the screen. For additional information, please refer to the user manual of the Fluorescence Immunoassay Device.

Linearity range of the PLAC Test (Lp-PLA2) is 10-1000 ng/mL.

QUALITY CONTROL

Each PLAC FIA Rapid Test Device contains an internal control that satisfies routine quality control requirements. This internal control is performed each time a sample is tested. This control indicates that the test device was inserted and read properly by the Biopanda Fluorescence Immunoassay Device. An invalid result from the internal control causes an error message on the analyzer indicating that the test should be repeated. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

LIMITATIONS

1. The PLAC FIA Rapid Test Device is for professional *in vitro* diagnostic use, and should only be used for the quantitative detection of Lp-PLA2.
2. The PLAC FIA Rapid Test Device will only indicate the presence of Lp-PLA2 in the specimen and should not be used as the sole criterion for evaluating Coronary disease.
3. As with all diagnostic tests, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.
4. High concentrations of Lp-PLA2 may produce a dose hook effect, resulting in incorrect interpretation of Lp-PLA2 levels.
5. The hematocrit level of the whole blood should be between 25% and 65%.
6. The results of PLAC FIA Rapid Test Device are based on measuring the levels of Lp-PLA2 in a specimen. It should not be used as the sole criterion for treatment decisions. If the result is positive, other clinical findings and alternative test methods are recommended to reach proper medical treatments.

EXPECTED RESULTS

Concentrations	Clinical Reference
≤250 ng/ml	Not indicative of Coronary disease
≥250 ng/ml	Indicative of Coronary disease

PERFORMANCE CHARACTERISTICS

1. **ACCURACY:** The test deviation is ≤±15%.
2. **SENSITIVITY:** The Biopanda PLAC FIA Test Device can detect levels of Lp-PLA2 as low as 10 ng/ml in whole blood, serum or plasma.
3. **DETECTION RANGE:** 10~1000 ng/ml
4. **LINEARITY RANGE:** 10~1000 ng/ml.
5. **PRECISION**

Intra-lot precision

Within-run precision has been determined by using 10 replicates of 2 different concentrations of Lp-PLA2 specimens. C.V. is ≤ 15%.

Inter-lot precision

Between-run precision has been determined by using 10 replicates for each of three lots using 2 different concentrations of Lp-PLA2 specimens. C.V. is ≤ 15%.

CROSS-REACTIVITY

Cross-reactivity studies were carried out with following analytes: HBsAg, HBsAb, HBeAg, HBeAb, HbCAb, syphilis, anti-HIV, anti-*H.pylori*, MONO, anti-CMV, anti-Rubella and anti-Toxoplasmosis positive specimens. The results showed no cross-reactivity.

INTERFERING SUBSTANCES

The following potentially interfering substances were added to cTnI negative and positive specimens.

Acetaminophen: 20 mg/dl	Caffeine: 20 mg/dL
Acetylsalicylic Acid: 20 mg/dL	Gentisic Acid: 20 mg/dL
Ascorbic Acid: 20mg/mL	Albumin: 10,500mg/dL
Creatin: 200 mg/dL	Hemoglobin 1,000 mg/dL
Bilirubin: 1,000mg/dL	Oxalic Acid: 600mg/dL
Cholesterol: 800mg/dL	Triglycerides: 1,600mg/dL

None of the substances at the concentration tested interfered in the assay.

METHOD COMPARISON

The product was evaluated with 115 clinical samples compared with commercial CLIA test kit. The correlation coefficient(r) is 0.9813.

REFERENCES

1. Dimitroglou Y, Sakalidis A, Mavroudis A, Kalantzis C, Valatsou A, Andrikou I, Christofi A, Mantzouranis E, Kachrimanidis I, Bei E, Lazarou E, Tsioufis C, Tousoulis D, Lazaros G. Lipoprotein-associated Phospholipase A2 in Coronary Artery Disease. *Curr Top Med Chem.* 2022;22(28):2344-2354.
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3. Younus A, Humayun C, Ahmad R, Ogunmoroti O, Kandimalla Y, Aziz M, Malik R, Saand AR, Valdes C, Badlani R, Younus MA, Ali SS, Chen Y, Nasir K. Lipoprotein-associated phospholipase A2 and its relationship with markers of subclinical cardiovascular disease: A systematic review. *J Clin Lipidol.* 2017 Mar-Apr;11(2):328-337.
4. Maiolino G, Pedon L, Cesari M, Frigo AC, Wolfert RL, Barisa M, Pagliani L, Rossitto G, Seccia TM, Zanchetta M, Rossi GP. Lipoprotein-associated phospholipase A2 activity predicts cardiovascular events in high risk coronary artery disease patients. *PLoS One.* 2012;7(10):e48171.

Index of Symbols

	Manufacturer		Tests per kit		Do not reuse test
	<i>In vitro</i> diagnostic medical device		Expiration date		Catalogue number
	Storage temperature		Lot Number		Consult instructions for use
	Do not use if package is damaged				

Thank you for purchasing Biopanda's PLAC FIA Rapid Test. Please read this manual carefully before operating to ensure proper use.



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