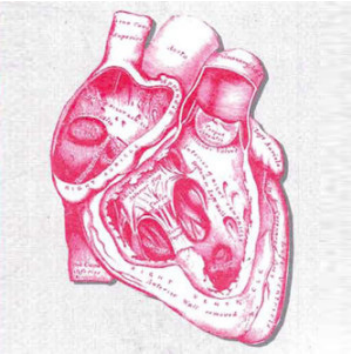
Advanced ImmunoChemical Inc.
Providing for the Advancement of Medical Science

ASSAY NOTES
Product Information

Courtesy of HyTest, Ltd

Lipoprotein-associated phospholipase A2 (Lp-PLA2)



Reagents for the development of quantitative LpPLA2 immunoassays

Advanced ImmunoChemical offers several human Lp-PLA2-specific murine monoclonal antibodies (MAbs) which can be used for the development of immunoassays that enable the detection of Lp-PLA2. In addition, we offer recombinant human Lp-PLA2 protein.

Antibodies are suitable for developing a quantitative immunoassay that measures the amount of the biomarker in mass units (ng/ml).

Monoclonal antibodies specific to Lp-PLA2

Advanced ImmunoChemical offers five well-characterized human Lp-PLA2- specific MAbs for the detection of Lp-PLA2 from plasma samples. The antibodies were developed against a recombinant human Lp-PLA2 expressed in a mammalian cell line.

A quantitative sandwich immunoassay for Lp-PLA2

Two MAb combinations are recommended for the development of a sandwich immunoassay to measure Lp-PLA2 in human plasma samples: PL42cc–PL46cc and PL26cc–PL4cc. Also other pairs are possible. All MAb combinations are capable of detecting native Lp-PLA2 in human serum or plasma.

All pair recommendations are listed in Table 1. Meanwhile, the calibration curve for the combination PL42cc–PL46cc is provided in Figure 1.

Capture	Detection	Limit of detection ng/ml
PL42cc	PL46cc	0.2
PL26cc	PL46cc	0.2
PL26cc	PL4cc	0.5
PLA32cc	PL4cc	0.5

Table 1. The most sensitive capture-detection pairs. *Data is based on the results obtained using in-house fluoroimmunoassay.*

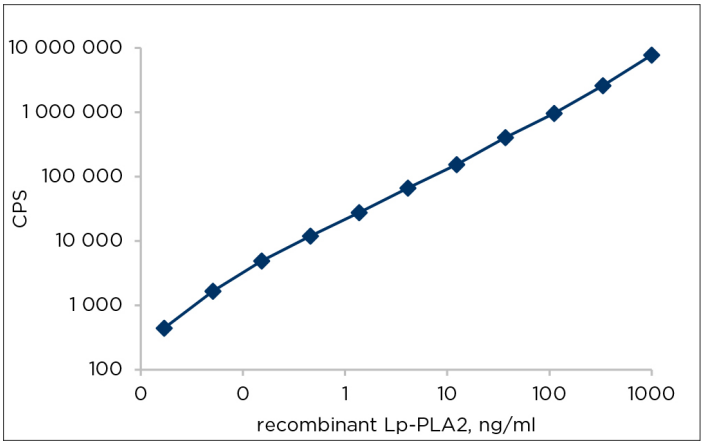


Figure 1. Calibration curve for the MAb combination PL42cc– PL46cc. *The capture antibody PL42cc was coated onto the wells of a Costar EIA/RIA plate and the plate was blocked with a buffer that contained 1% casein and 0.05% Tween 20 at room temperature for 15 minutes. Recombinant human Lp-PLA2 (Cat.# 8-Lp-PLA2) and the Eu3+-labeled detection MAb PL46cc were diluted in an assay buffer and incubated in coated plate wells for 2.5 hours at 37°C. Note: Due to the lipid binding properties of Lp-PLA2 it has a tendency to non- specifically adsorb onto plastic surfaces. To avoid this, 1% casein was added to the assay buffer to 0.5% (v/v) final concentration.*

Upon serial dilutions, recombinant Lp-PLA2 and endogenous Lp-PLA2 in normal human serum showed the same pattern of signal decrease in sandwich fluoroimmunoassays employing the MAb combination PL42cc–PL46cc (see Figure 2). This demonstrates that the immunochemical properties of recombinant Lp-PLA2 are similar to those of native Lp-PLA2.

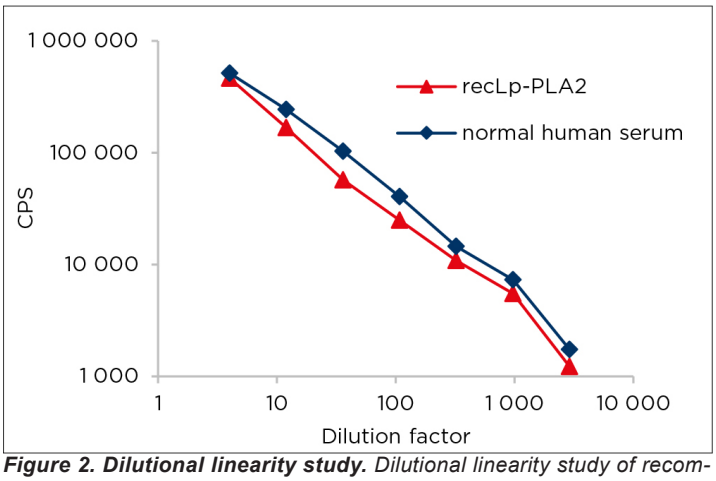


Figure 2. Dilutional linearity study. *Dilutional linearity study of recombinant Lp-PLA2 and native Lp-PLA2 (normal human serum from an apparently healthy volunteer) studied using the MAb combination PL42cc–PL46cc. The protocol is described in the Figure 1 caption. The initial concentration of the recombinant human Lp-PLA2 was 111 ng/ml.*

All antibodies are suitable for labeling with horseradish peroxidase. Figure 3 provides examples of sandwich ELISA assays that utilize HRP-labeled detection antibodies.

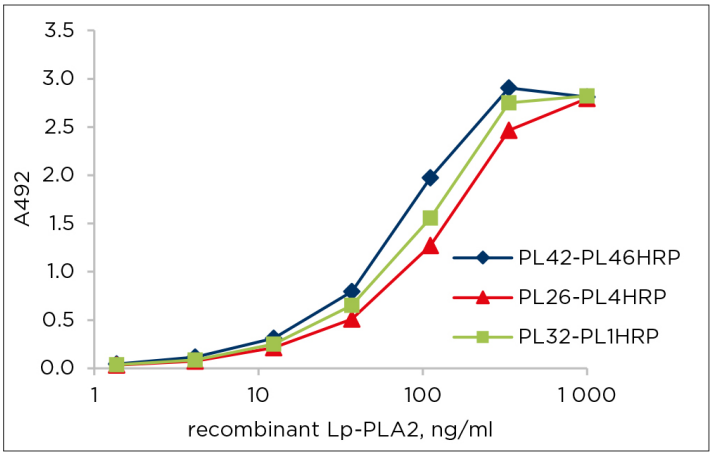


Figure 3. *The titration curve of recombinant Lp-PLA2 (Cat.# 8-Lp-PLA2) in sandwich ELISA using HRP-labelled antibodies. Plate wells coated with coating antibodies (1 µg per well) were blocked with 1% casein to prevent non-specific adsorption of antigen onto the plate surface. Recombinant Lp-PLA2 was added in indicated concentrations followed by HRP-linked detection antibodies (0.4 µg per well). Note: The MAb PL1 is an in-house clone.*

Measuring patient samples

In order to conduct preliminary clinical studies, serum samples from patients diagnosed with AMI at admission (N=13) as well as from apparently healthy volunteers (N=13) were obtained. Figure 4 shows the box-whisker plots of immunoassays that utilize MAbs PL26cc–PL4cc (A) and PL42cc–PL46cc (B) or a commercially available diagnostic assay (C) (see Figure 4).

MAb pair PL26cc–PL4cc detected native Lp-PLA2 in a manner that was very similar to that of the commercially available ELISA assay. Meanwhile, the PL42cc–PL46cc assay detected native Lp-PLA2 in a slightly different way.

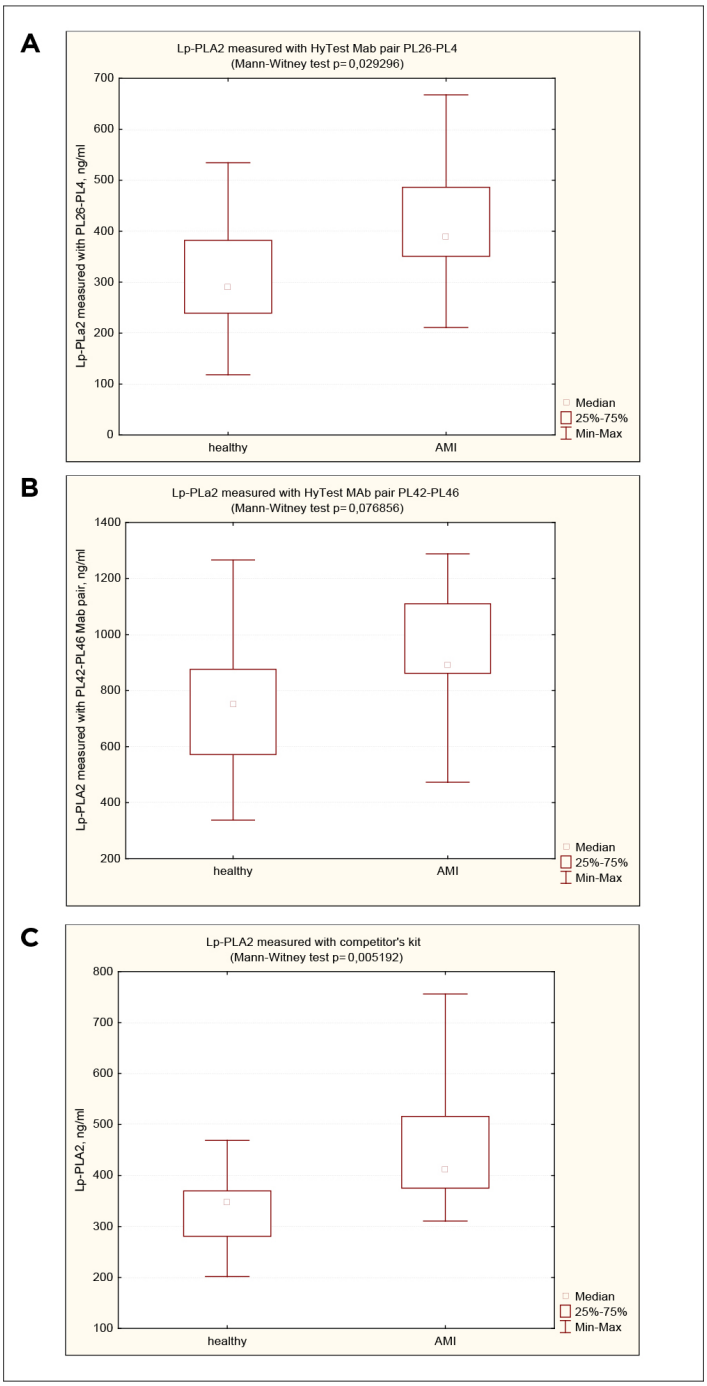


Figure 4. Detection of native Lp-PLA2 in serum samples. *Lp-PLA2 was detected in serum of acute myocardial infarction patients and healthy volunteers using fluoroimmunoassay with the MAb pair PL26cc–PL4cc (A) and PL42cc–PL46cc (B) or by using a commercially available ELISA kit (C). Serum samples were diluted 1:30 with an assay buffer (A and B) or according to the manufacturer's instructions (C). Samples were incubated for 2.5 hours at 37°C (A and B) or for 3 hours at room temperature (C).*

Recombinant human Lp-PLA2

Advanced Immunochemical offers recombinant human Lp-PLA2 (recLp-PLA2) that is expressed in a mammalian cell line. The protein contains 6×His tag on its C-terminus linked with a GG spacer. The calculated molecular mass and isoelectric point of recLp-PLA2 are 49,000 Da and 7.1, respectively. RecLp-PLA2 contains N-linked glycans like its native counterpart (data not shown).

RecLp-PLA2 is purified from the conditioned media of the mammalian cell line with several chromatographic procedures. Isolated recLp-PLA2 is substantially free of contaminants and has purity >75% as determined by densitography following SDS-gel electrophoresis in reducing conditions (data not shown).

Recombinant Lp-PLA2 associates with plasma fractions like endogenous Lp-PLA2

In gel-filtration, the immunoreactivity of native Lp-PLA2 is distributed over the entire elution profile that forms three distinct peaks. Elution volumes of the peaks are in good accordance with those for VLDL, LDL and HDL, i.e. lipoprotein particles with which the native Lp-PLA2 forms complexes [1]. RecLp-PLA2 spiked in normal human serum showed that the elution profile of the recombinant protein was similar to that of endogenous Lp-PLA2 (see Figure 5). The peaks coincide with those of the native protein which indicates that recLp-PLA2 binds to the same moieties in the serum as native Lp-PLA2.

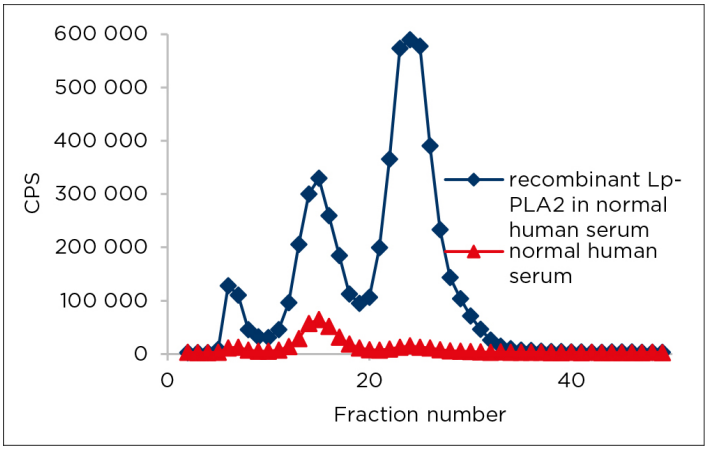


Figure 5. Gel-filtration studies of the association of recombinant human Lp-PLA2 with lipoproteins in normal human serum. *150 µl of normal human serum or 150 µl of serum spiked with 3 µg of recLp-PLA2 were applied onto a Superose 6 gel-filtration column. Immunoreactivity in fractions was determined by a fluoroimmunoassay with the MAb combination PL42cc–PL46cc. RecLp-PLA2 appears to be binding to the same lipoproteins as endogenous Lp-PLA2 when added to native serum.*

Recovery of recombinant Lp-PLA2 in human serum

When recLp-PLA2 is spiked in normal human serum, the resulting signal represents a summary of a signal with recLp-PLA2 alone in an assay buffer and native Lp-PLA2 in serum over a wide concentration range (see Figure 6).

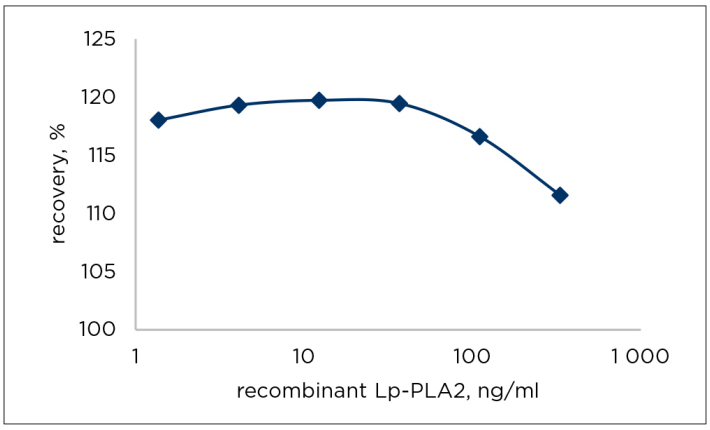


Figure 6. Recovery studies of recLp-PLA2 in normal human serum with the MAb combination PL42–PL46. *recLp-PLA2 was spiked into normal human serum to concentrations indicated. The same concentrations of recLp-PLA2 were used for measurement in an assay buffer. Serum dilutions were prepared with the assay buffer and used for the native Lp-PLA2 immunoreactivity measurement.*

Stability of recombinant human Lp-PLA2

The recombinant human Lp-PLA2 is sold as a lyophilized product. However, a protein preparation in a storage buffer retained its immunoreactivity when stored at different temperatures (see Figure 7). Based on the results, recLp-PLA2 retained approximately 80% of its immunoreactivity for at least two weeks when stored at room temperature. Meanwhile, when stored at 37°C, 50% of the immunoreactivity was lost after four days.

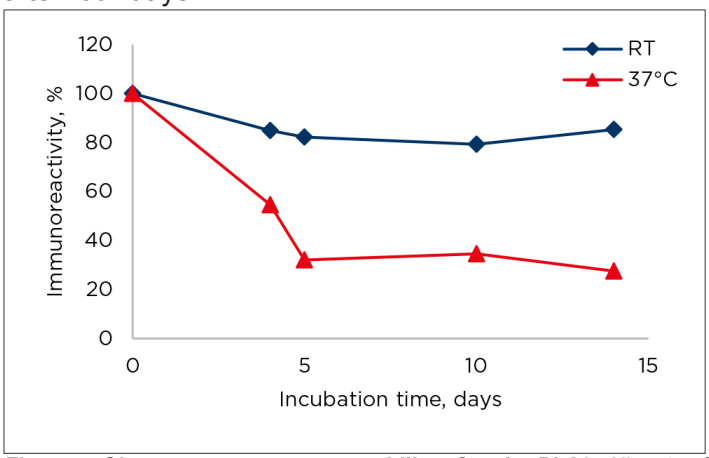


Figure 7. Short-term temperature stability of recLp-PLA2. *Aliquots of recLp-PLA2 solution were incubated at room temperature or at 37°C for two weeks. At indicated time points, aliquots were diluted to an assay buffer and recLp-PLA2 immunoreactivity was measured using the MAb combination PL42cc–PL46cc.*

The immunoreactivity of recLp-PLA2 measured with the MAb combination PL42cc–PL46cc does not change significantly over 15 freeze-thaw cycles (see Figure 8).

1. Innis-Whitehouse, W., et al., An efficient chromatographic system for lipoprotein fractionation using whole plasma. J Lipid Res, 1998. 39(3): p. 679-90.

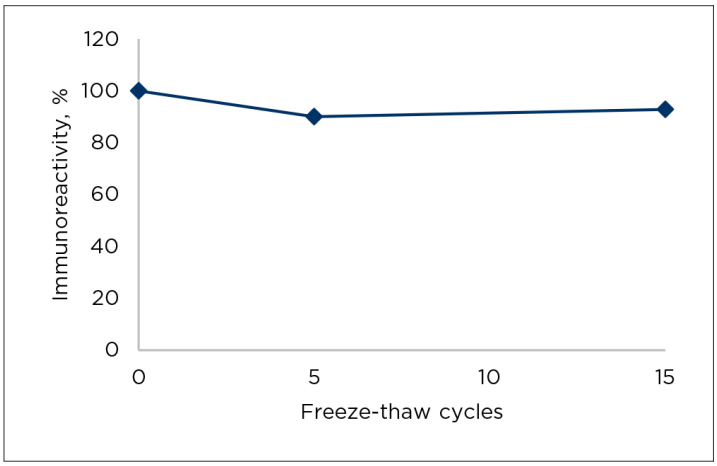


Figure 8. Freeze-thaw stability of recLp-PLA2. *recLp-PLA2 was subjected to repeated freeze-thaw procedures. Following an indicated amount of cycles, samples were taken and analyzed in a sandwich fluoroimmunoassay using the MAb combination PL42cc-PL46cc along with control aliquot stored at -20°C. The results indicate that the protein is not very sensitive to repeated freezing and thawing.*

Ordering Information:

MONOCLONAL ANTIBODIES

Product Name	Cat #	MAb	Subclass	Remarks
Human lipoprotein-associated phospholipase A2 (Lp-PLA2), *In vitro*	2-PLA2	PL4cc	IgG1	EIA, WB
		PL26cc	IgG1	EIA, WB
		PL32cc	IgG1	EIA, WB
		PL42cc	IgG1	EIA, WB
		PL46cc	IgG1	EIA, WB

ANTIGEN

Product Name	Cat #	Purity	Source
Human lipoprotein-associated phospholipase A2 (Lp-PLA2), recombinant	8-Lp-PLA2	>75%	Recombinant

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