Human PLA2G7/PAF-AH/Lp-PLA2 ELISA Kit (DEIA2940)

Cat.No: DEIA2940
Lot. No. (See product label)

Size

96T

Intended use

For the quantitative determination of human Phospholipase A2 Group VII (PLA2G7) concentrations in cell culture supernates, serum, and plasma.

General Description

Phospholipase A2 Group VII (PLA2G7), also known as lipoprotein-associated phospholipase (Lp-PLA2) and platelet-activating factor acetylhydrolase (PAF-AH), is a secreted 55-67 kDa calcium-independent glycoprotein that belongs to the phospholipase A2 superfamily (1-3). The active site of the 420 amino acid mature human PLA2G7 is composed of a serine, histidine, and aspartic acid triad, which catalyzes the hydrolysis of the sn-2 ester bond of phospholipids (4-6). Substrates of PLA2G7 are limited to PAF (1-O-alkyl-2-acetyl-snglycero-3-phosphocholine), a potent pro-inflammatory phospholipid mediator, and PAF-like molecules that are generated upon oxidation of phospholipids on low density lipoprotein (LDL) particles (2,7,8). PLA2G7 is produced mainly by hematopoietic cells such as monocytes/ macrophages, lymphocytes, mast cells, megakaryocytes and platelets (9). Secreted PLA2G7 contains asparagine-conjugated carbohydrate containing sialic acid residues, which prevents association of the enzyme with high density lipoprotein (HDL) particles. In humans, circulating PLA2G7 is mainly bound to LDL (80%), a smaller amount is also bound to HDL (20%) (2, 10-12).

PLA2G7 activity has been suggested to have a dual role in atherogenesis. Because it degrades and inactivates PAF and PAF-like pro-inflammatory phospholipid mediators, it is suggested to play an anti-atherogenic/anti-inflammatory role (2, 7, 8). However, LDL bound PLA2G7 also has a crucial role in the formation of atherosclerotic plaques (9, 11, 13-16). When LDL is trapped in the subendothelial space at the site of new plaque formation (as a result of the interaction between its basic apolipoprotein B100 molecules and the negatively-charged subendothelial proteoglycans), the associated lipids are subjected to oxidative modifications. LDL associated PLA2G7 then hydrolyzes the oxidized phospholipids to release lysophosphatidylcholine and oxidized non-esterified fatty acid, both of which are pro-inflammatory and can stimulate the production of cytokines, upregulate adhesion molecules, recruit macrophages, and ultimately lead to foam cell formation. The balance between the pro- and anti-inflammatory roles of PLA2G7 may depend on the concentration of the available substrates. In humans, multiple recent studies have pointed to a pro-atherogenic role of PLA2G7. Increased plasma levels of the enzyme have been shown to be a good predictor of cardiovascular disease and have a strong association with atherosclerosis (17-20).

The Quantikine Human PLA2G7/PAF-AH/Lp-PLA2 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human PLA2G7 in cell culture supernates, serum, and plasma. It contains E. coli-expressed recombinant human PLA2G7 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human PLA2G7 showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for naturally occurring human PLA2G7.

Principle Of The Test

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This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human PLA2G7 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any PLA2G7 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human PLA2G7 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of PLA2G7 bound in the initial step. The color development is stopped and the intensity of the color is measured.

Reagents And Materials Provided

1. Human PLA2G7 Microplate: 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human PLA2G7.
Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8°C.

2. Human PLA2G7 Standard: 2 vials of recombinant human PLA2G7 in a buffer with preservatives; lyophilized. Refer to the vial label for reconstitution volume.
Use a new Standard for each assay. Discard after use.

3. Human PLA2G7 Conjugate: 21 mL of polyclonal antibody specific for human PLA2G7 conjugated to horseradish peroxidase with preservatives.
4. Assay Diluent: 11 mL of a buffered protein solution with preservatives. Assay Diluent RD1-9 may contain a precipitate. Warm to room temperature, and mix gently to dissolve. If the precipitate does not completely dissolve, mix well during use.
5. Calibrator Diluent: 21 mL of a concentrated buffered protein base with preservatives.
6. Wash Buffer Concentrate: 21 mL of a 25-fold concentrated solution of buffered surfactant with preservatives. May turn yellow over time.
7. Color Reagent A: 12.5 mL of stabilized hydrogen peroxide.
8. Color Reagent B: 12.5 mL of stabilized chromogen (tetramethylbenzidine).
9. Stop Solution: 6 mL of 2 N sulfuric acid.
May be stored for up to 1 month at 2-8°C.*

10. Plate Sealers: 4 adhesive strips.

Materials Required But Not Supplied

• Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
• Pipettes and pipette tips.
• Deionized or distilled water.
• Squirt bottle, manifold dispenser, or automated microplate washer.
• 100 mL and 500 mL graduated cylinders.
• Horizontal orbital microplate shaker (0.12' orbit) capable of maintaining a speed of 500 ± 50 rpm.
• Test tubes for dilution of standards and samples.
• Human PLA2G7 Controls (optional).

Specimen Collection And Preparation

SAMPLE COLLECTION & STORAGE

1. Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -

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20°C. Avoid repeated freeze-thaw cycles.

2. Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

3. Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Note: Grossly lipemic samples are not suitable for measurement of PLA2G7 with this assay. Citrate plasma has not been validated for use in this assay.

SAMPLE PREPARATION

1. Cell culture supernate samples require a 2-fold dilution. A suggested 2-fold dilution is 75 mL of sample + 75 mL of Calibrator Diluent (diluted 1:4)*.

2. Serum and plasma samples require a 20-fold dilution. A suggested 20-fold dilution is 10 mL of sample + 190 mL of Calibrator Diluent (diluted 1:4).

SAMPLE VALUES

1. Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human PLA2G7 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=35)	113	21.2-167	30.3
EDTA plasma (n=35)	121	26.7-183	32.9
Heparin plasma (n=35)	131	31.0-206	35.6

2. Cell Culture Supernates - Aliquots of cell culture supernate from 14 various cell lines were assayed for levels of human PLA2G7. No detectable levels were observed.

Reagent Preparation

Bring all reagents to room temperature before use.

1. Wash Buffer: If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

2. Substrate Solution: Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 mL of the resultant mixture is required per well.

3. Calibrator Diluent (diluted 1:4): Add 20 mL of Calibrator Diluent to 60 mL of deionized or distilled water to prepare 80 mL of Calibrator Diluent (diluted 1:4).

4. Human PLA2G7 Standard: Refer to the vial label for reconstitution volume. Reconstitute the Human PLA2G7 Standard with

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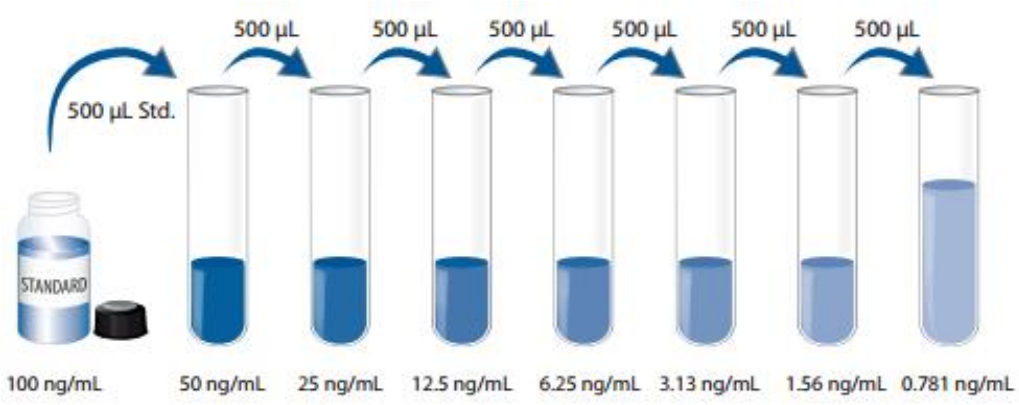
Calibrator Diluent (diluted 1:4). This reconstitution produces a stock solution of 100 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes. Mix well prior to making dilutions.

5. Pipette 500 mL of Calibrator Diluent (diluted 1:4) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 50 ng/mL standard serves as the high standard. Calibrator Diluent (diluted 1:4) serves as the zero standard (0 ng/mL).



Assay Procedure

Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.

2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.

3. Add 100 mL of Assay Diluent to each well. May contain a precipitate. Warm to room temperature, and mix gently to dissolve. If the precipitate does not completely dissolve, mix well during use.

4. Add 50 mL of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12' orbit) set at 500 ± 50 rpm.

5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 mL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

6. Add 200 mL of Human PLA2G7 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.

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7. Repeat the aspiration/wash as in step 5.

8. Add 200 mL of Substrate Solution to each well. Protect from light. Incubate for 30 minutes at room temperature on the benchtop.

9. Add 50 mL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples require dilution. See Sample Preparation section.

Calculation

1. Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

2. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human PLA2G7 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

3. Since samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

CALIBRATION
This immunoassay is calibrated against a highly purified E. coli-expressed recombinant human PLA2G7 produced at CD.

Typical Standard Curve

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

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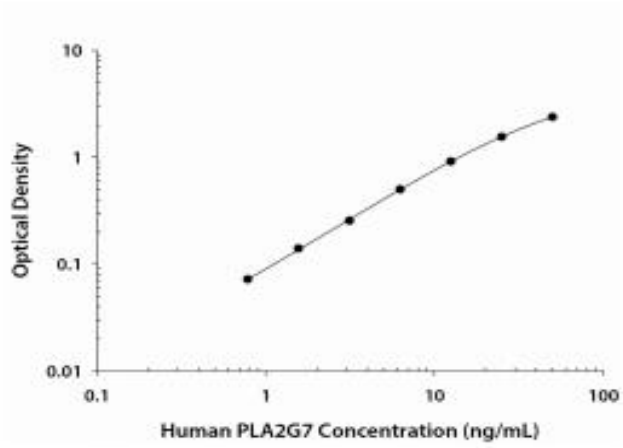

(ng/mL)	O.D.	Average	Corrected
0	0.022 0.026	0.024	—
0.781	0.091 0.101	0.096	0.072
1.56	0.162 0.165	0.164	0.140
3.13	0.270 0.287	0.279	0.255
6.25	0.509 0.537	0.523	0.499
12.5	0.934 0.942	0.938	0.914
25	1.573 1.577	1.575	1.551
50	2.355 2.453	2.404	2.380

Reference Values

Precision

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/mL)	1.69	3.52	11.7	2.35	4.34	13.0
Standard deviation	0.115	0.192	0.272	0.226	0.300	0.676
CV (%)	6.8	5.5	2.3	9.6	6.9	5.2

Sensitivity

Twenty assays were evaluated and the minimum detectable dose (MDD) for human PLA2G7 ranged from 0.025-0.284 ng/mL. The mean MDD was 0.074 ng/mL.

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The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity

This assay recognizes natural and recombinant human PLA2G7.
The factors listed below were prepared at 500 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 500 ng/mL in a mid-range recombinant human PLA2G7 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:
Apolipoprotein A-I
Apolipoprotein A-II
Apolipoprotein B
Apolipoprotein B100
Apolipoprotein C1
Apolipoprotein C2
Apolipoprotein D
Apolipoprotein E
PLA2G2A
PLA2G4A
PLA2G1B (mature)
PLA2G1B (Pro)

Recombinant mouse:
PLA2G2A
PLA2R1

Natural proteins:
human Apolipoprotein A-I
human Apolipoprotein A-II
human Low Density Lipoprotein

Linearity

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human PLA2G7 were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay. Samples were diluted prior to assay as directed in the Sample Preparation section.

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		Cell culture media (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	101	103	106	100
	Range (%)	98-107	101-105	105-108	96-103
1:4	Average % of Expected	102	106	108	107
	Range (%)	98-106	104-108	99-114	104-111
1:8	Average % of Expected	99	110	111	109
	Range (%)	90-112	109-111	108-114	106-114
1:16	Average % of Expected	95	111	109	108
	Range (%)	87-105	107-114	104-115	102-114

Recovery

The recovery of human PLA2G7 spiked to levels throughout the range of the assay in various matrices was evaluated. Samples were diluted prior to assay as directed in the Sample Preparation section.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	92	85-105%
Serum (n=4)	106	91-115%
EDTA plasma (n=4)	101	91-112%
Heparin plasma (n=4)	102	92-106%

Precautions

TECHNICAL HINTS

• When mixing or reconstituting protein solutions, always avoid foaming.
• To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
• To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
• When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
• Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
• Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution. Some components in this kit contain a preservative which may cause

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an allergic skin reaction. Avoid breathing mist. Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.
Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

Limitations

LIMITATIONS OF THE PROCEDURE
• FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
• The kit should not be used beyond the expiration date on the kit label.
• Do not mix or substitute reagents with those from other lots or sources.
• If samples generate values higher than the highest standard, further dilute the samples with Calibrator Diluent and repeat the assay.
• Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
• This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

References

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