HUMAN PHOSPHOLIPASE A2, GROUP VII (PLA2G7) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN PLA2G7 CONCENTRATIONS IN PLASMA AND SERUM

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PRODUCT INFORMATION:

ELISA NAME	HUMAN PLA2G7 ELISA KIT
Catalog No.	SK00532-08F*
Lot No.	20112300
Formulation	5 X 96 T
Standard range	195-12500 pg/mL
Sensitivity	30 pg/mL
Sample Volume	100 µl
Dilution Factor	4~ 8 (Optimal dilutions should be determined by each laboratory for each application)
Sample Type	Serum, EDTA Plasma
Specificity	Human
Calibration	Human PLA2G7/PAFA Recombinant
Intra-assay Precision	4-6%
Inter-assay Precision	8-10%
Storage	2-8°C

*SK00532-08F : 5 kits of SK00532-08

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DESCRIPTION

This Human PLA2G7 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human PLA2G7 from serum and EDTA plasma in a sandwich ELISA format.

This immunoassay contains recombinant human PLA2G7 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural PLA2G7 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human PLA2G7. The capture antibody can bind to the human PLA2G7 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human PLA2G7 is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of PLA2G7 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
PLA2G7 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified IgG against human PLA2G7	**532-08-01**	**1 plate**
PLA2G7 Standard – 100ng/vial of recombinant human PLA2G7 in a buffered protein base with preservative; lyophilized.	**532-08-02**	**1 vial**
Detection Antibody – 12 mL/vial, 10-fold concentrate of biotinylated purified IgG against human PLA2G7 with preservative; lyophilized.	**532-08-03**	**1 vial**
Positive Control – one vial of recombinant human PLA2G7; lyophilized.	**532-08-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservative.	**DB06**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB40**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution – 11 mL of 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 8 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20 or -70°C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard and Detection Antibody Concentrate Solution SHOULD BE STORED at -20°C or -70°C for

up to one month. Streptavidin-HRP Conjugate 100-fold concentrated solution and TMB Substrate Solution can be stored at 2 – 8 °C for up to 8 months (**DO NOT FREEZE** and **PROTECT FROM LIGHT**). All other components may be stored at 2 – 8 °C for up to 8 months.

Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 - 8°C after opening.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes 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.

Plasma - Collect plasma using EDTA, heparin, or citrate 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.

Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Serum and plasma samples may need a 4-8 fold or high dilution. A suggested 4 fold dilution is 60 µL sample + 180 µL Dilution Buffer to make a 4 fold dilution. A suggested 8 fold dilution is 30 µL sample + 210 µL Dilution Buffer to make a 8 fold dilution.

Optimal dilutions should be determined by each laboratory for each application with a sample pretest.

Use polypropylene test tubes.

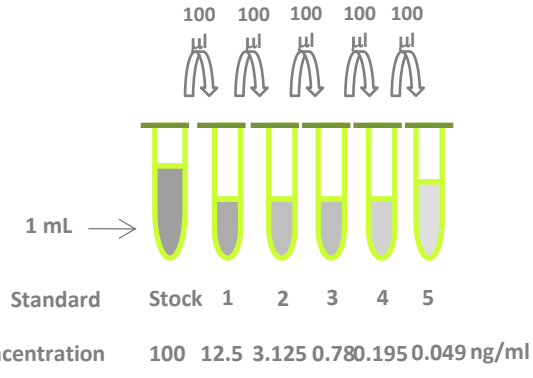
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

PLA2G7 Standard - Reconstitute the Human PLA2G7 Standard with 1 ml of Dilution Buffer. This reconstitution produces a stock solution of 100 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 300 µL of Dilution Buffer into tubes #1 to #5. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 12500 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	100000 pg/ml
# 1	100 µl of stock	300 µl	12500 pg/ml
# 2	100 µl of 1	300µl	3125 pg/ml
# 3	100µl of 2	300µl	780 pg/ml
# 4	100µl of 3	300µl	195 pg/ml
# 5	100µl of 4	300µl	49 pg/ml



Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.2 mL of Dilution Buffer (**DB06**) to produce a 10-fold concentrated stock solution. Pipette 10.8 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.2 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of **HRP Diluent Solution (DB40)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. *Note: 1x working solution of Streptavidin-HRP Conjugate should be used within a few days (protect from light).*

Positive Control - Reconstitute the Positive Control with 2.0 mL of Dilution Buffer. Note: Positive Control could be used within a few days if stored at -20 °C or -70 °C.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack and seal.
3. Add 100 µL per well of Dilution Buffer to Blank wells.
4. Add 100 µL of Standard, sample, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) 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 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 40 min on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 8-12 minutes on microplate shaker at room temperature. **Protect from light.**
11. Add 100 µL 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 if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

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

TYPICAL DATA

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

STANDARD (PG/ML)	AVERAGE OD450NM (CORRECTED)
Blank	0 (0.078)
49 (optional)	0.022
195	0.084
781	0.300
3125	0.952
12500	1.869
50000 (optional)	3.588

Lot No.: 20112300
Positive Control: 2-5 ng/ml

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human PLA2G7	100
Human CRP	0
Human MPO	0
Human VDBP	0
Human IL-6	0
Human MCP-1	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µL of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Detection Antibody working solution to each well. Incubate 2 hour on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Streptavidin-HRP conjugate working solution to each well. Incubate 40 min on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µL Substrate Solution to each well. Incubate 8-12 min on the plate shaker at RT. **Protect from light.**
↓
Add 100 µL Stop Solution to each well. Read 450nm within 15 min.