Datasheet for ABIN424287

PLA2G4A ELISA Kit

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Overview

Quantity:	96 tests
Target:	PLA2G4A
Reactivity:	Mouse
Method Type:	Sandwich ELISA
Detection Range:	62.5 pg/mL - 4000 pg/mL
Minimum Detection Limit:	62.5 pg/mL
Application:	ELISA

Product Details

Purpose:	The ELISA kit is an enzyme immunoassay for the in vitro quantitative measurement of mouse cPLA2 in tissue homogenates and cell lysates
Sample Type:	Tissue Homogenate, Cell Lysate
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of Phospholipase A2, Cytosolic (cPLA2). No significant cross-reactivity or interference between Phospholipase A2, Cytosolic (cPLA2) and analogues was observed.
Cross-Reactivity (Details):	No significant cross-reactivity or interference was observed.
Sensitivity:	24.9 pg/mL
Characteristics:	The test principle applied in this kit is Sandwich enzyme immunoassay. The microtiter plate provided in this kit has been pre-coated with an antibody specific to Phospholipase A2, Cytosolic (cPLA2). Standards or samples are then added to the appropriate microtiter plate

Product Details

	wells with a biotin-conjugated antibody specific to Phospholipase A2, Cytosolic (cPLA2). Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain Phospholipase A2, Cytosolic (cPLA2), biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of Phospholipase A2, Cytosolic (cPLA2) in the samples is then determined by comparing the O.D. of the samples to the standard curve.
Components:	• Pre-coated, ready to use 96-well strip plate, flat buttom • Plate sealer for 96 wells • Reference Standard • Standard Diluent • Detection Reagent A • Detection Reagent B • Assay Diluent A • Assay Diluent B • Reagent Diluent (if Detection Reagent is lyophilized) • TMB Substrate • Stop Solution • Wash Buffer (30 x concentrate) • Instruction manual
Material not included:	• Microplate reader with 450nm filter. • Precision single or multi-channel pipettes and disposable tips. • Eppendorf Tubes for diluting samples. • Deionized or distilled water. • Absorbent paper for blotting the microtiter plate. • Container for Wash Solution

Target Details

Target:	PLA2G4A
Alternative Name:	Phospholipase A2, Cytosolic (cPLA2) (PLA2G4A Products)
Pathways:	Inositol Metabolic Process, G-protein mediated Events, VEGF Signaling

Application Details

Comment:	The standard curve concentrations used for the ELISA's were 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.625 ng/mL, 0.313 ng/mL, 0.156 ng/mL

	Information on standard material: The standard might be recombinant protein or natural protein, that will depend on the specific kit. Moreover, the expression system is E.coli or yeast or mammal cell. There is 0.05% proclin 300 in the standard as preservative. Information on reagents: The stop solution used in the kit is sulfuric acid with concentration of 1 mol/L. And the wash solution is TBS. The standard diluent contains 0.02 % sodium azide, assay diluent A and assay diluent B contain 0.01% sodium azide. Some kits can contain is BSA in them. Information on antibodies: The provided antibodies and their host vary in different kits.
Sample Volume:	100 µL
Assay Time:	4.5 h
Plate:	Pre-coated
Protocol:	1. Prepare all reagents, samples and standards 2. Add 100 µL standard or sample to each well. Incubate 2 hours at 37 °C 3. Aspirate and add 100 µL prepared Detection Reagent A. Incubate 1 hour at 37 °C 4. Aspirate and wash 3 times 5. Add 100 µL prepared Detection Reagent B. Incubate 30 minutes at 37 °C 6. Aspirate and wash 5 times 7. Add 90 µL Substrate Solution. Incubate 15-25 minutes at 37 °C 8. Add 50 µL Stop Solution. Read at 450 nm immediately.
Reagent Preparation:	1. Bring all kit components and samples to room temperature (18-25 °C) before use. If the kit will not be used up in one time, please only take out strips and reagents for present experiment, and leave the remaining strips and reagents in required condition. 2. Standard - Reconstitute the Standard with 1.0 mL of Standard Diluent, keep for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 32,000pg/mL. Firstly dilute the stock solution to 4,000pg/mL and the diluted standard serves as the highest standard (4,000pg/mL). Then prepare 7 tubes containing 0.5 mL Standard Diluent and use the diluted standard to produce a double dilution series. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 4,000pg/mL, 2,000pg/mL, 1,000pg/mL, 500pg/mL, 250pg/mL, 125pg/mL, 62.5pg/mL, and the last microcentrifuge tube with Standard Diluent is the blank as 0pg/mL. 3. Detection Reagent A and Detection Reagent B - If lyophilized reconstitute the Detection

Reagent A with 150µL of Reagent Diluent, keep for 10 minutes at room temperature, shake gently (not to foam). Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute them to the working concentration 100-fold with Assay Diluent A and B, respectively.
4. Wash Solution - Dilute 20 mL of Wash Solution concentrate (30x) with 580 mL of deionized or distilled water to prepare 600 mL of Wash Solution (1x).
5. TMB substrate - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Note:

1. Making serial dilution in the wells directly is not permitted.
2. Prepare standards within 15 minutes before assay. Please do not dissolve the reagents at 37 °C directly.
3. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals are completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10µL for one pipetting.
4. The reconstituted Standards, Detection Reagent A and Detection Reagent B can be used only once.
5. If crystals have formed in the Wash Solution concentrate (30x), warm to room temperature and mix gently until the crystals are completely dissolved.
6. Contaminated water or container for reagent preparation will influence the detection result.

Sample Collection:	**Serum:** Allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000 × g. Assay immediately or store samples in aliquot at -20°C or -80°C. Avoid repeated freeze/thaw cycles. **Plasma:** Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 × g within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C. Avoid repeated freeze/thaw cycles. **Biological Fluids:** Centrifuge samples for 20 minutes at 1000 × g. Remove particulates and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.
Calculation of Results:	Average the duplicate readings for each standard, control, and samples and subtract the average zero standard optical density. Construct a standard curve by plotting the mean O.D. and concentration for each standard and draw a best fit curve through the points on the graph or create a standard curve on log-log graph paper with the target concentration on the y-axis and absorbance on the x-axis. Draw the best fit straight line through the standard points and it can be determined by regression analysis.Using some plot software, for instance, curve expert

Application Details

	1.30, is also recommended. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
Assay Precision:	**Intra-assay Precision (precision within an assay):** Three samples with low, medium and high levels of the target antigen were tested twenty times on one plate, respectively. **Inter-assay Precision (precision between assays):** Three samples with low, medium and high levels of the target antigen were tested on three different plates, eight replicates in each plate. **CV (%)** = SD/mean X 100 • Intra-assay: CV less than 10 % • Inter-assay: CV less than 12 %
Restrictions:	For Research Use only

Handling

Precaution of Use:	The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.
Handling Advice:	The stability of kit is determined by the loss rate of activity. The loss rate of this kit is less than 5 % within the expiration date under appropriate storage condition. To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.
Storage:	4 °C/-20 °C
Storage Comment:	• For unopened kit: All the reagents should be kept according to the labels on vials. The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20°C upon receipt while the others should be at 4°C. • For opened kit: When the kit is opened, the remaining reagents still need to be stored according to the above storage condition. Besides, please return the unused wells to the foil pouch containing the desiccant pack, and reseal along entire edge of zip-seal. Note: It is highly recommended to use the remaining reagents within 1 month provided this is within the expiration date of the kit. • For ELISA kit, 1 day storage at 37°C can be considered as 2 months at 4°C, which means 3 days at 37°C equaling 6 months at 4°C.
Expiry Date:	6 months

Publications

Product cited in:	Meng, Du, Li, Gao, Song, Lu, Tu, Jiang, Guo: "The synergistic mechanism of total saponins and

Publications

flavonoids in Notoginseng-Safflower pair against myocardial ischemia uncovered by an integrated metabolomics strategy." in: **Biomedicine & pharmacotherapy**, Vol. 130, pp. 110574, (2020) (PubMed).

Onyimba, Coronado, Garton, Kim, Bucek, Bedja, Gabrielson, Guilarte, Fairweather: "The innate immune response to coxsackievirus B3 predicts progression to cardiovascular disease and heart failure in male mice." in: **Biology of sex differences**, Vol. 2, pp. 2, (2011) (PubMed).

Images

ELISA

Image 1.