(For scientific research use only, not for clinical diagnosis!)

Porcine F2-Isoprostane (iso-PGF2α) ELISA

Kit Instructions for Use Specifications:

48T/96T

Please read the instructions carefully before use. If you have any questions, please contact us through the following methods: Official hotline: 025-5229-8998 Sales department phone: 13914481711 Technical phone: 15950492658 Contact email: 3224949330@qq.com Company website: www.byabscience.cn For specific shelf life, please see the reagents Box packaging label. Please use the kit within the shelf life.

When contacting us, please provide the product number and production date (see box label) so that we can serve you more efficiently.

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Website: www.byabscience.cn **Official hotline: 025-5229-8998** **Supervision phone number:**

Kit performance Detection range: 125 pg/mL-2000 pg/mL.

Sensitivity: The lowest detectable dose is less than 10 pg/mL.

Precision: intra-batch variation coefficient CV% is less than 10%; inter-batch variation coefficient CV% is less than 15%.

Recovery rate: The recovery rate is between 85%-115%.

Specificity: This kit recognizes natural and recombinant porcine F2-isoprostane (iso-PGF2α) and has no crossover with structural analogs. Stability: Stored at 2℃-8℃, validity period is 6 months.

Purpose: Used to detect the concentration of porcine F2-isoprostane (iso-PGF2α) in samples such as serum, plasma, cell culture supernatant and tissue.

Shelf life: Stored at 2℃-8℃, valid for 6 months.

Experimental principle

试剂盒采用酶联免疫分析方法。采用生物素标记 iso-PGF2α，纯化的抗 iso-PGF2α抗体包被微孔板，在竞争抑制反应中，一定量的固相抗体与生物素标记 iso-PGF2α及非标记抗原（校准品或标本）进行抑制竞争反应，抗体与生物素标记的 iso-PGF2α结合量受非标记抗原量所抑制，非标记抗原量多,抗体与生物素标记的 iso-PGF2α结合就少，反之结合就多；反应平衡后，形成固相抗体-生物素化 iso-PGF2α，再加入酶标记的亲和素，形成固相抗体-生物素化 iso-PGF2α-酶标-亲合素复合物。经加底物显色后，用酶标仪在 450nm 波长下测定吸光度（OD 值）。随着 iso-PGF2α浓度的升高，OD 值逐渐下降呈良好的线性关系。本试剂盒具有灵敏度高、特异性强、重复性好、操作简单、快速等特点，对血清中 iso-PGF2α的减少或升高有可靠的检出性能。

试剂盒组分与保存 未开封的试剂盒保存在 2-8 度，不得使用过期试剂盒。

组分	**48 孔配置**	**96 孔配置**	开封后储存
预包被酶标板	48T	96T	**2-8℃14 天**
标准品	0.3mL*6 管	0.3mL*6 管	**2-8℃14 天**
样本稀释液	3 ml	6 ml	2-8℃180 天
生物素化抗原	3 ml	6 ml	**2-8℃14 天**
HRP 标记亲和素	3 ml	6 ml	2-8℃180 天
显色底物 A	3 ml	6 ml	2-8℃180 天
显色底物 B	3 ml	6 ml	2-8℃180 天
终止液	3 ml	6 ml	2-8℃180 天
20×洗液	15 ml	25 ml	2-8℃180 天
封板膜	2 张	2 张	
说明书	1 份	1 份	
自封袋	1 个	1 个	

校准品浓度依次为：2000 、1000 、500 、250 、125 、0 pg/mL。

注意：1：使用前请检查试剂盒中试剂的标签和数量与表格是否一致。

2：如果试剂盒的组份需要再次使用，请确保上一次使用之后没有被污染。

3：酶标板单次未使用完，要谨记密封放到 2-8℃保存。

试验所需自备试验器材 (不提供，但可协助购买)

1) Microplate reader capable of detecting absorbance at 450 nm 2) Pipette, pipette tip, and sample addition tank 3) 37°C incubator or water bath 4) Test tubes, centrifuge tubes, measuring cylinders, etc. for preparing reagents 5) Distilled water or deionized water Ionized water

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6) Vortex oscillator and microplate oscillator

Notes: 1) For scientific research use only,

not for clinical diagnosis.

2) Use within the validity period marked on the kit. Expired products must not be used.

3) Do not mix with kits or components from other manufacturers. Use the sample diluent provided with the kit.

4) If the sample value is higher than the highest standard concentration value, please dilute the sample appropriately and then re-measure.

5) Human anti-mouse and other heterophilic antibodies present in the sample to be tested will interfere with the test results. Please eliminate this factor before testing.

6) The test results obtained by other methods are not directly comparable to the test results of this kit.

7) Please wear a lab coat and latex gloves for protection during the test. Especially when testing blood or other body fluid samples, please follow the national biological laboratory safety protection regulations.

8) Carry out incubation strictly according to the specified time and temperature to ensure accurate results. All reagents must reach room temperature 20-25°C before use. Store reagents refrigerated immediately after use.

9) Improper plate washing can lead to inaccurate results. Make sure to absorb as much liquid as possible from the wells before adding substrate. Do not allow the microwells to dry out during incubation.

10) Eliminate residual liquid and fingerprints on the bottom of the plate, otherwise it will affect the OD value.

11) The substrate chromogenic solution should be colorless or very light in color.

12) Avoid cross-contamination of reagents and specimens to avoid erroneous results.

13) Avoid direct exposure to strong light during storage and incubation.

14) The microplate reader used for detection needs to be equipped with a filter capable of detecting a wavelength of 450±10nm, and the optical density range is between 0-3.5. It is recommended to preheat 15 minutes in advance before use.

15) The EP tubes and suction tips used in the test are single-use and are strictly prohibited from mixing.

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Sample preparation and storage

The following lists only general guidelines for sample collection and preservation. During the collection and storage of all samples, sodium azide shall not be used as a preservative. If the sample is not analyzed immediately, it should be aliquoted and stored frozen, and repeated freezing and thawing should be avoided.

Cell culture supernatant - centrifuge to remove precipitate, analyze immediately or aliquot and store frozen at -20°C.

Serum - Collect blood in a clean test tube, coagulate at room temperature for 30 minutes, centrifuge at 2000×g for 20 minutes, and collect serum. Analyze immediately or aliquot and store frozen at -20°C.

Plasma—anticoagulate with heparin, citrate, or EDTA, and centrifuge at 2000×g for 20 minutes at 2-8°C within 30 minutes of blood draw. To eliminate the influence of platelets, it is recommended to further centrifuge at 10,000 × g for 10 minutes at 2-8°C. Analyze immediately or aliquot and store frozen at -20°C.

Cell lysis buffer - For adherent cells, remove the culture medium and wash with PBS, normal saline or serum-free culture medium. Add an appropriate amount of lysis solution and pipet several times with a gun to fully contact the lysate and cells. Typically after 10 seconds, cells are lysed. For suspended cells, collect the cells by centrifugation and wash them with PBS, physiological saline or serum-free culture medium. Add an appropriate amount of lysis solution, blow the cells with a gun, and flick them with your fingers to fully lyse the cells. After full lysis, centrifuge at 10000-14000×g for 3-5 minutes and take the supernatant. Analyze immediately or aliquot and store frozen at -20°C.

Tissue homogenate - rinse the tissue with pre-cooled PBS (0.01M, pH=7.4) to remove residual blood (lysed red blood cells in the homogenate will affect the measurement results), weigh and cut the tissue into pieces. Mix the minced tissue with the corresponding volume of PBS (generally

at a weight-to-volume ratio of 1:9, for example, 1g of tissue sample corresponds to 9mL of PBS. The specific volume can be adjusted appropriately according to experimental needs and recorded. It is recommended to add Protease inhibitor) was added to a glass homogenizer and ground thoroughly on ice. In order to further lyse tissue cells, the homogenate can be sonicated or repeatedly frozen and thawed. Finally, centrifuge the homogenate at 5000 × g for 5 to 10 minutes, and take the supernatant for detection.

Urine - Collect in sterile tubes and centrifuge at 2000×g for 20 minutes. Carefully collect the supernatant. If a precipitate forms, centrifuge again.

Reagent preparation 1. Before use, all components must be rewarmed for at least 60 minutes to ensure sufficient rewarming to room temperature.

2. Concentrated washing liquid: The concentrated washing liquid taken out from the refrigerator will produce crystals. This is a normal phenomenon. Heating in a water bath will completely dissolve the crystals. Concentrated detergent and distilled water, dilute 1:20, that is, 1 part of concentrated detergent, add 19 parts of distilled water.

Operating procedures: Return all reagents and components to room temperature first. It is recommended to do duplicate holes for standards, quality control materials and samples.

1. Prepare the working solution of various components of the kit according to the method described in the previous instructions.

2. Take out the required slats from the aluminum foil bag, seal the remaining slats in a ziplock bag and return it to the refrigerator.

3. Take out the pre-coated plate from the sealed bag and set up a blank control well without adding any liquid; set up 2 wells for each calibrator and add 50 µl of the corresponding calibrator into each well; add the serum to be tested directly to each of the remaining detection holes. Or 50µl of quality control product.

4. Add 50 µl of biotinylated antigen to all wells except the blank well, mix well, attach sealing film, and incubate at 37°C for 60 minutes.

5. Manual plate washing: discard the liquid in the wells, fill each well with washing solution, let stand for 10 seconds and spin dry, repeat 3 times and pat dry. Wash the plate with a plate washer: select the washing program 3 times and pat dry after washing the plate.

(Tip: In order to obtain ideal experimental results, the residual liquid must be completely removed. After washing the plate, please proceed to the next step immediately and do not let the microplate dry.) 6. Add 50 µl of enzyme-labeled avidin to each well (blank (Excluding control wells), mix well, affix sealing film, and incubate at 37°C for 30 minutes.

7. Manual plate washing: discard the liquid in the wells, fill each well with washing solution, let stand for 10 seconds and spin dry, repeat 3 times and pat dry. Wash the plate with a plate washer: select the washing program 3 times and pat dry after washing the plate.

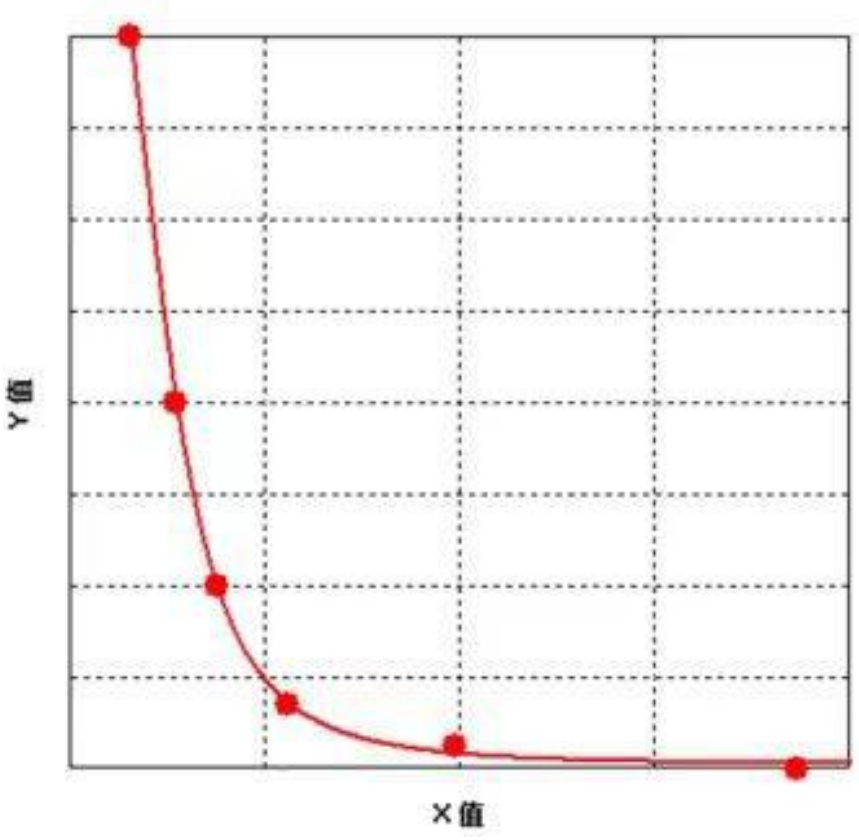
8. Add 50 µl of chromogen A and 50 µl of chromogen B to each well. After shaking and mixing, place at 37℃ to develop color in the dark for 15 minutes. Add 50 µl of stop solution to each well.

9. Use a microplate reader to read, take the wavelength of 450nm, first use the blank control well to adjust the zero point, and then measure the optical density value (OD value) of each well.

Result calculation

9. Use the concentration of the standard substance as the abscissa and the corresponding absorbance (OD value) as the ordinate. Use computer software and four-parameter Logistic curve fitting (4-pl) to create a standard curve equation. Through the absorbance (OD value) of the sample value), use the equation to calculate the concentration value of the sample. [Calculation using ELISA Calc software]

10. If the sample is diluted, the concentration value measured by the above method must be multiplied by the dilution factor to obtain the final concentration of the sample. Note: Experimenters need to establish a standard curve based on their own experiments. For each test, a standard curve must be established for each enzyme plate. The following curves are for reference only!



(Schematic diagram of the music, for reference only)

[Problem Analysis] If the experimental results are not good, please take pictures of the color development results in time, save the experimental data, keep the used strips and unused reagents, and then contact our company's technical support to solve the problem for you. At the same time, you can also refer to the following information:

[Questions and Answers]

Problem description	Possible reasons	Corresponding countermeasures Corresponding countermeasures
standard curve gradient difference	Incorrect liquid aspiration or	Check pipettes and tips
	Equilibration time is too short	Ensure sufficient balancing time
	Incomplete washing	Ensure the washing time and number of washings and the amount of liquid
Very weak or colorless	Incubation time too short	Ensure adequate incubation time
	The experimental temperature is incorrect	Use recommended experimental temperatures
	Insufficient reagent volume or missing addition	Check the liquid aspirating and adding process to ensure that all reagents are added in order and in
	Incorrect dilution	
	Enzyme label inactivation or substrate failure	Mix enzyme conjugate and substrate and check by rapid color development
Reading value is low	Microplate reader settings are incorrect	Check the wavelength and filter
		Turn on the microplate reader and preheat it in advance
Large coefficient of variation	Adding fluid incorrectly	Check the filling situation
High background value	The working concentration of the	Use the recommended dilution
	Incomplete washing of enzyme plate	Ensure that each step of cleaning is complete; if using an automatic plate washer, please check whether all outlets are blocked;
	The lotion is contaminated	Prepare fresh lotion
Low sensitivity	Improper storage of ELISA kits	Store relevant reagents according to
	Not terminated before reading	Stop solution should be added to