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

Bovine Progesterone (P4)

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.

Nanjing BYabscience technology Co.,Ltd

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

Kit performance Detection range: 0.938 ng/mL–30 ng/mL.

Sensitivity: The lowest detectable dose is less than 0.1 ng/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 bovine progesterone (P4) and has no crossover with structural analogs.

Stability: Stored at 2℃-8℃, validity period is 6 months.

Purpose: Used to detect the concentration of bovine progesterone (P4) in samples such as serum, plasma, cell culture supernatant and tissue. Shelf life: Stored at 2℃-8℃, valid for 6 months.

Experimental principle

The progesterone (P4) content in the samples was detected using enzyme-linked immunosorbent competition method. First, coat the microplate with goat anti-rabbit to make a solid-phase secondary antibody. Then add the sample to be tested, horseradish peroxidase-labeled progesterone (P4) and anti-progesterone (P4) antibody to form a coating. Secondary antibody-anti-progesterone (P4) antibody-progesterone (P4) (HRP) complex, the binding amount of labeled progesterone (P4) is inversely proportional to the amount of [Chinese name] in the sample. After color development, measure the absorbance value (OD value) on a microplate reader, fit the concentration-absorbance curve through a computer or drawing, and back-calculate the progesterone (P4) content in the serum to be tested.

Kit components and storage: Store unopened kits at 2-8 degrees Celsius. Do not use expired kits.

Components	**48-well configuration**	**96-well configuration**	Store after opening
Pre-coated enzyme plate	48T	96T	**2-8℃14 days**
Standard product	0.3mL*6 tubes	0.3mL*6 tubes	**2-8℃14 days**
样本稀释液	3 ml	6 ml	2-8℃180 天
抗体	3 ml	6 ml	**2-8℃14 天**
HRP 标记抗原	3 ml	6 ml	**2-8℃14 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：30 、12 、6 、4 、0.938 、0 ng/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）能够检测 450 nm 吸光度的酶标仪 2）移液器及枪头、加样槽 3）37℃恒温箱或水浴锅 4）准备试剂用的试管、离心管、量筒等 5）蒸馏水或去离子水

6）涡旋振荡器、微孔板振荡器

注意事项 1）仅供科研使用，不得用于临床诊断。

2）在试剂盒标示的有效期内使用，过期产品不得使用。

3）跟其他厂家的试剂盒或者组分不能混用，使用试剂盒配套的样品稀释液。

4）如果样本值高于最高标准品浓度值，请将样本适当稀释后，再重新测定。

5）待测样本中存在的人抗鼠等异嗜抗体会干扰检测结果，检测前，请排出该因素。

6）通过其他方法得到的检测结果，与本试剂盒测定结果不具有直接的可比性。

7) 试验中请穿着实验服并戴乳胶手套做好防护工作。特别是检测血液或者其他体液样品时，请按国家生物试验室安全防护条例执行。

8) 严格按照规定的时间和温度进行温育以保证准确结果。所有试剂都必须在使用前达到室温 20-25℃。使用后立即冷藏保存试剂。

9) 洗板不正确可以导致不准确的结果。在加入底物前确保尽量吸干孔内液体。温育过程中不要让微孔干燥掉。

10) 消除板底残留的液体和手指印，否则影响 OD 值。

11）底物显色液应呈无色或很浅的颜色。

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.

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 must 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 effect 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 once 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.

3. Substrate: Substrate solutions A and B, mix thoroughly at a volume of 1:1 before use, and use within 15 minutes after mixing.

Operating procedures: Return all reagents and components to room temperature first. For standards, quality control materials and samples, it is recommended to make duplicate holes.

1. Prepare the working solutions 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. Before each experiment, according to the experimental detection quantity, take 1/10 of the volume of the enzyme-labeled antigen and mix it with the enzyme-labeled antigen to make an enzyme-labeled antigen mixture. For example: take 3ml of enzyme-labeled antigen, add 0.3ml of antibody and mix to make a mixed solution.

4. Take out the pre-coated plate from the sealed bag, and set up a blank control well without adding any liquid; set up two holes for each calibrator in turn, and add 50 μl of the corresponding calibrator to each

well; add quality directly to each of the remaining detection holes. 50μl of control substance or serum to be tested.

5. Add 50 μl of enzyme label mixture to each well (except the blank control well), mix thoroughly, attach a sealing film, and incubate at 37℃ for 1 hour.

6. 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.

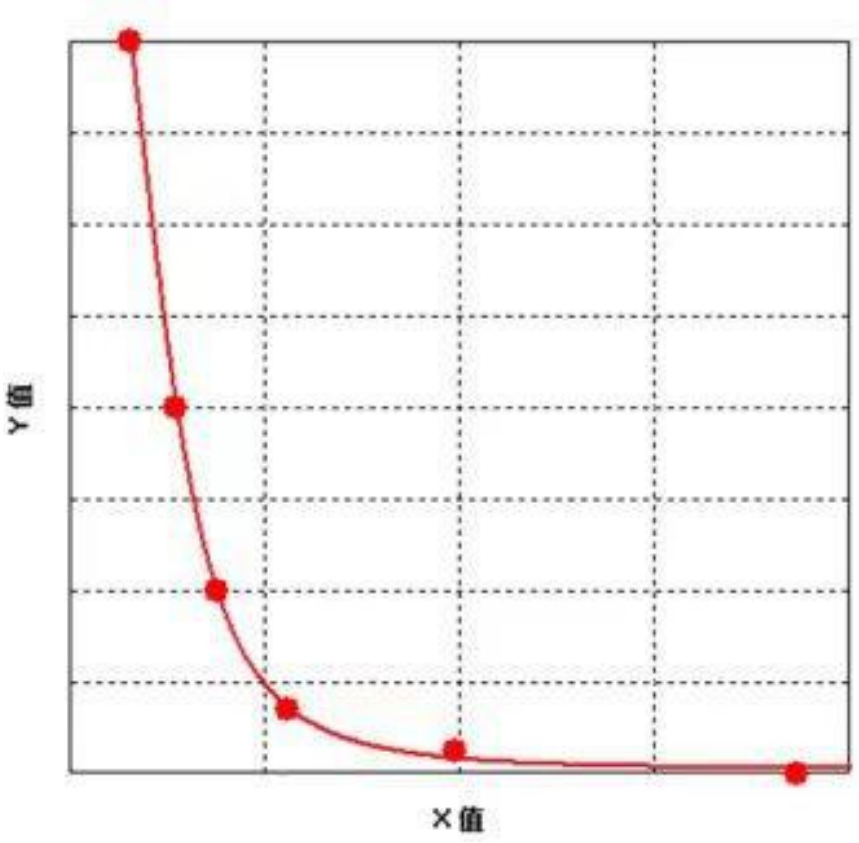
7. Add 50 μl of developer solution A and 50 μl of developer solution B to each well. After shaking and mixing, place at 37℃ to develop color in the dark for 15 minutes. Add 50 μl of stop solution to each well.

8. Use a microplate reader to read. For a 450nm single-wavelength microplate reader, you need to first adjust the zero point with a blank control hole, and then measure the absorbance 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