（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Progesterone (P) ELISA Kit

Catalog No: E-HD-E185

96T/96T*5

Version Number:	V1.0
Replace version:	/
Revision Date:	2025.09.03

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.vetassay-elab.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

This ELISA kit uses Competition-ELISA as the principle to detect the progesterone (P) in human serum. The ELISA Microtiter plate provided in this kit has been pre-coated with goat anti-rabbit antibody. Sample are added to the ELISA Microtiter plate wells, then add HRP Conjugate and Antibody working solution in sequentially, to form "goat anti-rabbit antibody- anti-P antibody-HRP Conjugate" compound. The substrate reagent is added to initiate the color developing reaction. There is a negative correlation between the OD value of samples and the concentration of P. The concentration of P in the samples can be calculated by comparing the OD of the samples to the standard curve.

Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	0.5/0.5*5 mL each (0, 0.2, 0.6, 1.8, 5.4, 20.0 ng/mL)
HRP Conjugate	6/6*5 mL
Antibody Working Solution	6/6*5 mL
Substrate Reagent A	7/7*5 mL
Substrate Reagent B	7/7*5 mL
Stop Solution	7/7*5 mL
20×Concentrated Wash Buffer	15/15*5 mL
Plate Sealer	3/15 pieces
Sealed Bag	1/5 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Other materials required but not supplied

Microplate Reader with 450 nm wavelength filter or dual-wavelength (450/630 nm)
High-precision transferpettor, EP tubes and disposable pipette tips
37℃ Incubator
Deionized or distilled water
Absorbent paper

Notes

1. Please read the manual carefully before use, changes of operation may result in unreliable results.
2. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly executed. All the waste should be handled as contaminant.
3. The stop solution is corrosive, it should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contact it carelessly.

4. The ELISA Microtiter plate obtained from cold storage conditions should be adjusted to room temperature before use. The unused plate should be kept in a sealed bag with desiccant.
5. Concentrated washing liquid at low temperature condition is easy to crystallization, it should be adjusted to room temperature in order to dissolve completely before use.
6. The results shall depend on the readings of the micro-plate Reader.
7. **Each reagent is optimized for use in the E-HD-E185. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-HD-E185 with different lot numbers.**
8. All the samples and waste material should be treated as infective material according to the relevant rules of biosafety.

Storage and expiry date

Store at 2-8℃. Avoid freeze.

Please store the opened plate at 2-8℃, the shelf life of the opened kit is up to 1 month.

Expiry date: expiration date is on the packing box.

Sample preparation

1. **Serum:** Human serum can be used as detected sample. Fresh collected samples should be fully centrifuged, then take clear liquid for test. Suspended fibrous protein may cause a false positive. Samples can be stored at 2-8℃ for one week and stored at -20℃ for more than a week. Avoid freeze-thaw cycles. Freezing samples should be mixed fully before test.
2. **Wash Buffer:** The **20×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, and then dilute it with deionized water at 1:19.

Assay procedure

Restore all reagents and samples to room temperature (25℃) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8℃.

1. **Number:** number the sample, standard in order (multiple well), and keep a record of standard wells and sample wells. Set 1 well for blank control (without adding any liquid). Blank well is not necessary for dual-wavelength detection.
2. **Add Sample:** Add 50 μL of sample and standard into each numbered wells.
3. **HRP conjugate:** add 50 μL of **HRP Conjugate** to each well except the blank control well, then add 50 μL of **Antibody working solution** to each well in sequentially, mix fully.
4. **Incubate:** gently tap the plate to mix thoroughly. Cover the ELISA Microtiter plate with sealer. Incubate for 1 h at 37℃ in shading light.
5. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately fully fill **Wash Buffer** to each well and wash. Repeat the wash procedure for 3 times, 10s intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).

6. **Add substrate:** add 50 µL of **Substrate Reagent A** and 50 µL of **Substrate Reagent B** to each well. Gently tap the plate to mix thoroughly. Cover with a new plate sealer. Incubate for 15 min at 37℃ in shading light.
7. **Stop reaction:** add 50 µL of **Stop Solution** to each well, gently tap the plate to mix thoroughly.
8. **OD Measurement:** set the Microplate Reader wavelength at 450 nm (it is recommended to set the dual wavelength at 450 nm/630 nm) to detect A value of each well. Blank well is not essential when using dual wavelength 450 nm/630 nm for detection.

Result analysis

1. The dual wavelength enzyme-linked immunosorbent assay (ELISA) reader does not require a blank control well or zero point adjustment. A single wavelength enzyme-linked immunosorbent assay (ELISA) reader must be equipped with a blank control well. First, zero the blank control well, and then measure. If there is no zero adjustment, the absorbance value of each hole needs to be subtracted from the absorbance value of the blank control hole.
2. Absorbance(%)=$A/A_0 \times 100\%$

   A: Average absorbance of standard or sample

   A_0: Average absorbance of 0 ppb Standard
3. Drawing and calculation of standard curve

   Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot. Add average absorbance value of sample to standard curve to get corresponding concentration.

Reference value

1. Male: 0.1 - 3.5 ng/mL
2. Female: Follicular phase 0.1 - 2.1 ng/mL; Luteal phase 1.5 - 35 ng/mL; Menopause 0.1 - 1.5 ng/mL
3. By measuring the serum of 200 healthy individuals, statistical processing (normal distribution, 95% confidence interval) was conducted to determine the reference range. Due to individual differences among people, as well as variations in region, age and gender, the determined reference ranges may differ. Therefore, the reference ranges provided in this manual are for reference only. Each laboratory should establish its own reference ranges based on the normal population in their respective regions.

Limitations of test method

1. Severe hemolysis and chylous blood may affect the test results.