



Technical Manual

Human Progesterone (P) ELISA Kit

- Catalogue Code: HDES0117
- Antibody ELISA Kit
- Research Use Only

1. Test principle

Enzyme linked immunosorbent assay (ELISA) was used to detect the content of progesterone (P) in serum. Firstly, a sheep anti rabbit coated microplate was used to prepare a solid-phase secondary antibody. Then, the test serum, horseradish peroxidase labeled progesterone, and anti-progesterone antibody were added to form a coated secondary antibody anti progesterone antibody progesterone (HRP) complex. The binding amount of labeled progesterone was negatively correlated with the amount of progesterone in the serum. After color development, the absorbance value (OD value) was measured using an enzyme-linked immunosorbent assay (ELISA) reader, and the concentration absorbance curve was fitted using a computer or graph to calculate the progesterone content in the serum to be tested.

2. Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	0.5 mL each (0, 1, 3, 10, 30, 70 ng/mL)
HRP Conjugate	6 mL
Antibody	6 mL
Quality Control Solution	1 mL*2 Level 1 is (1.8~4.2ng/mL), Level 2 is (18~42ng/mL)
Substrate Reagent A	7 mL
Substrate Reagent B	7 mL
Stop Solution	7 mL
20×Concentrated Wash	15 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

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

3. 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° C Incubator or water bath
- Deionized or distilled water
- Absorbent paper

4. 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 HDES0117. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other HDES0117 with different lot numbers.**
8. All the samples and waste material should be treated as infective material according to the relevant rules of biosafety.

5. Storage and expiry date

Store at 2-8° C. Avoid freeze.

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

Expiry date: expiration date is on the packing box.

6. Sample preparation

1. Collect venous **Serum** and store it at 2-8° C for 2 days. It should be stored below -15° C for 3 months.
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.

7. Assay procedure

Restore all reagents and samples to room temperature (25° C) 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° C.

1. Remove the pre-packaged board from the sealed bag, set a blank control hole, and do not

- add any liquid; Two wells are set up for each standard substance, and 50 μ L of the **Standard Liquid** is added to each well; Add 50 μ L of **Quality Control Solution** or test **Serum** directly to each of the remaining detection wells.
2. Add 50 μ L of **HRP Conjugate** to each well (except for blank control wells), then add 50 μ L of **Antibody** to each well in the same order, mix thoroughly, apply a sealing plate membrane, and incubate at 37 ° C for 1 hour.
 3. Remove the plate sealer and aspirate the liquid of each well. Repeat the washing procedure for 3 times with **Wash Buffer** and immerse for 30-60 sec each 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).
 4. Add 50 μ L of **Substrate Reagent A** and 50 μ L of **Substrate Reagent B** to each well. Gently tap the plate to mix thoroughly. Cover with a new plate sealer. incubate at 37° C for 15 min in shading light.
 5. Add 50 μ L of **Stop Solution** to each well, gently tap the plate to mix thoroughly.
 6. **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.

8. 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. Calculate the percentage binding rate: Divide the OD value of the calibration standard or sample by the OD value of S0 (0 ng/mL of **Standard Liquid**) to obtain the percentage binding rate.
3. Hand working diagram: Use a logit In coordinate paper with the concentration of the calibration standard as the horizontal axis and the corresponding percentage binding rate as the vertical axis to draw a smooth straight line. Find the concentration value on the curve based on the percentage binding rate of the serum to be tested.
4. Computer: The concentration is calculated by the computer.

9. Limitations of test method

1. Severe hemolysis and chyle blood may affect the test results.
2. The sample tested for this product with a concentration of 3200 μ IU/mL did not exhibit a "hook effect".

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