Version 21-04/24

User's Manual

Progesterone ELISA Kit

REF **DEIA284**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

Enzyme Immunoassay for the Quantitative Determination of Progesterone in Plasma and Serum.

General Description

Progesterone is a sexual hormone and belongs to the group of gestagens. These female sexual hormones are produced in the placenta and adrenal cortex. The female sexual hormones, of which progesterone is the main representative, influence the female sexual organs together with the estrogens. The special function of the gestagens, especially progesterone lies in the regulation of the ovary nidation, the support of the pregnancy and the coordination of the body temperature (1,2,3). The secretion of the gestagens is controlled by the pituitary gland. In the blood, only 2-10% of progesterone appears unbound. The remainder is coupled to corticoidbinding globulin (CBG), sex-hormone-binding globulin (SHBG) and albumin. Normally the serum concentration of the hormone is low in animal blood. In plasma of pregnant animals, however, or in plasma of animals illegally treated with hormones, significantly higher levels can be found. The Progesterone ELISA is a quick, economical and sensitive method to detect progesterone in animal plasma or serum. After an appropriate sample preparation, 40 samples can be tested in duplicate within 140 minutes.

Principles of Testing

The Progesterone quantitative test is based on the principle of the enzyme linked immunosorbent assay. A progesterone conjugate is bound on the surface of a microtiter plate. Progesterone containing samples or standards and an antibody directed against progesterone are given into the wells of the microtiter plate. Immobilized and free progesterone compete for the antibody binding sites. After one hour incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A peroxidase conjugate directed against the progesterone antibody is given into the wells and after another hour incubation, the plate is washed again. Then a substrate solution is added and incubated for 20 minutes, resulting in the development of a blue colour. The colour development is inhibited by the addition of a stop solution, and the colour turns yellow. The yellow colour is measured photometrically at 450 nm. The concentration of progesterone is indirectly proportional to the colour intensity of the test sample.

Reagents And Materials Provided

The kit contains reagents for 96 determinations. They have to be stored at 2-8°C. Expiry data are found on the labels of the bottles and the outer package.

1. Microtiter plate consisting of 12 strips with 8 breakable wells each, coated with progesterone conjugate.
2. Progesterone Standards (0; 1; 5; 10; 50; 100 ng/mL): 6 vials with 1.0 mL each, ready-touse.
3. Anti-Progesterone Antibody (mouse): 6 mL, dyed red, ready-to-use.
4. Conjugate (anti-rabbit-IgG-HRP): 15 mL, dyed red, readyto-use.
5. Substrate Solution (TMB): 15 mL; ready-to-use.
6. Stop Solution (0.5 M H_2SO_4): 15 mL; ready-touse.

7. Sample Diluent (PBS): 2 × 50 mL, ready-to-use. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.
8. Washing Solution (PBS + Tween 20): 60 mL as 10× concentrate, dyed blue. Dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.
9. Two plastic foils to cover the strips during the incubation.
10. Plastic bag to store unused microtiter strips.
11. Instruction Manual.

Materials Required But Not Supplied

1. 50, 100 and 1000 µL-micropipets
2. Microtiter plate shaker
3. ELISA reader (450 nm)
4. Freezer (-25/-60°C)
5. TBME (tertiary butyl-methyl-ether)
6. Petrol ether (boiling range 30-50°C)

Storage

Store kit at 2-8°C. It is stable up to the expiry date stated on the label of the box. Do not use kit beyond its expiry date.

Specimen Collection And Preparation

Serum/Plasma

To 1 mL plasma or serum 5 mL ether mixture (TBME/petrol ether 30/70(v/v), boiling range 30-50°C) is added in a glass vial. The vial is heavily shaken for 20 minutes. Afterwards the mixture is put into a freezer for 60 minutes at -25°C (or 30 minutes at -60°C) and the supernatant is decanted. The supernatant is evaporated to dryness at 60°C in a water bath. The residue is reconstituted by adding 0.5 mL sample diluent. It is heavily shaken for 1 minute and warmed to 37°C for 5 minutes. The last step is repeated two more times.

Assay Procedure

1. Prepare samples as described above.
2. Pipet 100 µL ready-to use standards or prepared samples in duplicate into the appropriate wells of the microtiter plate. Immediately add 50 µL progesterone antibody into each well.
3. Cover the microtiter plate with a plastic foil and incubate for 60 minutes at room temperature on a microtiter plate shaker (or 90 minutes without shaker).
4. Wash the plate three times as follows: Discard the contents of the wells (dump or aspirate). Pipet 300 µL of diluted washing solution into each well. After the third repetition empty the wells again and remove residual liquid by striking the plate against a paper towel. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbencies.

5. Pipet 100 µL of conjugate (anti-rabbit-IgG-HRP) into each well.
6. Cover the microtiter plate with a plastic foil and incubate for 60 minutes at room temperature on a microtiter plate shaker (or 90 minutes without shaker).
7. Wash the plate as outlined in 4.
8. Pipet 100 µL of substrate solution into each well.
9. Allow the reaction to develop in the dark (e.g. cupboard or drawer; the chromogen is light-sensitive) for 20 minutes at room temperature.
10. Stop enzyme reaction by adding 100 µL of stop solution (0.5 M H_2SO_4) into each well. The blue colour will turn yellow upon addition.
11. After thorough mixing, measure absorbance at 450 nm (reference wavelength 620 nm), using an ELISA reader. The colour is stable for 30 minutes.

Calculation

1. Calculate the average optical density (OD 450 nm) for each set of reference standards or samples.
2. Construct a standard curve by plotting the mean optical density obtained for each reference standard against its concentration in ng/mL on semilog graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis.
3. Using the mean optical density value for each sample, determine the corresponding concentration of progesterone in ng/mL from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. The diluted samples must be further converted by the appropriate dilution factor (0.5 for the above described extraction). The factor is dependent on the sample preparation procedure employed.

Evaluation

The following table contains an example for a typical standard curve. The binding is calculated as percent of the absorption of the 0 ng/mL standard. These values are only an example and should not be used instead of the standard curve which has to be measured in every new test.

Progesterone (ng/mL)	(% binding of 0 ng/mL)
0	100
1	67
5	49
10	37
50	15
100	11

Precision

The intra-assay variation of the testosterone test was determined to 3%.

Sensitivity

The sensitivity of the Progesterone ELISA is 0.2 ng/mL (based on the standard curve).

Specificity

Cross-reactivity relative to progesterone (=100%)

17α-Hydroxyprogesterone	5.0%
Hydroxycortison	0.3%
Pregnenolone	1.1%
Pregnanolone	0.5%

References

1. Hierholzer, K. et al; Endokrinologie, Bd. 2 (1977).
2. Reinboth, R; Vergleichende Endokrinologie (1980).
3. Davies, I. J, Ryan, K. J; Vitam. and Horm. 30, 223 (1971).