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17-OH Progesterone ELISA

Catalog No.: PG338S (96 Tests)

INTENDED USE

The Calbiotech, Inc. 17- hydroxylprogesterone (17-OHP) ELISA kit is intended for the quantitative determination of 17- OHP in serum. **For Research Use Only. For professional use only. Not for use in diagnostic procedures.**

SUMMARY AND EXPLANATION

Measures 17-hydroxyprogesterone, a key intermediate in cortisol and androgen biosynthesis. Applied in research on adrenal steroidogenesis, enzyme deficiencies (e.g., 21-hydroxylase), and hormonal flux during the menstrual cycle and pregnancy.

PRINCIPLE OF THE TEST

The Calbiotech 17 α -OH Progesterone (17-OHP) is based on the principle of competitive binding between 17-OHP in the test specimen and 17-OHP-HRP for a constant amount of Rabbit anti-17-OHP antibody. In the assay, goat anti-rabbit IgG-coated wells are incubated with 25 μ l 17-OHP standards, 50 μ l 17-OHP-HRP conjugate, and 50 μ l rabbit anti-17-OHP. HRP-labeled-17-OHP competes with 17-OHP in the standards and sample for a fixed number of binding sites of the specific anti-17-OHP antibody. Thus, the amount of 17-OHP peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of 17-OHP in the specimen increases. Unbound 17-OHP is then removed by the washing steps. TMB Substrate is added, resulting in the development of blue color. The color development is stopped with the addition of stop solution, and the absorbance is spectrophotometrically measured at 450nm. A standard curve is prepared relating color intensity to the concentration of 17-OHP.

| MATERIALS PROVIDED | | 96 TESTS |
|--------------------|--|----------|
| 1. | Microwells coated with Goat anti-Rabbit IgG | 12x8x1 |
| 2. | 17-OHP Standards: 6 vials (ready to use) | 0.5 mL |
| 3. | 17-OHP Controls: 2 vials (ready to use) | 0.5 mL |
| 4. | 17-OHP Antibody Reagent: 1 bottle (ready to use) | 7 mL |
| 5. | 17-OHP Assay Diluent: 1 bottle | 7 mL |
| 6. | 17-OHP 20X Enzyme Conjugate: 1 vial | 0.45 mL |
| 7. | TMB Substrate: 1 bottle (ready to use) | 12 mL |
| 8. | Stop Solution: 1 bottle (ready to use) | 12 mL |
| 9. | Wash concentrate 20X: 1 bottle | 25 mL |

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY

1. Store the kit at 2-8oC.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light.



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WARNINGS AND PRECAUTIONS

Potential biohazardous materials:

1. The standards contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. It is recommended that standards, control and serum samples be run in duplicate
5. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION AND HANDLING

This assay is designed for use with human serum or plasma samples obtained in compliance with applicable laws, regulations, and institutional policies. Handle and store samples using procedures appropriate for research use. Samples may be stored refrigerated (2–8 °C) for up to seven days, or frozen (–20 °C or below) for up to six months. Avoid repetitive freeze–thaw cycles.

REAGENT PREPARATION

1. **Working Enzyme Conjugate:** Prepare 1X working dilution at 1:20 with assay diluents as needed, e.g. 0.1ml of the stock conjugate in 1.9ml of assay diluent for 40 wells. The diluted conjugate has to be used on the same day.
2. **Wash Buffer:** Prepare 1X Wash Buffer by adding the contents of the bottle (25ml, 20X) to 475ml of distilled water. Store at room temperature (20-25°C).

ASSAY PROCEDURE

1. Place the desired number of coated strips into the holder.
2. Dispense 25µl 17-OHP standards, controls, and samples into appropriate wells.
3. Add 50µl of working dilution 17-OHP enzyme conjugate to all the wells.
4. Add 50 µl of 17-OHP Antibody Reagent to all wells.
5. Shake the microplate gently for 20-30 seconds to mix.
6. Incubate for 60 minutes at room temperature.
7. Briskly shake out the contents of the wells. Rinse the wells 3 times with 1X wash buffer. Strike the wells sharply on absorbent paper to remove residual water droplets.
8. Add 100µl of TMB substrate to each well.
9. Cover the microplate and incubate for 15 minutes at room temperature.
10. Add 50µl of stop solution to each well and gently mix until a uniform color, in each well, is obtained.
11. Read the absorbance in each well at 450nm within 15 minutes after adding the stop solution.

CALCULATION OF RESULTS

A standard curve is constructed as follows:

1. Calculate the average absorbance values for each set of standards and patient samples
2. To construct the standard curve, plot the mean absorbance of each 17-OHP standards (vertical axis) against its concentration in ng/ml (horizontal axis)
3. Draw the best-fit curve through the plotted points.
4. Read the absorbance for each unknown sample from the curve to determine the corresponding concentration of 17-OHP.

Example of a Typical Standard Curve

| | OD450nm | Conc. (ng/mL) |
|--------------|---------|---------------|
| Std 1 | 2.378 | 0 |
| Std 2 | 1.804 | 0.1 |
| Std 3 | 0.670 | 0.5 |
| Std 4 | 0.400 | 1 |
| Std 5 | 0.180 | 2.5 |
| Std 6 | 0.053 | 10 |