9051 Mira Mesa Blvd, #910094
San Diego, CA 92191
Tel: 760-390-3989

17-OH Progesterone (17-OHP) ELISA Kit

Name	17-OH Progesterone (17-OHP) ELISA Kit
INTENDED USE	The 17- hydroxylprogesterone (17-OHP) ELISA kit is intended for the quantitative determination of 17- OHP in serum.
PRINCIPLE OF THE TEST	The 17α-OH Progesterone (17-OHP) kit 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 25µl 17-OHP standards, 50µl 17-OHP-HRP conjugate, and 50µl 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
Microwells coated with Goat anti-Rabbit IgG	12x8x1
17-OHP Standards: 6 vials (ready to use)	0.5 ml
17-OHP Antibody Reagent: 1 bottle (ready to use)	7 ml
17-OHP Assay Diluent: 1 bottle	7 ml

17-OHP 20X Enzyme Conjugate: 1 vial	0.45 ml
TMB Substrate: 1 bottle (ready to use)	12 ml
Stop Solution: bottle (ready to use)	12 ml
Wash concentrate 20X: 1 bottle	25 ml

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MATERIALS NOT PROVIDED

Distilled or deionized water
Precision pipettes
Disposable pipette tips
ELISA reader capable of reading absorbance at 450 nm
Absorbance paper or paper towel
Graph paper

STORAGE AND STABILITY

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

WARNINGS AND PRECAUTIONS

Potential biohazardous materials:
The calibrator and controls 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, 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.
This kit is designed for research use only.
Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.

	The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
SPECIMEN COLLECTION AND HANDLING	Collect blood specimens and separate the serum immediately. Specimens may be stored refrigerated at (2-8C) for 5 days. If storage time exceeds 5 days, store frozen at (-20 C) for up to one month. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well. Do not use grossly lipemic specimens.
REAGENT PREPARATION	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. 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	Place the desired number of coated strips into the holder. Dispense 25µl 17-OHP standards, controls, and samples into appropriate wells. Add 50µl of working dilution N-17-OHP enzyme conjugate to all the wells. Add 50 µl of N-17-OHP Antibody Reagent to all wells. Shake the microplate gently for 20-30 seconds to mix. Incubate for 60 minutes at room temperature. 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. Add 100µl of TMB substrate to each well. Cover the microplate and incubate for 15 minutes at room temperature. Add 50µl of stop solution to each well and gently mix until a uniform color, in each well, is obtained. Read the absorbance in each well at 450nm within 15

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minutes after adding the stop solution.

CALCULATION OF RESULTS

A standard curve is constructed as follows:

Calculate the average absorbance values for each set of standards and patient samples

To construct the standard curve, plot the mean absorbance of each 17-OHP standards (vertical axis) against its concentration in ng/ml (horizontal axis)

Draw the best-fit curve through the plotted points.

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

EXPECTED VALUES

We recommend each laboratory to establish its own normal ranges, for the population it serves. Until then, literature values may be used as guidelines.

Sensitivity

The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 20 times in the same run.

Serum	No. of Replicates	Mean (ng/mL)	Standard Deviation	Mean + 2SD (Sensitivity)
Zero Standard	24	0.024	0.01	0.04

Correlation with a Reference ELISA kit:

Serum samples were tested by this ELISA kit and a reference ELISA kit. Results were as follows:

Correlation	Slope	Intercept
0.985	1.02	0.22

Precision

Intra-Assay

Serum	No. of Replicates	Mean (ng/mL)	Standard Deviation	Coefficient of Variation (%)
1	24	0.4	0.01	2.93
2	24	2.0	0.07	3.60
3	24	7.8	0.27	3.41

Inter-assay

Serum	No. of Replicates	Mean ng/ml	Standard Deviation	Coefficient of Variation (%)
1	24	0.4	0.01	4.68
2	24	2.0	0.11	5.45
3	24	8.0	0.29	3.67

Linearity

Two different patient samples were diluted with the sample diluent to 1/2, 1/4 and 1/8. 17-OHP values were calculated and results were corrected with the dilution factor.

	Original Value	Percentage of Recovery	Percentage of Recovery	Percentage of Recovery
Serum	ng/ml	1/2	1/4	1/8
1	7.8	90%	92%	92%
2	4.6	95%	101%	97%