

CALDON BIOTECH INC. Progesterone ELISA

Catalog No. PG072S
(96 tests)

INTENDED USE

For the quantitative determination of Progesterone concentration in human serum.

SUMMARY AND EXPLANATION

Progesterone is a C21 steroid which is synthesized from both tissue and circulating cholesterol. Cholesterol is transformed to pregnenolone which is then converted via a combined dehydrogenase and isomerase to progesterone. The principle production sites are the adrenals and ovaries and the placenta during pregnancy. The majority of this steroid is metabolized in the liver to pregnanediol and conjugated as a glucuronide prior to excretion by the kidneys. Progesterone exhibits a wide variety of end organ effects. The primary role of progesterone is exhibited by the reproductive organs. In males, progesterone is a necessary intermediate for the production of corticosteroids and androgens. In females, progesterone remains relatively constant throughout the follicular phase of the menstrual cycle. The concentration then increases rapidly following ovulation and remains elevated for 4-6 days and decreases to the initial level 24 hours before the onset of menstruation. In pregnancy, placental progesterone production rises steadily to levels of 10 to 20 times those of the luteal phase peak. Progesterone measurements are thus performed to determine ovulation as well as to characterize luteal phase defects. Monitoring of progesterone therapy and early stage pregnancy evaluations comprise the remaining uses of progesterone assays. Progesterone EIA kits are designed for the measurement of total progesterone in human serum or plasma.

PRINCIPLE OF THE TEST

progesterone EIA is based on the principle of competitive binding between progesterone in the test specimen and progesterone-HRP conjugate for a constant amount of rabbit anti-progesterone. In the incubation, goat anti-rabbit IgG-coated wells are incubated with 25 μ l progesterone standards, controls, patient samples, 100 μ l progesterone-HRP Conjugate Reagent and 50 μ l rabbit anti-progesterone reagent at room temperature (18-25°C) for 90

minutes. During the incubation, a fixed amount of HRP-labeled progesterone competes with the endogenous progesterone in the standard, sample, or quality control serum for a fixed number of binding sites of the specific progesterone antibody. Thus, the amount of progesterone peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of progesterone in the specimen increases. Unbound progesterone peroxidase conjugate is then removed and the wells washed. Next, a solution of TMB Reagent is then added and incubated at room temperature for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of 1N HCl, and the absorbance is measured spectrophotometrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled progesterone in the sample. A standard curve is obtained by plotting the concentration of the standard versus the absorbance. The progesterone concentration of the specimens and controls run concurrently with the standards can be calculated from the standard curve.

MATERIALS PROVIDED

1. Microwells coated with Goat Anti-Rabbit IgG (12x8x1 wells). 96 wells.
2. Standard: 6 vials (0.5 mL each). Ready to use.
3. Rabbit Anti-Progesterone Reagent (7 mL) Ready to use.
4. Enzyme Conjugate: 1 bottle (12 mL). Ready to use.
5. Controls: 1& 2 (0.5 mL each) Ready to use.
6. TMB Substrate: 1 bottle (11 mL). Ready to use.
7. Stop Solution: 1 bottle (11 mL). Ready to use.

STORAGE AND STABILITY

1. Store the kit at 2 - 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.

WARNINGS AND PRECAUTIONS

1. 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, 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. This test kit is designed for in vitro diagnostic use only.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that serum samples be run in duplicate.
6. 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 HANDLING

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20°C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed.
5. Do not use grossly lipemic specimens.
6. **Please note:** Samples containing sodium azide should not be used in the assay.

REAGENTS PREPARATION

1. All reagents should be allowed to reach room temperature (18-25 °C) before use.
2. Reconstitute each lyophilized standard with 2.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. Reconstituted standards should be stored sealed and are stable for 30 days at 2-8°C.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.

2. Dispense 25µl of standards, specimens and controls into appropriate wells.
3. Dispense 100µl of Progesterone-HRP Conjugate Reagent into each well.
4. Dispense 50µl of rabbit anti-progesterone reagent to each well. Thoroughly mix for 30 seconds. It is very important to mix completely.
5. Incubate at room temperature (18-25°C) for 90 minutes.
6. Rinse and flick the microwells 5 times with distilled or deionized water. (Please do not use tap water.)
7. Dispense 100µl of TMB Reagent into each well. Gently mix for 5 seconds.
8. Incubate at room temperature (18-25°C) for 20 minutes.
9. Stop the reaction by adding 100 µl of Stop Solution to each well.
10. Gently mix 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
11. Read absorbance at 450 nm with a microtiter well reader within 15 minutes.

CALCULATION OF RESULTS

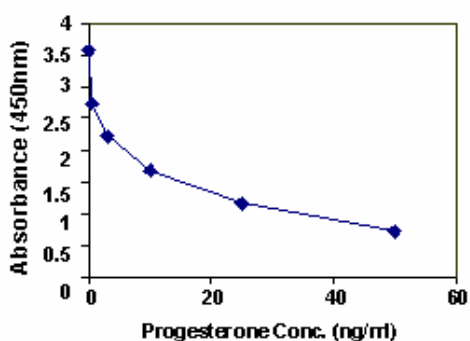
1. Calculate the mean absorbance value (A₄₅₀) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on a linear-linear graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
3. Use the mean absorbance values for each specimen to determine the corresponding concentration of Progesterone in ng/ml from the standard curve.
4. Any values obtained for diluted samples must be further converted by applying the appropriate dilution factor in the calculations.

EXAMPLE OF THE STANDARD CURVE

Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against Progesterone concentrations shown in the X axis. **Note:** This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each laboratory must provide its

own data and standard curve in each experiment.

Progesterone (ng/ml)	Absorbance (450 nm)
0	3.576
0.5	2.730
3	2.215
10	1.679
25	1.148
50	0.718



EXPECTED VALUES

Each laboratory should establish its own normal range based on the patient population. The Progesterone ELISA was performed on randomly selected outpatient clinical laboratory samples. The results of these determinations are as follows:

Males:
 adult 0.05-1.25 ng/ml
 Prepubertal (children) 0 - 0.68 ng/ml

Females:
 follicular phase 0.10 - 1.60 ng/ml
 luteal phase 2.50 – 32.0 ng/ml
 post menopausal 0.06 – 1.60 ng/ml
 pregnancy >250 ng/ml

SENSITIVITY

The lowest detectable level of progesterone in this test is 0.05 ng/ml.

SPECIFICITY

The following materials have been checked for cross reactivity. The percentage indicates cross reactivity at 50% displacement compared to Progesterone.

Data on the cross-reactivity for several endogenous and pharmaceutical steroids are summarized in the following table:

$$\text{Cross-reactivity (\%)} = \frac{\text{Observed Progesterone Concentration}}{\text{Steroid Concentration}} * 100$$

Steroid	Cross-Reactivity
Progesterone	100%
Androsterone	0.086%
Corticosterone	0.74%
Cortisone	0.11%
Cholesterol	<0.08%
Estradiol	<0.01%
Estrone	0.08%
Estriol	<0.024%
Prednisolone	0.075%
Testosterone	0.1%

LIMITATIONS OF THE TEST

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

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