Diagnostic Automation / Cortez Diagnostics, Inc.
IMMUNO DIAGNOSTICS

AccuDiag™ Progesterone ELISA Kit

Cat# 2077-18

IVD See external Label 2°C 8°C Σ 96 Tests

Test	Progesterone ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Competitive Immunoassay
Detection Range	0-50 ng/mL
Sample	25µL Serum/Plasma
Specificity	97%
Sensitivity	0.06ng/mL
Total Time	~ 110 min
Shelf Life	12-14 Months from the manufacturing date

INTENDED USE

For the quantitative determination of Progesterone concentration in human serum or plasma

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.

Diagnostic Automation, Inc. Progesterone EIA kits are designed for the measurement of total progesterone in human serum or plasma.

TEST PRINCIPLE

The Diagnostic Automation, Inc. 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 µl progesterone standards, controls, patient samples, 100 µl progesterone-HRP Conjugate Reagent and 50 µl 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 Stop Solution, 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.

SPECIMEN COLLECTION AND PREPARATION

1. Serum should be used in the test.
2. No special pretreatment of sample is necessary.
3. Serum samples may be stored at 2-8°C for up to 24 hours, and should be frozen at –20°C or lower for longer periods. Avoid grossly hemolytic (bright red), lipemic (milky), or turbid samples.
4. **Please note:** Samples containing sodium azide should not be used in the assay.

PROCEDURAL NOTES

1. Manual Pipetting: It is recommended that no more than 32 wells be used for each assay run. Pipetting of all standards, samples, and controls should be completed within 3 minutes.
2. Automated Pipetting: A full plate of 96 wells may be used in each assay run. However, it is recommended that pipetting of all standards, samples, and controls be completed within 3 minutes.
3. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
4. It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. *Antibody-Coated Wells (1 plate, 96 wells)*
   Microtiter wells coated with goat anti-rabbit IgG
2. *Reference Standard Set (0.5 ml/vial)*
   Contains 0, 0.5, 3.0, 10, 25, and 50 ng/ml of progesterone in human serum with preservatives; liquid, ready to use
3. *Rabbit Anti-Progesterone Reagent (7 ml)*
   Contains rabit anti-progesterone in phosphate buffer with preservatives
4. *Progesterone Conjugate Concentrate (11x), (1.3 ml)*
   Contains progesterone conjugated to horseradish peroxidase with Preservatives

5. *Progesterone Conjugate Diluent (13 ml)*
   Contains bovine serum albumin buffer wih preservatives
6. *Pregesterone Control 1 and 2 (0.5 ml/vial)*
   Contains approximately 5 and 30 ng/ml progesterone, respectively, in human serum
7. *TMB Reagent (11 ml)*
   Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution
8. *Stop Solution -1N HCl (11 ml)*
   Diluted hydrochloric acid

Materials required but not provided

1. Precision pipettes: 25 µl, 50 µl, 100 µl, 200 µl, and 1.0 ml.
2. Disposable pipette tips.
3. Distilled or deionized water.
4. Vortex mixer or equivalent.
5. Absorbent paper or paper towel.
6. Linear-linear graph paper.
7. Microtiter plate reader.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) before use.
2. To prepare Working Progesterone-HRP Conjugate Reagent, add 0.1 ml of Progesterone-HRP Conjugate Concentrate (11x) to 1.0 ml of Progesterone-HRP Conjugate Diluent (1:10 dilution) and mix well. The amount of conjugate diluted depends on your assay size. Discard the excess after use.
3. Samples with expected progesterone concentrations over 50 ng/ml may be quantitated by dilution with diluent available from Diagnostic Automation, Inc.

ASSAY PROCEDURE

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

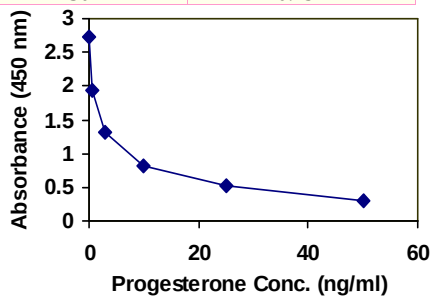
RESULTS

1. Calculate the mean absorbance value (A_{450}) 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 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	2.719
0.5	1.937
3	1.391
10	0.828
25	0.528
50	0.291



EXPECTED VALUES

Each laboratory should establish its own normal range based on the patient population. Diagnostic Automation, Inc. Progesterone EIA was performed on randomly selected outpatient clinical laboratory samples. The following information is cited from reference #9.

Males:	adult	0.13 – 0.97 ng/ml
	Prepubertal (children)	0.07 – 0.52 ng/ml
Females:	follicular phase	0.15 – 0.70 ng/ml
	luteal phase	2.00 – 25.0 ng/ml
	post menopausal	0.06 – 1.60 ng/ml

Pregnancy:

1ST TRIMESTER	10.3–44.0 NG/ML
2ND TRIMESTER	19.5–82.5 NG/ML
3RD TRIMESTER	65.0–229 NG/ML

PERFORMANCE CHARACTERISTICS

I. Accuracy

A statistical study using 109 human serum samples demonstrated good correlation with a commercially available kit as shown below.

Comparison between the Diagnostic Automation, Inc. Progesterone EIA and the DRG Progesterone kit provided the following data:

N = 109
Correlation coefficient = 0.977
Slope = 0.867
Intercept = 0.727
DAI Mean = 5.3 ng/ml
DRG Mean = 3.9 ng/ml

II. Sensitivity

The minimum detectable concentration of the Diagnostic Automation, Inc. Progesterone ELISA assay as measured by 2 SD from the mean of a zero standard is estimated to be 0.0625 ng/ml.

III. Precision

a. Intra-Assay Precision

Within-run precision was determined by replicate determinations of four different serum samples in one assay. Within-assay variability is shown below:

Samples	1	2	3	4
# Replicates.	24	24	24	24
Mean Progesterone(ng/ml)	1.8	8.0	21.7	44.8
S.D.	0.1	0.2	0.7	1.1
C.V. (%)	7.1	2.6	3.3	2.4

b. Inter-Assay Precision

Between-run precision was determined by replicate measurements of six different serum samples over a series of individually calibrated assays. Between-assay variability is shown below:

Samples	1	2	3	4
# Replicates	20	20	20	20
Mean Progesterone (ng/ml)	1.7	7.9	21.0	44.6
S.D.	0.2	0.4	1.2	1.1
C.V. (%)	12.6	4.5	5.9	2.6

IV. Linearity Study

Four patient samples were serially diluted to determine linearity. The mean linearity was 105.9%.

#	Dilution	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Expected
1.	Undiluted	-----	44.4	-----
	1:2	22.2	24.9	112.0
	1:4	11.1	12.8	115.3
	1:8	5.6	7.1	127.7
	1:16	2.8	3.5	125.2
	1:32	1.4	1.8	126.7
	1:64	0.7	0.8	108.5
	1:128	0.3	0.3	81.0
				Mean = 113.8%
2.	Undiluted	-----	40.6	-----
	1:2	20.3	22.6	111.2
	1:4	10.2	9.8	96.9
	1:8	5.1	6.0	118.9
	1:16	2.5	2.6	102.0
	1:32	1.3	1.2	96.4
	1:64	0.6	0.4	70.4
	1:128	0.3	0.3	89.1
				Mean = 97.9%
3.	Undiluted	-----	30.5	-----
	1:2	15.2	16.6	108.6
	1:4	7.6	8.3	108.3
	1:8	3.8	4.5	117.9
	1:16	1.9	2.1	112.1
	1:32	1.0	0.5	54.4
	1:64	0.5	0.3	69.5
				Mean = 95.1%
4.	Undiluted	-----	41.9	-----
	1:2	20.9	24.7	118.1
	1:4	10.5	12.1	115.3
	1:8	5.2	7.1	135.6
	1:16	2.6	3.7	142.1
	1:32	1.3	1.8	135.8
	1:64	0.7	0.5	72.7
	1:128	0.3	0.3	99.0
				Mean = 116.9%

V. Recovery Study

Various patient serum samples of known Progesterone levels were combined and assayed in duplicate. The mean recovery was 111.3%.

PAIR NO.	EXPECTED [Progesterone] (ng/ml)	OBSERVED [Progesterone] (ng/ml)	% RECOVERY
1	41.5	43.1	103.9
2	43.1	45.7	106.1
3	19.9	19.8	99.1
4	18.0	19.2	106.4
5	3.8	4.3	115.4
6	7.3	8.7	118.6
7	0.8	0.7	80.4

VI. 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:

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

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%

CLINICAL APPLICATION

Information is cited from reference #10

1. **Documentation of Ovulation:**
   Monitor the progesterone concentration during the menstrual cycle is useful in the documentation of ovulation. **Progesterone concentration > 3.0 ng/ml will be a strong presumptive evidence of ovulation.**
2. **Normal vs. Abnormal Progesterone Levels:**
   Greater-than-normal levels may indicate pregnancy. High level can also indicate adrenal cancer or ovarian cancer, a molar pregnancy, or overproduction of hormones by the adrenal glands. However, levels of progesterone are higher during a multiple pregnancy than during a single pregnancy.
   Lower-than-normal levels may indicate amenorrhea. Abnormally low levels of progesterone can also indicate problems with ovulation. **In a pregnant woman, progesterone levels fall to < 5 ng/mL may indicate a threatened miscarriage.**
3. **Ectopic Pregnancy:**
   Progesterone can also be useful in ectopic pregnancy diagnosis. For values < 25 ng/ml during pregnancy, fetus viability need to be established by ultrasound. However, progesterone < 5 ng/ml in the first trimester indicates a nonviable pregnancy regardless of location of the fetus.

QUALITY CONTROL

1. Good laboratory practice requires that quality control specimens (controls) be run with each calibration curve to verify assay performance. To ensure proper performance, control material should be assayed repeatedly to establish mean values and acceptable ranges.
2. We recommend using Bio-Rad Lyphochek Immunoassay Control Sera as controls. The Diagnostic Automation, Inc. Progesterone EIA kit also is provided with internal controls, Levels 1 and 2.
3. Controls containing sodium azide cannot be used.

LIMITATIONS OF THE PROCEDURE

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. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. 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.

PRECAUTIONS

1. **CAUTION:** This kit contains human material. The source material used for manufacture of this kit tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling and disposal should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.[21]
2. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
3. Do not use the reagent when it becomes cloudy or contamination is suspected.
4. Do not use the reagent if the vial is damaged.
5. Replace caps on reagents immediately. Do not switch caps.
6. Each well can be used only once.
7. Do not pipette reagents by mouth.
8. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
9. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
10. For in vitro diagnostic use.

STORAGE

1. Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. The opened and used reagents are stable until the expiration date if stored properly at 2-8°C.
3. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

INSTRUMENTATION

A microtiter well reader with a bandwidth of 10 nm or less and an optical density range (OD) of 0 to 3 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

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ISO 13485
ISO 9001

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Date Adopted	Cat # 2077-18
2010-05-02	AccuDiag™- Progesterone ELISA-2013
EC REP	CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands. www.cepartner4u.eu
Revision Date: 10-09-2013	