

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

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%

5. **Recovery**

Known quantities of progesterone were added to a serum that contained a low concentration of progesterone.

Expected Value (ng/ml)	Recovered (ng/ml)	Percentage of Recovery
2.5	2.51	100.4
10	9.16	91.6
20	19.1	95.5

REFERENCES

- Radwanska, E., Frankenberg, J., and Allen, E., Plasma progesterone levels in normal and abnormal early human pregnancy. *Fertility and Sterility*, 1978; 30, 398-402.
- Aultre, M.B., and Benson, H., Progesterone: An overview and recent advances, *J. Par. Sci.*, 1976; 65: 783-800.
- March, C.M., Goebelsmann, U., Nakamura, R.M., and Mishell, D.R. Jr., Roles of estradiol and progesterone in eliciting the midcycle luteinizing hormone and follicle-stimulating hormone surges, *J. Clin. Endo. Metab.*, 1979; 49, 507-513.
- Ross, G.T., Vande Wiele, R.L., and Frantz, A.G., The Ovaries and the breasts. In: Williams, R.H., ed., *Textbook of Endocrinology*. Saunders Company, Philadelphia; 1981: 355-411.
- Chattoraj, S.C., Endocrine function. In: Tietz, N.W., ed., *Fundamentals of Clinical Chemistry*. Saunders Company, Philadelphia; 1976: 699-823.
- Shepard, M.K., and Senturia, Y.D., Comparison of serum progesterone and endometrial biopsy for confirmation of ovulation and evaluation of luteal function. *Fertility and Sterility*, 1977; 28: 541-548.
- Johansson, E.D.B., and Jonasson, L.-E., Progesterone levels in amniotic fluid and plasma from women: I. Levels during normal pregnancy. *Acta Obstet. Gynec. Scand.*, 1971; 50: 339-343.
- USA Center for Disease Control/National Institute of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.
- Tietz, N.W. ed., *Clinical Guide to Laboratory Tests*, 3rd Edition, W.B. Saunders, Co., Philadelphia, 1995: 509-512.
- ICN *Guide to Endocrine Testing*. Diagnostic Division, ICN Biomedicals, Inc. pp. 2:20-27.

2008-01-14

Warning

All of BQ Kits ELISA kits have not been tested for clinical use and are not approved in the United States by the FDA for diagnostic clinical use. They are components or reagents made solely for research use, further manufacturing and export use. It is the commitment of BQ Kits customers to receive its products solely for the purpose of exportation or research, and not for the purposes of clinical diagnostic use.

BQ KITS, INC. DOES NOT MAKE ANY OTHER WARRANTY OR REPRESENTATION WHATSOEVER, WHETHER EXPRESS OR IMPLIED, WITH RESPECT TO THESE PRODUCTS. IN PARTICULAR BQ KITS, INC. DOES NOT MAKE ANY WARRANTY OF SUITABILITY, NON-INFRINGEMENT, MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE OF ANY PRODUCT

BQ Kits

Progesterone ELISA

Catalog No. BQ 129S (96 tests)

INTENDED USE

The Progesterone ELISA kit is used for the quantitative measurement of Progesterone 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 Progesterone 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

The Progesterone is a solid phase competitive ELISA. The samples and Progesterone enzyme conjugate are added to the wells coated with anti-Progesterone monoclonal antibody. Progesterone in the patient's sample competes with a Progesterone enzyme conjugate for binding sites. Unbound Progesterone and Progesterone enzyme conjugate is washed off by washing buffer. Upon the addition of the substrate, the intensity of color is inversely proportional to the concentration of Progesterone in the samples. A standard curve is prepared relating color intensity to the concentration of the Progesterone.

MATERIALS PROVIDED		96 tests
1.	Microwell coated with Progesterone MAb	12x8x1
2.	Progesterone Standard set: 6 vials (ready to use)	0.25 ml
3.	Enzyme Conjugate (20X)	1.2 ml
4.	Assay Diluent	24 ml
5.	TMB Substrate: 1 bottle (ready to use)	12 ml
6.	Stop Solution: 1 bottle (ready to use)	12 ml
7.	Wash concentrate (20X): 1 bottle	25 ml

MATERIALS NOT PROVIDED

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

STORAGE AND STABILITY

- Store the kit at 2 – 8° C.
- 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 contains 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 test kit is USA FDA exempt product.
- 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.
- It is recommended that standards, control and serum samples be run in duplicate.
- 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

- It is recommended to collect serum samples with commercially available equipments. The serum samples should be completely colorless even the slight red color shows blood contamination.
- Specimens may be stored refrigerated at (2-8° C) for 1 days. Store frozen at (-20° C) for up to one month.
- Avoid multiple freeze-thaw cycles.
- Prior to assay, frozen serum samples should be completely thawed and mixed well.

REAGENTS PREPARATION

- Working Reagent A Progesterone-enzyme Conjugate Solution**
Dilute the Progesterone enzyme conjugate 1:21 with assay diluent in a suitable container. For example, dilute 100µl of conjugate with 2ml of assay diluent buffer for 10 wells (A slight excess of solution is made).
- Wash Buffer**
Prepare 1X Wash Buffer by adding the contents of the bottle (25ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature.

ASSAY PROCEDURE

Prior to assay, bring all reagents to room temperature. Gently mix all reagents before use.

- Place the desired number of coated strips into the holder
- Pipet 10 µl of Progesterone standards, control and patient's serum samples.
- Add 200µl of Progesterone Enzyme Conjugate to all wells.
- Incubate for 60 minutes at room temperature (18-26° C).
- Remove liquid from all wells. Wash wells three times with 300 ml of 1X wash buffer. Blot on absorbent paper towels.
- Add 100 µl of TMB substrate to all wells.
- Incubate for 15 minutes at room temperature.
- Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
- Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stop solution.

CALCULATION OF RESULTS

The standard curve is constructed as follows:

- Check Progesterone standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.
- To construct the standard curve, plot the absorbance for Progesterone standards (vertical axis) versus Progesterone standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
- Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Example of a Standard Curve

Standard	OD (450 nm)
Standard 1 (0 ng/ml)	2.20
Standard 2 (2.5 ng/ml)	1.32
Standard 3 (5 ng/ml)	0.92
Standard 4 (10 ng/ml)	0.65
Standard 5 (20 ng/ml)	0.42
Standard 6 (40 ng/ml)	0.23

EXPECTED VALUES

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values for Progesterone were established by and may be used as initial guideline ranges only:

Classification	ng/ml
AM-PM	<50

LIMITATIONS OF THE TEST

- The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

PERFORMANCE CHARACTERISTICS

- Correlation with a Reference ELISA kit:**
A total of 86 samples were tested by Progesterone ELISA and a reference ELISA kit. Results were as follows:

Correlation	Slope	Intercept
0.94	1.1	0.8

- Precision**

Intra-Assay Precision was determined by assaying 10 replicates of each of three samples; low, normal, and high.

sample	No. of Replicates	Mean ng/ml	Standard Deviation	Coefficient of Variation (%)
1	10	1.72	0.092	5.36
2	10	10.1	0.16	1.62
3	10	19.7	0.4	2.04

Inter assay Precision was determined by assaying duplicates of three samples pools in 10 separate runs, using a standard curve constructed for each run.

Sample	No. of Runs	Mean ng/ml	Standard Deviation	Coefficient of Variation (%)
1	10	2.16	0.21	9.68
2	10	9.73	0.85	8.8
3	10	19.1	1.2	6.3

- Sensitivity**

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

Sample	No. of Replicates	Mean ng/ml	Standard Deviation	Mean + 2SD (Sensitivity)
Zero Standard	20	0.04	0.09	0.22 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: