Progesterone

Enzyme Immunoassay Kit

Catalog No. 5081P
96 Well Kit

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FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

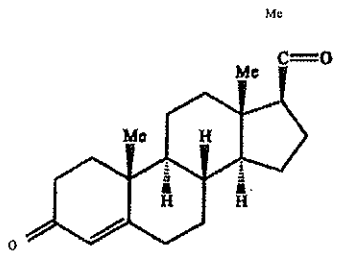
Description

Abraxis Progesterone kit is a competitive immunoassay for the quantitative determination of Progesterone in biological fluids. Please read the complete kit insert before performing this assay. The kit for the quantitative measurement of Progesterone uses a polyclonal antibody to Progesterone to bind, in a competitive manner, Progesterone in a sample or an alkaline phosphatase molecule which has Progesterone covalently attached to it. After a simultaneous incubation at room temperature the excess reagents are washed away and substrate is added. After a short incubation time the enzyme reaction is stopped and the yellow color generated is read on a microplate reader at 405 nm. The intensity of the bound yellow color is inversely proportional to the concentration of Progesterone in either standards or samples. The measured optical density for the samples is used to calculate the concentration of Progesterone in the sample. For further explanation of the principles and practice of immunoassays please see the excellent books by Chard[1] or Tijssen[2].

Introduction

Progesterone is the major female sex hormone. This steroid is responsible for reproductive-related activities such as breast glandular development, the endometrial aspects of the menstrual cycle, and the establishment and maintenance of pregnancy. In addition, progesterone also directs pregnancy-support physiology including changes in carbohydrate, protein and lipid metabolism, thermoregulation, sodium reabsorption in renal tubules and the reduction of alveolar and atrial carbon dioxide partial pressures (P_{CO_2})[3-5]. Progesterone is involved in cell cycle progression, acts as a neurosteroid to promote remylination of nerve axons and is used therapeutically to treat menopause-related symptoms in women[6-8]. Progesterone is secreted in large amounts by the corpus luteum and by the extracellular conversion of cholesterol, cholestryl esters, adrenal steroids, prenenolone and pregnenolone sulfate. Small quantities are also secreted directly from the adrenal glands. Since sex steroids are not stored, progesterone is quickly cleared from circulation by extracellular conversion to androgens or estrogen, or to preganediol which is conjugated to glucuronic acid in the liver and excreted in urine. Only a small portion of circulating plasma progesterone is free (2.4%) with the remaining steroid bound to serum proteins. Methods to determine progesterone in urine and blood typically involve gas chromatography, radioimmunoassay or enzyme immunoassay[10,11].

Progesterone



Precautions

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1. Some kit components contain azide, which may react with lead or copper plumbing. When disposing of reagents always flush with large volumes of water to prevent azide build-up.
2. Stop Solution is a solution of trisodium phosphate. This solution is caustic; care should be taken in use.
3. The activity of the alkaline phosphatase conjugate is dependent on the presence of Mg^{2+} and Zn^{2+} ions. The activity of the conjugate is affected by concentrations of chelators (>10 mM) such as EDTA and EGTA.
4. We test this kit's performance with a variety of samples, however it is possible that high levels of interfering substances may cause variation in assay results.
5. The Progesterone Standard provided, Catalog No. 80-0117, is supplied in ethanolic buffer at a pH optimized to maintain Progesterone integrity. Care should be taken handling this material because of the known and unknown effects of steroids on biological tissue.

Storage

All components of this kit are stable at 4 °C until the kit's expiration date.

Materials Supplied

1. **Goat anti-Mouse IgG Plate, Catalog No. 80-0050**
   A plate using break-apart strips coated with goat antibody specific to mouse IgG.
2. **Progesterone Alkaline Phosphatase Conjugate, 6 mL, Catalog No. 80-0115** A blue solution of alkaline phosphatase conjugated with progesterone.
3. **Progesterone Monoclonal Antibody, 6 mL, Catalog No. 80-0116** A yellow solution of a monoclonal antibody to progesterone.
4. **Assay Buffer, 30 mL, Catalog No. 80-0010**
   Tris buffered saline containing proteins and sodium azide as preservative.
5. **Wash Buffer Concentrate, 30 mL, Catalog No. 80-1286**
   Tris buffered saline containing detergents.
6. **Progesterone Standard, 0.5 mL, Catalog No. 80-0117**
   A 100,000 pg/mL solution of progesterone.
7. **Steroid Displacement Reagent, 1 mL, Catalog No. 80-0120**
8. **pNpp Substrate, 20 mL, Catalog No. 80-0075**
   A solution of p-nitrophenyl phosphate in buffer. Ready to use.
9. **Stop Solution, 5 mL, Catalog No. 80-0247**
   A solution of trisodium phosphate in water. Keep tightly capped. Caution: **Caustic.**

Progesterone Assay Layout Sheet, 1 each, Catalog No. 30-0023

Plate Sealer, 1 each, Catalog No. 30-0012

Materials Needed but Not Supplied

1. Deionized or distilled water.
2. Precision pipets for volumes between 5 µL and 1,000 µL.
3. Repeater pipets for dispensing 50 and 200 µL.
4. Disposable beaker for diluting Wash Buffer.
5. Graduated cylinders.
6. A microplate shaker.
7. Adsorbent paper for blotting.
8. Microplate reader capable of reading at 405 nm, preferably with correction between 570 and 590 nm.

Sample Handling

Abraxis kit is compatible with Progesterone samples in a wide range of matrices. Samples diluted sufficiently into Assay Buffer can be read directly from the standard curve. Please refer to recovery data on page 11 for suitable dilutions necessary for other samples. However, the end user **must verify** that the recommended dilutions are appropriate for their samples. Included with the kit is the Steroid Displacement Reagent which should be added to serum, plasma and other samples containing steroid binding proteins. Samples should then be diluted with 1 part of the Reagent for every 99 parts of sample, i.e., 1:100. **Samples containing mouse IgG may interfere with the assay.**

Samples in the majority of tissue culture media can also be read in the assay after being diluted, provided the standards have been diluted into the tissue culture media instead of Assay Buffer. **Tissue Culture Media must be diluted 1:10 in provided Assay Buffer prior to use.** There will be a small change in the binding associated with running the standards and samples in media. Users should only use standard curves generated in media or buffer to calculate concentrations of Progesterone in the appropriate matrix.

Some samples may have very low levels of Progesterone present and extraction may be necessary for accurate measurement. A suitable extraction procedure is outlined below:

Materials Needed

1. Progesterone Standard to allow extraction efficiency to be accurately determined.
2. ACS Grade Diethyl Ether.

Procedure

1. Add sufficient Progesterone to a typical sample for determination of extraction efficiency.
2. In a fume hood add 1 mL of Diethyl Ether for every mL of sample. Stopper and shake sample.
3. Allow layers to separate. Carefully pipet off the top ether layer and place in a clean test tube.
4. Repeat steps 1 and 2 twice more, combining the ether layers.
5. Evaporate the ether to dryness under nitrogen.
6. Dissolve the extracted Progesterone with at least 250 µL of Assay Buffer. Vortex well, then allow to sit for five minutes at room temperature. Repeat twice more.
7. Run the reconstituted samples in the assay immediately. If analysis is to be delayed, store samples evaporated and desiccated at or below -20 °C.

Procedural Notes

1. Do not mix components from different kit lots or use reagents beyond the kit expiration date.
2. Allow all reagents to warm to room temperature for at least 30 minutes before opening.
3. Standards can be made up in either glass or plastic tubes.
4. Keep unused plate strips sealed in bag with desiccant.
5. Pre-rinse the pipet tip with reagent, use fresh pipet tips for each sample, standard and reagent.
6. Pipet standards and samples to the bottom of the wells.
7. Add the reagents to the side of the well to avoid contamination.
8. This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4 °C in the sealed bag provided. The wells should be used in the frame provided.
9. **Care must be taken to minimize contamination by endogenous alkaline phosphatase.** Contaminating alkaline phosphatase activity, especially in the substrate solution, may lead to high blanks. Care should be taken not to touch pipet tips and other items that are used in the assay with bare hands.
10. **Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.**

Reagent Preparation

1. **Progesterone Standard**

   Allow the 100,000 pg/mL Progesterone Standard solution to warm to room temperature. Label six 12 x 75 mm glass tubes #1 through #6. Pipet 2 mL of standard diluent (Assay Buffer or Tissue Culture Media) into tube #1. Pipet 500 µL of standard diluent into tubes #2 through #6. Remove 10 µL of diluent from tube #1. Add 10 µL of the 100,000 pg/mL standard to tube #1. Vortex thoroughly. Add 500 µL of tube #1 to tube #2 and vortex thoroughly. Add 500 µL of tube #2 to tube #3 and vortex. Continue this for tubes #4 through #6.

   The concentration of Progesterone in tubes #1 through #6 will be 500, 250, 125, 62.5, 31.25 and 15.62 pg/mL, respectively. See the Progesterone Assay Layout Sheet for dilution details.

2. **Wash Buffer**

   Prepare the Wash Buffer by diluting 5 mL of the concentrate supplied with 95 mL of deionized water. This can be stored at room temperature until the kit expiration or for 3 months, whichever is earlier.

Assay Procedure

Bring all reagents to room temperature for at least 30 minutes prior to opening.

All standards and samples should be run in duplicate.

1. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells with the desiccant back into the pouch and seal the ziploc. Store unused wells at 4 °C.
2. Pipet 100 µL of standard diluent (Asssay Buffer or Tissue Cuture Media) into the NSB and the Bo (0 pg/mL Standard) wells.
3. Pipet 100 µL of Standards #1 through #6 into the appropriate wells.
4. Pipet 100 µL of the Samples into the appropriate wells.
5. Pipet 50 µL of Assay Buffer into the NSB wells.
6. Pipet 50 µL of blue Conjugate into each well, except the Total Activity (TA) and Blank wells.
7. Pipet 50 µL of the yellow Antibody Solution into each well, except the Blank, TA and NSB wells.

NOTE: Every well used should be **Green** in color except the NSB wells which should be **Blue.** The Blank and TA wells are empty at this point and have no color.

8. Tap the plate gently to mix. Incubate the plate at room temperature on a plate shaker for 2 hours at ~500 rpm. The plate may be covered with the plate sealer provided, if so desired.
9. At the end of the first incubation, empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 2 more times for a total of **3 Washes.**
10. After the final wash empty or aspirate the wells and firmly tap the plate dry on a lint free paper towel to remove any remaining wash buffer.
11. Add 5 µL of the blue Conjugate to the TA wells.
12. Add 200 µL of the pNpp Substrate solution to every well. Incubate at room temperature for 45 minutes without shaking.
13. Add 50 µL of Stop Solution to every well. This stops the reaction and the plate should be read immediately.
14. Blank the plate reader against the Blank wells, read the optical density at 405 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all readings.

Calculation of Results

Several options are available for the calculation of the concentration of Progesterone in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve fitting program. If this sort of data reduction software is not readily available, the concentration of Progesterone can be calculated as follows:

1. Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average NSB OD from the average OD bound:

   Average Net OD = Average OD - NSB OD

2. Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo), using the following formula:

$$\text{Percent Bound} = \frac{\text{Net OD}}{\text{Net Bo OD}} \times 100$$

3. Using Logit-Log paper plot Percent Bound versus Concentration of Progesterone for the standards. Approximate a straight line through the points. The concentration of Progesterone in the unknowns can be determined by interpolation.

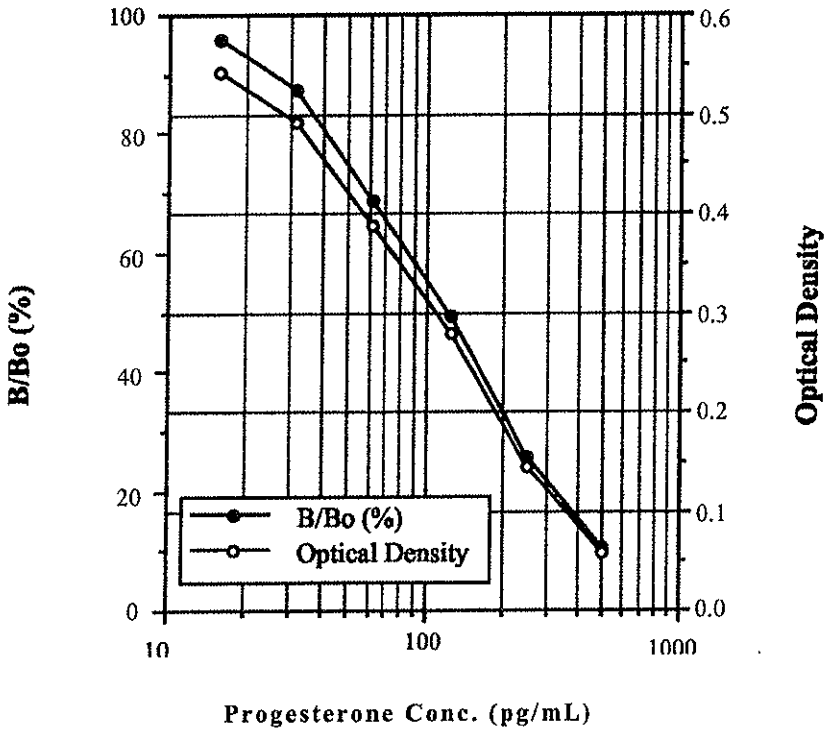
Typical Results

The results shown below are for illustration only and **should not** be used to calculate results from another assay.

Sample	Mean OD(-Blank)	Average Net OD	Percent Bound	**Progesterone (pg/mL)**
Blank OD	(0.080)			
TA	0.410			
NSB	0.000	0.000	0.00%	
Bo	0.562	0.562	100%	**0**
S1	0.06 1	0.06 1	10.9%	**500**
S2	0.147	0.147	26.2%	**250**
S3	0.280	0.280	49.8%	**125**
S4	0.389	0.389	69.2%	**62.5**
S5	0.494	0.494	87.9%	**31.25**
S6	0.543	0.543	96.6%	**15.62**
Unknown 1	0.373	0.373	66.4%	**71.7**
Unknown 2	0.132	0.132	23.5%	**282.2**

Typical Standard Curve

A typical standard curve is shown below. This curve **must not** be used to calculate Progesterone concentrations; each user must run a standard curve for each assay.



Typical Quality Control Parameters

Total Activity Added	=	0.410 x 10 = 4.10
%NSB %	=	0.0%
Bo/TA	=	13.8%
Quality of Fit	=	0.999 (Calculated from 4 parameter logistic curve fit)
20% Intercept	=	319 pg/mL
50% Intercept	=	118 pg/mL
80% Intercept	=	42 pg/mL

Performance Characteristics

The following parameters for this kit were determined using the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols[12].

Sensitivity

Sensitivity was calculated by determining the average optical density bound for twenty (20) wells run as Bo, and comparing to the average optical density for twenty (20) wells run with Standard #6. The detection limit was determined as the concentration of Progesterone measured at two (2) standard deviations from the zero along the standard curve.

Average Optical Density for the Bo	=	0.593 ± 0.017 (2.92%)
Average Optical Density for Standard #6	=	0.531 ± 0.013 (2.50%)
Delta Optical Density (0-15.63 pg/mL)	=	0.062
2 SD's of the Zero Standard = 2 x 0.017	=	0.034

$$\text{Sensitivity} = \frac{0.034 \times 15.63 \text{ pg/mL}}{0.062} = \mathbf{8.57\ pg/mL}$$

Linearity

A sample containing 340.9 pg/mL Progesterone was diluted 4 times 1:2 in the kit Assay Buffer and measured in the assay. The data was plotted graphically as actual Progesterone concentration versus measured Progesterone concentration.

The line obtained had a slope of 1.015 and a correlation coefficient of 0.995.

Precision

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of Progesterone and running these samples multiple times (n=1 2) in the same assay. Inter-assay precision was determined by measuring three samples with low, medium and high concentrations of Progesterone in multiple assays (n=8).

The precision numbers listed below represent the percent coefficient of variation for the concentrations of Progesterone determined in these assays as calculated by a 4 parameter logistic curve fitting program.

	Progesterone (pg/mL)	Intra-assay %CV	Inter -assay %CV
Low	23.1	7.6	
Medium	118.4	5.4	
High	325.9	4.9	
Low	20.4		6.8
Medium	107.7		8.3
High	314.1		2.7

Cross Reactivities

The cross reactivities for a number of related steroid compounds was determined by dissolving the cross reactant (purity checked by N.M.R. and other analytical methods) in Assay Buffer at concentrations from 10,000,000 to 10 pg/mL. These samples were then measured in the Progesterone assay, and the measured Progesterone concentration at 50% B/Bo was calculated. The % cross reactivity was calculated by comparison with the actual concentration of cross reactant in the sample and expressed as a percentage.

Compound	Cross Reactivity
Progesterone	100%
5á-Pregnane-3,20-dione	100%
17-OH-Progesterone	3.46%
5-Pregnen-3ß-o1-20-one	1.43%
Corticosterone	0.77%
4-Androstene-3, 1 7-dione	0.28%
Deoxycorticosterone	0.056%
DHEA	0.013%
1 7ß-Estradiol	<0.001%
Estrone	<0.001%
Estriol	<0.001%
Testosterone	<0.001%
Hydrocortisone	<0.001%
5á-Pregnane-3á,20á-diol	<0.001%
Danazol	<0.001%

Progesterone Sample Recoveries

Please refer to pages 4 and 5 for Sample Handling recommendations and Standard preparation.

Progesterone concentrations were measured in a variety of different samples including tissue culture media, human saliva, and serum. Progesterone was spiked into the undiluted samples of these media which were then diluted with the appropriate diluent and then assayed in the kit. The following results were obtained:

Sample	% Recovery*	Recommended Dilution*
Tissue Culture Media	105.0	1:10
Human Saliva	98.1	1:10
Human Serum	106.4	1:10

* See Sample Handling instructions on page 4 for details.

References

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12. National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.

LIMITED WARRANTY

Abraxis warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

Abraxis must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if Abraxis is not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

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PROGESTERONE ASSAY LAYOUT SHEET

• DILUTION TABLE FOR MAKING STANDARDS 1-6:

Std.	Assay Buffer Vol. (µL)	Vol. Added (µL)	Progesterone Conc. (pg/mL)
1	1,990	10, Stock	500
2	500	500, Std. 1	250
3	500	500, Std. 2	125
4	500	500, Std. 3	62.5
5	500	500, Std. 4	31.25
6	500	500, Std. 5	15.62

• ASSAY PROTOCOL FLOW CHART:

Well I.D.:	Blank A1, B1	TA C1, D1	NSB E1, F1	Bo G1, H1	Stds. A2 - D3	Samples E3 - H12
Standard Diluent	---	---	100 µL	100 µL	---	---
Assay Buffer	---	---	50 µL	---	---	---
Std. and/or Sample	---	---	---	---	100 µL	100 µL
Conjugate	---	---	50 µL	50 µL	50 µL	50 µL
Antibody	---	---	---	50 µL	50 µL	50 µL
Incub. 2 hours @ RT, shaking	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 3 x 400 µL	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Conjugate	---	5 µL	---	---	---	---
Substrate	200 µL	200 µL	200 µL	200 µL	200 µL	200 µL
Incub. 45 min. @ RT	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Stop Solution	50 µL	50 µL	50 µL	50 µL	50 µL	50 µL

Catalog No. 30-0023

August 4, 2004
Printed on Recycled Paper

• **PROGESTERONE PLATE LAYOUT:**

A1 Blank	A2 Std 1	A3 Std 5	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1 Blank	B2 Std 1	B3 Std 5	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1 TA	C2 Std 2	C3 Std 6	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1 TA	D2 Std 2	D3 Std 6	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1 NSB	E2 Std 3	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1 NSB	F2 Std 3	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1 Bo	G2 Std 4	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1 Bo	H2 Std 4	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

Kit Lot No. _____ Exp. Date _____ Date _____ Tech. _____

1st Incub.: Start Time _____ Temp. _____

End Time _____ Temp. _____

2nd Incub.: Start Time _____ Temp. _____

End Time _____ Temp. _____

Notes: _____

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