Progesterone ELISA Kit

Catalog Number KA0299

96 assays

Version: 05

Intended for research use only

www.abnova.com

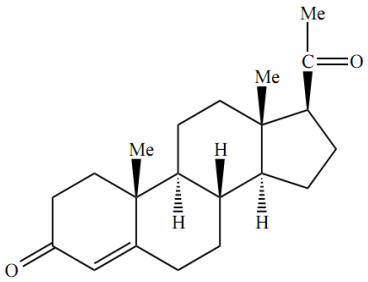
Table of Contents

Introduction

Background

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 (PCO2)[3-5]. Progesterone is involved in cell cycle progression, acts as a neurosteroid to promote re-mylination 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, pregnenolone 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 pregnanediol 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[9]. Methods to determine progesterone in urine and blood typically involve gas chromatography, radioimmunoassay or enzyme immunoassay[10, 11].

Progesterone



Principle of the Assay

Progesterone ELISA 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 uses a polyclonal antibody to Progesterone to bind, in a competitive manner, Progesterone in a sample or Progesterone which has an alkaline phosphatase molecule 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].

General Information

Materials Supplied

List of component

Component	Description	Amount
Goat anti-Mouse IgG Plate	A plate using break-apart strips coated with goat antibody specific to mouse IgG.	96 well
Assay Buffer	Tris buffered saline containing proteins and sodium azide as preservative.	27 mL
Progesterone Alkaline Phosphatase Conjugate	A blue solution of progesterone conjugated to alkaline phosphatase.	5 mL
Progesterone Monoclonal Antibody	A yellow solution of a monoclonal antibody to progesterone.	5 mL
Progesterone Standard	A 100,000 pg/mL ethanolic solution of progesterone.	0.5 mL
Wash Buffer Concentrate	Tris buffered saline containing detergents.	27 mL
Steroid Displacement Reagent	A specially formulated reagent that aids in the disassociation of steroid from steroid binding proteins.	1 mL
pNpp Substrate	A solution of p-nitrophenyl phosphate in buffer. Ready to use.	20 mL
Stop Solution	A solution of trisodium phosphate in water. Keep tightly capped. Caution: Caustic.	5 mL
Plate Sealer	-	1 slice

Note: Activity of conjugate is affected by concentration of chelators > 10 mM (such as EDTA and EGTA).

Storage Instruction

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

Materials Required but Not Supplied

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

Precautions for Use

- Safety Warnings & Precautions

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

✓ 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.

✓ Stop Solution is a solution of trisodium phosphate. This solution is caustic; care should be taken in use.

✓ 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.

✓ The performance of this kit has been tested using a variety of samples, however it is possible that high levels of interfering substances may cause variation in assay results.

✓ The Progesterone Standard provided, 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.

✓ Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.

✓ Avoid contamination by endogenous alkaline phosphatase.

✓ Do not expose reagents or supplies to bare skin.

Assay Protocol

Reagent Preparation

- 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 table below for dilution details.

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

- 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.

Sample Preparation

- Sample Handling

The Progesterone ELISA 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 for suitable dilutions necessary for other samples. Please note, 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. The Steroid Displacement reagent will disassociate the steroid from the binding protein allowing it to be detectable by the assay. The Steroid Displacement Reagent should be used to treat appropriate samples in the following manner: one part Steroid Displacement Reagent per 99 parts sample. Once the Steroid Displacement Reagent has been added to the neat sample, briefly vortex the sample, allow it to sit for approximately 5 minutes and then proceed with sample dilution. The Steroid Displacement Reagent needs to be added prior to any subsequent dilutions of the sample. 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. 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.

Please note that the tissue culture media may need to be diluted the Assay Buffer in order to avoid matrix interference. If samples required further dilution in Assay Buffer, the standard curve must be prepared in a like manner (e.g. 1:10 dilution of tissue culture media in Assay Buffer)

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. 1 ng/mL Progesterone Standard solution for determination of extraction efficiency in sample matrix. This solution can be made by diluting 5 µL of the supplied Standard with 500 µL of ethanol (ACS grade or equivalent).
2. ACS Grade Diethyl Ether.

- Procedure

1. Add sufficient Progesterone to a sample matrix for determination of extraction. For a typical experiment based on a one mL sample starting volume, add 25-50 pg/mL of the 1 ng/mL Progesterone solution from above. Continue with extraction procedure steps 2-7.
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 vortex step twice.
7. Run the reconstituted samples in the assay immediately. If analysis is to be delayed, store evaporated samples, desiccated, at or below -20°C.

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 Plate 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 (Assay Buffer or Tissue Culture 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.

Note 1: Pipet standards and samples to the bottom of the wells.

Note 2: Pipet the reagents to the sides of the wells to avoid possible contamination.

Note 3: Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and reagent.

Data Analysis

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. Such software is often supplied by plate reader manufacturers. Samples with concentrations outside of the standard curve range will need to be reanalyzed using a different dilution. 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:
   Percent Bound = (Net OD/ Net Bo OD) x 100

3. Graph the data points and the best-fit line through the points. The concentration of Progesterone in the unknowns can be determined by interpolation.

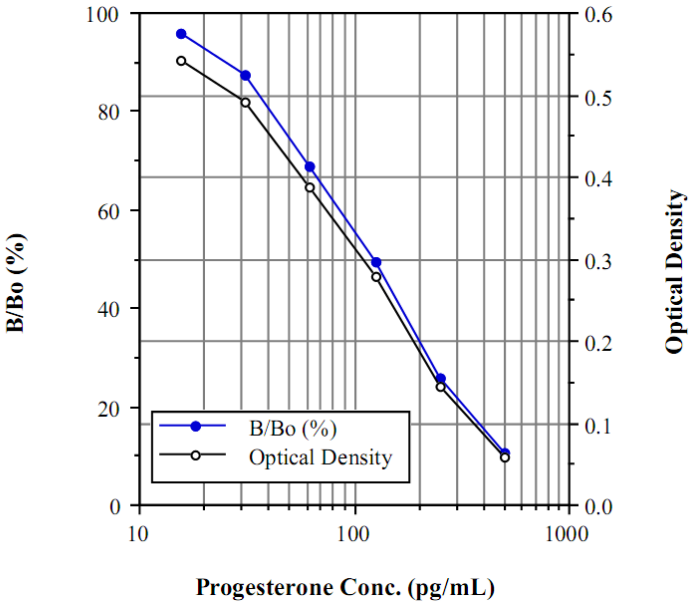
- Typical Results

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

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	0.061	0.061	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
Sensitivity	=	0.034 /0.062 x 15.63 pg/mL	=	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=12) 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%
5α-Pregnane-3,20-dione	100%
17-OH-Progesterone	3.46%
5-Pregnen-3ß-o1-20-one	1.43%
Corticosterone	0.77%
4-Androstene-3,17-dione	0.28%
Deoxycorticosterone	0.056%
DHEA	0.013%
17ß-Estradiol	<0.001%
Estrone	<0.001%
Estriol	<0.001%
Testosterone	<0.001%
Hydrocortisone	<0.001%
5α-Pregnane-3α,20α-diol	<0.001%
Danazol	<0.001%

- Sample Recoveries

Please refer to 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 for details.

Resources

References

1. P. Tijssen,"Practice & Theory of Enzyme Immunoassays", (1985) Amsterdam: Elsevier
2. T. Chard,"An Introduction to Radioimmunoassay & Related Techniques 4th Edition", (1990) Amsterdam: Elsevier.
3. C.W. Bardin, et al.,“Progesterone and Progestins”, (1983) NY: Raven Press.
4. F.S. Greenspan & P.H. Forsham, “Basic & Clinical Endocrinology 2nd Edition”, (1986) Norwalk, CN: Appleton-Century-Crofts, East.
5. M.B. Aufrere & H. Benson, J. Pharm. Sci., (1976) 65: 783.
6. T. Backstrom, “Non-reproductive Actions of Sex Steroids”, (1995) Ciba Foundation Symposium 191.
7. M. Schumacher & E-E. Baulieu, “Non-reproductive Actions of Sex Steroids”, (1995) Ciba Foundation Symposium 191.
8. R.L. Sutherland, et al., “Non-reproductive Actions of Sex Steroids”, (1995) Ciba Foundation Symposium 191.
9. J.F. Dunn, et al., J. Clin. Endocrin. Met., (1981) 53: 58.
10. B. Cook & G.H. Beastall, “Steroid Hormones: A Practical Approach”, (1987) Oxford: IRL Press Ltd.
11. N.W. Tietz, “Textbook of Clinical Chemistry”, (1986) Philadelphia, PA: W.B. Saunders Co.
12. National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Std 1	Std 5									
B	Blank	Std 1	Std 5									
C	TA	Std 2	Std 6									
D	TA	Std 2	Std 6									
E	NSB	Std 3										
F	NSB	Std 3										
G	Bo	Std 4										
H	Bo	Std 4										