Progesterone

NB-06-0500

Progesterone

#Cat: NB-06-0500 Size: 1x96 Tests

1. Introduction

Progesterone is a C-21 steroid hormone involved in the female menstrual cycle, pregnancy (supports gestation) and embryogenesis of humans and other species. Progesterone is the major naturally occurring human progestagen.

Progesterone is important for aldosterone (mineralocorticoid) synthesis, as 17-hydroxyprogesterone is for cortisol (glucocorticoid). Progesterone levels are relatively low in children and postmenopausal women. Adult males have levels similar to those in women during the follicular phase of the menstrual cycle.

In women, progesterone levels are relatively low during the preovulatory phase of the menstrual cycle, rise after ovulation, and are elevated during the luteal phase. If pregnancy occurs, progesterone levels are maintained at luteal levels initially. After delivery of the placenta and during lactation, progesterone levels are very low. The fall in progesterone levels following delivery is one of the triggers for milk production.

Progesterone is produced in the adrenal glands, the gonads (specifically after ovulation in the corpus luteum), the brain, and, during pregnancy, in the placenta.

Progesterone converts the endometrium to its secretory stage to prepare the uterus for implantation. If pregnancy does not occur, progesterone levels will decrease, leading, in the human, to menstruation.

Progesterone belongs to the group of neurosteroids that are found in high concentrations in certain areas in the brain and are synthesized there. Neurosteroids affect synaptic functioning, are neuroprotective, and affect myelinization.

Progesterone has multiple effects outside of the reproductive system. Progesterone is thermogenic, it reduces spasm and relaxes smooth muscle. Bronchi are widened and mucus regulated. Progesterone acts as an antiinflammatory agent and regulates the immune response. Progesterone also assists in thyroid function, in bone building by osteoblasts.

Measurement of serum progesterone concentrations have been used in evaluating ovarian function.

2. Intended use

Competitive immunoenzymatic colorimetric method for quantitative determination of Progesterone in serum or plasma.

3. Principle of the assay

Microtiter strip wells are precoated with anti-Progesterone antibodies (solid- phase). Progesterone in the sample competes with added horseradish peroxidase labelled Progesterone (enzyme-labelled antigen) for antibody binding. After incubation a bound/free separation is performed by solid-phase washing. The immune complex formed by enzyme-labelled antigen is visualizedby adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is **inversely** proportional to the amount of Progesterone in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorption at 450 nm is read using an ELISA microwell plate reader.

4. Materials

4.1. Reagents supplied

- **Anti-Progesterone IgG Coated Wells:** 12 breakapart 8-well snap-off stripscoated with anti-Progesterone IgG; in resealable aluminium foil.
- **Stop Solution**: 1 bottle containing 15 ml sulphuric acid, 0.15 mol/l (avoidany skin contact).
- **Progesterone-HRP Conjugate:** 1 bottle containing 22 ml of horseradish peroxidase labelled Progesterone.
- **TMB Substrate Solution**: 1 bottle containing 15 ml 3, 3´, 5, 5´-tetramethylbenzidine (H_2O_2-TMB 0.25 g/l) (avoid any skin contact).
- **Wash solution 10x conc**.: 1 bottle containing 50 ml of a 10x concentratedsolution of phosphate buffer 0.2 M, Proclin <0.0015%
- **Progesterone control**: 1 bottle containing 1 ml of a lot-specific controlsolution. The concentration is indicated on the label of the bottle.

- **Progesterone Standards:** 5 bottles, 1 ml each

☐	Standard 0:	0 ng/ml
☐	Standard 1:	0.2 ng/ml
☐	Standard 2:	1.0 ng/ml
☐	Standard 3:	8.0 ng/ml
☐	Standard 4:	40.0 ng/ml

4.2. Materials supplied

- 1 Strip holder
- 1 Cover foils
- 1 Test protocol
- 1 Distribution and identification plan

4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement ofabsorbance at 450 nm.
- Manual or automatic equipment for rinsing wells Pipettes to deliver volumes between 10 and 1000 µlDistilled water
- Timer

5. Stability and storage

The reagents are stable up to the expiry date stated on the label when stored at2...8 °C in the dark.

6. Reagent preparation

It is very important to bring all reagents, samples and standards to roomtemperature (22…28°C) before starting the test run!

6.1. Coated snap-off Strips

The ready to use break apart snap-off strips are coated with anti-Progesterone IgG antibodies. Store at 2…8°C. Open the bag only when it is at room temperature. *Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2…8 °C; stability until*

expiry date.

6.2. Progesterone-HRP Conjugate

Progesterone-HRP Conjugate is a ready to use solution.

6.3. Progesterone Standards

The standards are ready to use. Before using leave 5 min on a rotating mixer.After first opening, stable for another 6 months at 2...8°C.

6.4. TMB Substrate Solution

The bottle contains 15 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2...8°C in the dark. *The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away*.

6.5. Stop Solution

The bottle contains 15 ml 0.15 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2...8°C.

6.6. Wash Solution

Dilute the content of the concentrated Wash Solution with distilled water to a final volume of 500 ml prior to use. For smaller volumes respect the 1:10 dilution ratio. The diluted wash solution is stable for 30 days at 2…8°C. In concentrated wsh solution it is possible to observe the presence of crystals, in this case mix at room temperature until complete dissolution of crystals, for greater accuracy dilute the whole bottle of concentrated wash solution to 500ml on taking care also to transfer crystals with washing of the bottle, then mix until crystals are completely dissolved.

6.7. Stop Solution

The bottle contains 1 ml of a lot-specific control solution. The concentration is indicated on the label.

7. Specimen collection and preparation

The determination of Progesterone can be performed in plasma as well as in serum. Store the sample at -20°C if the determination is not performed on the same day as the sample collection. If samples are stored frozen, mix thawed samples well before

testing. *Avoid repeated freezing and thawing.*

8. Assay procedure

8.1. Test Preparation

Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and standards should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder. Please allocate at least:

1 well	(e.g. A1)	for the substrate blank
2 wells	(e.g. B1+C1)	for standard 0
2 wells	(e.g. D1+E1)	for standard 1
2 wells	(e.g. F1+G1)	for standard 2
2 wells	(e.g. H1+A2)	for standard 3
2 wells	(e.g. B2+C2)	for standard 4
2 wells	(e.g. D2+E2)	for control

It is recommended to determine standards and patient samples in duplicate.

Perform all assay steps in the order given and without any appreciable delays between the steps.

A clean, disposable tip should be used for dispensing each standard and eachpatient sample.

1. Dispense 20 µl standards and samples into their respective wells.
2. Add 200 µl Progesterone-HRP Conjugate to each well. Leave well A1 for substrate blank.
3. Cover wells with the foil supplied in the kit.
4. **Incubate for 1 hour at 37 °C.**

5. When incubation has been completed, remove the foil, aspirate thecontent of the wells and wash each well twice with 300 µl distilled wash solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.

6. Dispense 100 µl TMB Substrate Solution into all wells.
7. **Incubate for exactly 15 min at room temperature (22…28°C) in the dark.**
8. Dispense 100 µl Stop Solution into all wells in the same order and at thesame rate as for the TMB Substrate Solution.
   Any blue colour developed during the incubation turns into yellow.
9. Measure the absorbance of the specimen at 450 nm against a reference wavelength of 620-630 nm or against blank within 5 minutes.

8.2. Measurement

Adjust the ELISA Microwell Plate Reader **to zero** using the **substrate blank in well A1**.

If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard and patient sample in the distribution and identification plan.

Where applicable calculate the **mean absorbance values** of all duplicates.

9. Results

9.1. Calculation of results

Calculate the mean absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (Four Parameter Logistic).
Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in ng/ml.

9.2. Reference values

The following value for serum or plasma Progesterone should be considered as a guideline:

Men		<0.1 ng/ml	
Women	follicular phase	0.1 – 1.4 ng/ml	
	mid luteinic phase	4.0 – 25.0 ng/ml	
	menopause	<1.0 ng/ml	
	pregnancy	week	ng/ml
		18-21	53-76
		22-25	60-86
		26-29	71-133
		30-33	86-142
		34-37	104-175
		38-41	117-187

10. Quality control

Each laboratory should assay controls at normal, high and low levels range of Progesterone for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

11. Specific performance characteristics

11.1. Precision

Intra Assay Variation

Within run variation was determined by replicate determination (16x) of two different control sera in one assay. The within assay variability is 2.9 %.

Inter Assay Variation

Between run variation was determined by replicate measurements of three different control sera in 2 different lots. The between assay variability is 4.2 %.

11.2. Cross Reactivity

The cross reaction of the antibody calculated at 50% according to Abraham is:

Progesterone	100 %
17α OH Progesterone	0.29 %
17β Estradiol	0.0013 %
Estrone	0.00053 %
Testosterone	0.37 %
Estriol	< 0.0001 %
Cortisol	< 0.0001 %

11.3. Analytic Sensitivity

The lowest detectable concentration of Progesterone that can be distinguishedfrom the zero standard is 0.05 ng/ml at the 95 % confidence limit.

11.4. Accuracy

The recovery of 0.2 – 1.0 – 8.0 – 40.0 ng/ml Progesterone added to samplegave an average value (±SE) of 100.88% ± 8.29 % with reference to the original concentrations.

11.5. Method comparison

The Progesterone ELISA was compared to another commercially available Progesterone assay. Serum samples of 31 serum samplesaccording in both test systems.

The linear regression curve was calculated. y =

$0.97x + 0.04$ $r^2 = 0.887$

12. Precautions and warnings

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure

suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions andwarnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.

Only for in-vitro diagnostic use.

- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents ofthis test kit.
- Do not use reagents after expiry date stated on the label.Use
- only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and controlvials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
- The ELISA is only designed for qualified personnel who are familiar with good laboratory practice.
- Maximum precision is required for dispensation of the reagents.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- Avoid the exposure of TMB substrate to direct sunlight, metal or oxidants. The

Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.

For higher values, for example in pregnancy, dilute the sample; consider the dilution factor when calculating the result.

- Treatment of the patient with cortisone, natural or synthetic steroids can impair Progesterone determination.
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratoriesby assaying controls and/or pooled sera.
- Microbiologically contaminated samples should not be used in the assay.Highly lipemeic or haemolysed specimens should also not be used.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate.
- The incomplete or inaccurate liquid removal from the wells could influencethe assay precision and/or increase the background.
- Some reagents contain small amounts of Proclin 300® as preservative.Avoid contact with skin or mucosa.
- This method allows the determination of Progesterone from 0.2 ng/ml to 40ng/ml.

12.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or wastemanagement companies which will give advice on how to dispose hazardous waste.

13. Literature

Wisdom G.B. (1976) Clin. Chem. 22 (8), 1243 – 1255.

De Villa G.O. et al. (1972) J. Clin. Endoc. Metab. 35, 458.Joyce,B.G. et al. Steroids 29, no 6, 761, (1977)

Winkel P. et al. Clin. Chem. 22 (4), 422 (1976)
Rajkowski K.N et al. Steroids 29, no 5 (1977)

14. Ordering information

Prod. No.: NB-06-0500 Progesterone Determination (96 Determinations)

SCHEME OF THE ASSAY

Progesterone

Test Preparation

Prepare reagents and samples as described.
Establish the distribution and identification plan for all specimens and controls on the result sheet supplied in the kit.
Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

	Substrate blank	Standard 1– 4	Control	Sample
Standard 1 – 4		20 µl		
Control			20 µl	
Sample				20 µl
Conjugate		200 µl	200 µl	200 µl
Cover wells with foil supplied in the kit Incubate for 1 hour at 37 °C Wash each well three times with 300 µl diluted wash solution				
TMB Substrate	100 µl	100 µl	100 µl	100 µl
Incubate for exactly 15 min at room temperature (22…28°C) in the dark				
Stop Solution	100 µl	100 µl	100 µl	100 µl
Shake the microplate gently Photometric measurement at 450 nm				

For reference only

For Research Use Only. Not for Diagnostic or Therapeutic Use.

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