PROGESTERONE

Enzyme-immunoassay for the quantitative determination of Progesterone in human serum

CE

REF GD7270 00

IVD

INDICATION

Progesterone is a C-21 steroid hormone involved in the female menstrual cycle, pregnancy and embryogenesis of humans and other species.
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, in the gonads (specifically after ovulation in the corpus luteum), in 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 is thermogenic, it reduces spasm and relaxes smooth muscle, it is involved in bronchi dilation and mucus regulation. Progesterone acts as an anti inflammatory 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.

PRINCIPLE OF THE ASSAY

This test is based on "one step" competition enzyme immunoassay principle (ELISA). Tested specimen is placed into the microwells coated by specific anti-Progesterone antibodies simultaneously with Progesterone conjugated to Horseradish peroxidase (HRP). Progesterone from the specimen competes with the conjugated antigen for coated antibodies. After washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and quantified by addition of chromogen-substrate solution. The developed colour, detected at 450 nm, is inversely related to the quantity of Progesterone present in the specimen.
Progesterone concentration in the sample is calculated based on a series of standards.

KIT CONTENT

1. **Reagent A – Microplate**
   12x8 strips.
   8 wells breakable strips, coated with anti-Progesterone monoclonal antibodies. The strips are assembled on a plastic frame and contained in a sealed bag with desiccant. Bring the strips to room temperature before use, to prevent any moisture formation inside the bag.

2. **Reagent B – Enzymatic Tracer**
   1 vial of 12 ml.
   Ready to use solution containing Progesterone, conjugated with Horseradish peroxidase (HRP) in a proteic stabilized matrix with 0.004% Gentamycin sulphate, 0.1% ProClin 300 as preservatives.

3. **Reagent C – Washing Solution 25x**
   1 vial of 50 ml.
   Concentrated solution to be diluted 1:25 with distilled water. It contains a detergent in Phosphate buffer.

4. **Reagent D/E – Chromogen/Substrate**
   1 vial of 12 ml.
   Ready to use solution containing Tetramethylbenzidine (TMB) and H_2O_2 in Citric acid buffer.
   Avoid any skin contact and light exposure.

5. **Reagent F – Stop Solution**
   1 vial of 15 ml.
   Ready to use solution containing Sulphuric acid 0.2 M.
   Avoid any skin contact.

6. **Progesterone Standards:**
   6 vials of 0.5 ml each.
   Ready to use human serum based liquids containing Progesterone and 0.1% ProClin 300, 0.1% Phenol as preservatives.
   Approximately Progesterone concentrations are the following:
   S_0: 0 nmol/l, **S_1**: 1.6 nmol/l, **S_2**: 10.5 nmol/l,
   S_3: 26 nmol/l, **S_4**: 51 nmol/l, **S_5**: 105 nmol/l.
   For SI units: nmol/l x 0.314 = ng/ml.
   Actual concentrations to be used for calculation are stated on the labels of the vials.

7. **Progesterone Control:**
   1 vial of 0.5 ml.
   Ready to use liquid containing human serum with a defined quantity of Progesterone and 0.1% Proclin 300, 0.1% Phenol as preservatives.
   Refer to the vial label for acceptable range.

8. **Cardboard sealers**
   2 cardboard sealers to be used to cover the plate during the incubations.

9. **Package insert:** instruction for use GD7270 00 it/ing.

MICROBIOLOGICAL STATE AND CLEANING GRADE

1. All the materials of human origin resulted negative to HbsAg, HIV 1&2 and HCV FDA approved tests. Anyhow, as no test can guarantee the absolute absence of infective agents, handle reagents as potentially infected, especially standards, controls and samples. All objects come in direct contact with samples and all residuals of the assay should be treated or eliminated as potentially infected. Best procedures for inactivation are treatments with autoclave at 121°C for 30 minutes or with sodium hypochlorite at a final concentration of 2.5 % for 24 hours.
2. Avoid any contact with skin and mucous membrane, in particular for Chromogen/Substate and Stop Solutions.
3. Use protective disposable talk-free gloves.
4. Avoid contaminating reagents when taking them from the vials. We recommend to use automatic pipettes with disposable tips. When dispensing reagents, do not touch with tips the wall of wells in order to avoid cross-contaminations.
5. For the washing step, use only the Washing Solution provided in the kit and follow carefully the indications reported in "WASHING INSTRUCTION".
6. Avoid the substrate/chromogen to come in contact with oxidizing agents or metallic surfaces; avoid intense light exposure during incubation or reagent preparation.

STORAGE AND STABILITY OF THE KIT

1. The kit has to be stored at 2-8 °C and used before the expiry date stated on the label.
2. Unused strips have to be placed in the bag containing the desiccant and firmly sealed before restore at 2-8 °C. After opening the strips are stable up to the expiry date stated on the label.
3. All other reagents can be repeatedly used up to exhaustion if stored at 2-8 °C, provided that they are handled carefully to avoid any environment contamination. Under these conditions the reagents are stable up to the expiry date stated on the labels.

AUXILIARY MATERIALS

- Semi automatic pipettes of 25, 100 and 150 µl
- Vortex mixer and absorbent paper
- Chronometer
- Ultrapure Elisa grade water
- Thermoshaker at 37 (± 0.5) °C
- Photometric reader of microplates or microstrips, linear up to at least 2 OD and supplied with filter of 450 nm.
- Automatic microplates washing device or manual apparatus capable of aspirating and dispensing volumes of 300 µl.

SAMPLES

Serum only may be used. The kit is not calibrated for the determination of Progesterone in plasma, saliva or other specimens of human or animal origin. The blood should be collected in plain redtop venipuncture tube without additives and gel barrier. Separate serum as soon as possible to avoid any hemolysis. Samples can be stored at 2–8 °C for a short time (max three days). For longer storage the specimen should be frozen. Avoid repeated freezing and thawing. Highly lipemic, hemolysed, preserved by sodium azide or microbiologically contaminated samples should not be used in the assay.

REAGENTS PREPARATION

- WASHING SOLUTION: dilute 1:25 with distilled or ELISA grade water (e.g.: 20 ml of Reagent C + 480 ml of distilled water) and mix carefully before use. The diluted washing solution can be stored for 3 days at 2-8 °C. The concentrated solution may present a sediment that can be dissolved at 35-39 °C and shaking. It is recommended to store washing solution at room temperature for immediate use.

WASHING INSTRUCTION

A good washing procedure is essential to obtain correct and precise analytical results.
We therefore recommend to use a good quality ELISA microplate washer, maintained at a good level of washing mechanical performances.
Generally, 5 automatic washing cycles of 0.3 ml/well are sufficient to avoid false positive reactions and remove high background. Anyhow we recommend to calibrate the washing system on the kit itself so to match the declared analytical performances.
In case of manual washing, we suggest to perform 5 washing cycles, dispensing and aspirating 0.3 ml/well per cycle.
In any case the liquid washed out from the plates must be inactivated with a sodium hypochlorite solution at a final concentration of 2.5%, before being thrown away or autoclaved, as it must be considered as potentially infected.

ASSAY PROCEDURE

1. At least one hour before use, bring all reagents, standards, control and samples to room temperature (18-30 °C), mixing them carefully on vortex.
2. Do not mix reagents from different lots.
3. We recommend to distribute standards, control and samples in duplicate.
4. Distribution and incubation times must be the same for all wells in the same analysis.
5. Avoid long interruptions between each step of the assay procedure.
6. It is suggested to eliminate the excess of washing solution from the microplate after washing by blotting it gently on an absorbent paper pad.
7. The colour developed in the last incubation is stable for a maximum of 20 minutes. Otherwise, in case of reading after 10-15 min after dispensing stop solution, immediately place the strips **in the dark**.

ASSAY SCHEME

1. Put the desired number of microstrips into the frame.
2. Follow the scheme:

		Microplate wells coated with anti-Progesterone antibody	
	REAGENTS	**Standards, Control**	**Sample**
Immunological reaction	Standards, Control	25 µl	-
	Sample	-	25 µl
	Reagent B (Enzymatic Tracer)	100 µl	100 µl
	- Cover the strips with cardboard sealer - Incubate **90 minutes at room temperature (20-25 °C)**		
Washing	- Peel out the cardboard sealer and aspirate the reaction solution from all wells - Rinse 5 times with 300 µl of diluted washing solution, carefully aspirating off the remaining liquid		
Colorimetric reaction	Reagent D/E (Chromogen-Substrate)	100 µl	100 µl
	- Cover the strips with cardboard sealer - Incubate **20-30 minutes at room temperature** (**20-25** °C), avoiding light exposure		
	Reagent F (Stop Solution)	150 µl	150 µl
	- Gently mix for 5-10 seconds - Read the absorbance of each well at 450 nm.		

CALCULATION OF RESULTS

1. Calculate the mean of the absorbance values for each point of the standard curve, control and of each sample.
2. Draw a calibration curve on a linear graph paper with the mean optical densities on the Y-axis and the standards concentrations on the X-axis. If immunoassay software is being used, a 4-parameter curve is recommended.
3. Interpolate the values of the samples on the standard curve to obtain the corresponding values of concentration expressed in nmol/l.

VALIDITY OF THE TEST

For the test to be valid the following criteria must be met:

1. Standard 0 nmol/l OD 450 nm: ≥ 1.3
2. Calculated value of Control should be within the established range stated on the label.

EXPECTED VALUES

From data obtained by AMS the following reference ranges are suggested. Otherwise, it is recommended that each laboratory establishes its own normal and abnormal values according to the examined population.

Subjects	**Range (nmol/l)**
Male:	0.5-5.2
Female:	
follicular phase	0.5-6.5
luteal phase	8.0-87.0

Note:

- The results obtained with this kit should never be used as the sole basis for clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has to potential of causing interferences in immunological tests. For diagnostic purpose, results should be used in conjunction with other data; e.g. symptoms, results of other thyroid tests, clinical impressions, etc.

ANALYTICAL PERFORMANCES

Assay range

The range of the assay is 0 – 100 nmol/l.

Analytical Sensitivity

The lower detection limit is 0.5 nmol/l. The sensitivity was calculated by determining the variability of Standard 0 nmol/l and using the 2 SD (95% certainty) statistics.

Precision

a. Intra Assay Variation

Sample	**Mean, nmol/l**	**SD**	**%CV**
1	0.84	0.03	3.5
2	69.00	3.20	4.6

b. Inter Assay Variation

Sample	**Mean, nmol/l**	**SD**	**%CV**
1	0.84	0.03	3.5
2	68.70	3.60	5.3

Specificity

The following compounds were tested for cross-reactivity:

Substance	**Cross reactivity, %**
Progesterone	100
Testosterone	0.01
Estradiol	0.004
Cortisone	0.041
Cortisol	0.005
Corticosterone	0.100
DihydroProgesterone	0.041
Androstenedione	0.100
17-OH-Progesterone	2.900
Pregnandion	10.00

Recovery
Spiked samples were prepared by adding defined amounts of Progesterone to patient serum sample. The results are tabulated below:

Added (nmol/l)	Measured (nmol/l)	Expected (nmol/l)	Recovery (%)
-	25.0	25.0	-
200	112.5	110.0	97.7
100	62.5	62.8	100.5
50	37.5	35.2	93.8

Accuracy
The present kit was compared with a Chemiluminescent microparticle immunoassay as a reference test. 200 specimens were tested (values ranged from 0.3 to 170 nmol/l).
The following linear regression curve was calculated:

y = 0.9291x + 1.1575 nmol/l r = 0.958

PRECAUTIONS IN USE
The reagents are not considered harmful according to the 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.
The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management
Please refer to local legal requirements.

REFERENCES
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3. Matthews C.P., et al.:Obstet.Gynecol., 68:390 (1986).

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