# Progesterone ELISA

## *KAPD1561*

DIAsource ImmunoAssays S.A. - Rue du Bosquet, 2 - B-1348 Louvain-la-Neuve – Belgium

# History

**Summary of change :**

| **Previous Version :**<br>200224-1 | **Current Version :**<br>230718 |
|---|---|
| Old DiaSource logo | New DiaSource logo on the front page |

# Progesterone ELISA

en

**KAPD1561**

***IN VITRO DIAGNOSTIC USE***

DIAsource ImmunoAssays S.A. - Rue du Bosquet 2, B-1348 Louvain-la-Neuve, Belgium - Tel: +32 10 84 99 11 - Fax : +32 10 84 99 91

## 1 INTENDED USE

Progesterone ELISA is a test system for the quantitative determination of Progesterone in serum and plasma (EDTA, lithium heparin or citrate plasma). This assay is intended for in vitro diagnostic use only.

## 2 INTRODUCTION

Progesterone (pregn-4-ene-3, 20-dione) is a C21 steroid hormone containing a keto-group (at C-3) and a double bond between C-4 and C-5 (Δ4).
This steroid hormone is a female sex hormone which, in conjunction with estrogens, regulates the accessory organs during the menstrual cycle and it is particularly important in preparing the endometrium for the implantation of the blastocyte and in maintaining pregnancy.
In non-pregnant women progesterone is mainly secreted by the corpus luteum whereas in pregnancy the placenta becomes the major source. Minor sources are the adrenal cortex for both sexes and the testes for males.
Progesterone circulates in blood mainly bound to Corticosteroid Binding Globulin (CBG), Sex Hormone Binding Globulin (SHBG) and Albumin. Only 2-10% of the total concentration circulates as free hormone.
Blood progesterone concentrations vary widely according to the phases of menstrual cycle; they are lower than 1 ng/mL (3.2 nmol/L) in the follicular phase and around 10-20 ng/mL (32 -64 nmol/L) in the luteal phase.
The maximal levels are achieved 4-7 days after ovulation and remain elevated for 4-6 additional days prior to falling to the preovulatory levels 24 hours before the onset of menstruation.
Since the rise and fall of progesterone parallel the activity of ovarian follicle and corpus luteum, measurements of plasma progesterone are clinically used to confirm ovulation and normal function of the corpus luteum in non-pregnant women.
If ovulation does not occur the corpus luteum is not formed and no cyclical rise of progesterone in plasma is observed. Abnormal progesterone secretion has been implicated in premenstrual tension, irregular shedding of endometrium, dysmenorrhoea, and luteal insufficiency.
Progesterone concentration can vary not only from subject to subject but also in the same person from day to day or even from hour to hour. Consequently, in gynecological disorders or abnormal pregnancies serial measurements rather than single ones are recommended for a proper interpretation of results.
During pregnancy progesterone is widely produced by placenta, and plasma levels rise steadily achieving values as high as 200 ng/mL at term.

## 3 PRINCIPLE OF THE TEST

The DIAsource Progesterone ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding.
The microtiter wells are coated with a polyclonal (rabbit) antibody directed towards a unique antigenic site of the Progesterone molecule.
During the first incubation, the progesterone in the added sample competes with the added enzyme conjugate, which is a progesterone molecule conjugated to horseradish peroxidase, for binding to the coated antibody.
After a washing step, to remove all unbound substances, the solid phase is incubated with the substrate solution. The colorimetric reaction is abruptly stopped by addition of stop solution and optical density (OD) of the resulting yellow product is measured. The intensity of color is inversely proportional to the concentration of the analyte in the sample.
A standard curve is constructed by plotting OD values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

## 4 WARNINGS AND PRECAUTIONS

- This kit is for in vitro diagnostic use only.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
- All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- Avoid contact with Stop Solution containing 0.5 M $H_2SO_4$. It may cause skin irritation and burns.
- Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.
- Safety Data Sheets for this product are available upon request directly from DIAsource ImmunoAssays S.A.

## 5 REAGENTS

### 5.1 Reagents provided

1. **Microplate**
   One divisible microplate consisting of 12 modules of 8 wells each. Ready to use.
2. CAL | N — **Calibrator 0-6**, 7 vials, 1ml, ready to use
   Concentrations: 0; 0.3; 1.25; 2.5; 5; 15; 40 ng/mL Contain non-mercury preservative.
3. Ag | HRP — **Enzyme conjugate**, 1 vial, 25ml, ready to use
   Containing Progesterone conjugated to horseradish Peroxidase; Contain non-mercury preservative
4. CHROM | TMB — **TMB Substrate**, 1 vial, 25 ml, ready to use
5. STOP | SOLN — **Stop Solution**, 1 vial, 14 ml, ready to use
   Contains acid.
6. WASH | SOLN | CONC — **Wash buffer**, 1 vial, 30ml, 40x conc. see 8. PREPARATION OF REAGENTS

### 5.2 Materials required but not provided

- Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Calibrated variable precision micropipettes
- Absorbent paper
- Distilled water

## 6 SPECIMEN COLLECTION, STORAGE AND HANDLING

Serum or plasma (EDTA-, Heparin- or citrate plasma) can be used in this assay.

Note: Samples containing sodium azide should not be used in the assay.
In general it should be avoided to use haemolytic, icteric or lipaemic specimens. For further information refer to chapter “Interfering Sustances”.

### 6.1 Specimen Collection

**Serum:**
Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

**Plasma:**
Whole blood should be collected into centrifuge tubes containing anti coagulant and centrifuged immediately after collection.

### 6.2 Specimen Storage

Specimens should be capped and may be stored for up to 7 days at 2-8°C prior to assaying.
Specimens held for a longer time (up to 12 months) should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

### 6.3 Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted 10-fold or 100 fold with *Calibrator 0* and reassayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.
Example:
a) Dilution 1:10: 10 µL Serum + 90 µL Calibrator 0 (mix thoroughly)
b) Dilution 1:100: 10 µL dilution a) 1:10 + 90 µL Calibrator 0 (mix thoroughly).

## 7 STORAGE AND STABILITY

- Store test kit at 2 °C - 8 °C.
- Do not use reagents beyond this date.
- Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Once the foilbag has been opened, care should be taken to close it tightly again
- Opened kits retain activity for two months if stored as described above.

## 8 PREPARATION OF REAGENTS

Allow all reagents and required number of strips to reach room temperature prior to use.

**Wash Buffer**
Add deionized water to the 40X concentrated Wash Solution.
Dilute 30 mL of concentrated Wash Solution with 1170 mL deionized water to a final volume of 1200 mL.
The diluted Wash Solution is stable for 2 weeks at room temperature.

## 9 PROCEDURE

### 9.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each calibrator, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

### 9.2 Assay Procedure

Each run must include a calibration curve.

1. Secure the desired number of Microtiter wells in the holder.
2. Dispense 25 µL of each calibrator, control and samples with new disposable tips into appropriate wells.
3. Incubate for 5 minutes at room temperature.
4. Dispense 200 µL Enzyme Conjugate into each well. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
5. Incubate for 60 minutes at room temperature.
6. Rinse the wells **3 times** with 400 µL diluted Wash Solution per well, if a plate washer is used - or - Briskly shake out the contents of the wells.
   Rinse the wells **3 times** with 300 µL diluted *Wash Solution* per well for manual washing. Strike the wells sharply on absorbent paper to remove residual droplets.
   **Important note:**
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
7. Add 200 µL of Substrate Solution to each well.
8. Incubate for 15 minutes at room temperature.
9. Stop the enzymatic reaction by adding 100 µL of Stop Solution to each well.
10. Determine the optical density (OD) of the solution in each well at 450 nm (reading) and at 620 nm to 630 nm (background subtraction, recommended) with a microtiter plate reader.
    It is recommended that the wells be read within 10 minutes after adding the Stop Solution

### 9.3 Calculation of Results

1. Calculate the average absorbance values for each set of calibrators and patient samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each calibrator against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the calibration curve.
4. Automated method: The results in the Instructions for Use have been calculated automatically using a 4 Parameter curve fit. (4 Parameter Rodbard or 4 Parameter Marquardt are the preferred methods.) Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this calibration curve. Samples with concentrations higher than that of the highest calibrator have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.

#### 9.3.1 Example of Typical Calibration Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

| Calibrator | Optical Units (450 nm) |
|---|---|
| Calibrator 0 (0 ng/mL) | 1.52 |
| Calibrator 1 (0.3 ng/mL) | 1.17 |
| Calibrator 2 (1.25 ng/mL) | 0.88 |
| Calibrator 3 (2.5 ng/mL) | 0.69 |
| Calibrator 4 (5.0 ng/mL) | 0.55 |
| Calibrator 5 (15 ng/mL) | 0.35 |
| Calibrator 6 (40 ng/mL) | 0.13 |

## 10 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Employ appropriate statistical methods for analyzing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

## 11 PERFORMANCE

### 11.1 Assay Dynamic Range

The range of the assay is between 0.140 ng/mL – 40.0 ng/mL.

### 11.2 Specificity

The following substances were tested for cross reactivity of the assay:

| Steroid | Cross Reaction (%) |
|---|---|
| Progesterone | 100 |
| 17α OH Progesterone | 0.30 |
| Estriol | < 0.10 |
| Estradiol 17β | < 0.10 |
| Testosterone | < 0.10 |
| 11-Desoxycorticosterone | 1.10 |
| DHEA-S | < 0.02 |
| Cortisol | < 0.02 |
| Corticosterone | 0.20 |
| Pregnenolone | 0.35 |
| Cortison | < 0.10 |
| 11-Desoxycortisol | 0.10 |

### 11.3 Analytical Sensitivity

The analytical sensitivity was calculated from the mean minus two calibratordeviations of twenty (20) replicate analyses of *Calibrator 0* and was found to be 0.045 ng/mL.

The Limit of Blank (LoB) is 0.120 ng/mL.
The Limit of Detection (LoD) is 0.140 ng/mL.
The Limit of Quantification (LoQ) is 0.144 ng/mL.

### 11.4 Precision

#### 11.4.1 Intra Assay Variation

The within assay variability is shown below:

| Sample | n | Mean (ng/mL) | CV (%) |
|---|---|---|---|
| 1 | 20 | 0.6 | 5.4 |
| 2 | 20 | 4.7 | 7.0 |
| 3 | 20 | 10.8 | 6.9 |

#### 11.4.2 Inter Assay Variation

The between assay variability is shown below:

| Sample | n | Mean (ng/mL) | CV (%) |
|---|---|---|---|
| 1 | 12 | 0.6 | 10.0 |
| 2 | 12 | 4.6 | 4.3 |
| 3 | 12 | 10.7 | 5.6 |

#### 11.4.3 Inter-lot variation

The inter-assay (between-lots) variation was determined by repeated measurements of samples with 3 different kit lots.

| Sample | n | Mean (ng/mL) | CV (%) |
|---|---|---|---|
| 1 | 18 | 1.2 | 7.2 |
| 2 | 18 | 38.7 | 3.1 |

### 11.5 Recovery

Samples have been spiked by adding Progesterone solutions with known concentrations in a 1:1 ratio.
The expected values were calculated by addition of half of the values determined for the undiluted samples and half of the values of the known solutions. The % Recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100.

| Sample | Added Concentration 1:1 (v/v) (ng/mL) | Measured Conc. (ng/mL) | Expected Conc. (ng/mL) | Recovery (%) |
|---|---|---|---|---|
| | | 1.63 | | |
| | 40.0 | 20.35 | 20.82 | 97.8 |
| 1 | 15.0 | 8.45 | 8.32 | 101.6 |
| | 5.0 | 3.52 | 3.32 | 106.2 |
| | 2.5 | 2.31 | 2.07 | 112.0 |
| | | 4.17 | | |
| | 40.0 | 22.69 | 22.09 | 102.7 |
| 2 | 15.0 | 10.45 | 9.59 | 109.0 |
| | 5.0 | 4.42 | 4.59 | 96.3 |
| | 2.5 | 3.62 | 3.34 | 108.3 |
| | | 11.03 | | |
| | 40.0 | 26.94 | 25.52 | 105.6 |
| 3 | 15.0 | 12.44 | 13.02 | 95.6 |
| | 5.0 | 7.70 | 8.02 | 96.1 |
| | 2.5 | 6.15 | 6.77 | 90.9 |

### 11.6 Linearity

| Sample | Dilution | Measured Conc. (ng/mL) | Expected Conc. (ng/mL) | Recovery (%) |
|---|---|---|---|---|
| | None | 1.63 | 1.63 | |
| | 1:2 | 0.75 | 0.82 | 92.0 |
| 1 | 1:4 | 0.46 | 0.41 | 111.9 |
| | 1:8 | 0.20 | 0.20 | 99.1 |
| | 1:16 | 0.11 | 0.10 | 107.0 |
| | None | 4.17 | 4.17 | |
| | 1:2 | 2.30 | 2.09 | 110.3 |
| 2 | 1:4 | 1.09 | 1.04 | 104.8 |
| | 1:8 | 0.49 | 0.52 | 93.3 |
| | 1:16 | 0.23 | 0.26 | 87.8 |
| | None | 11.03 | 11.03 | |
| | 1:2 | 5.81 | 5.52 | 105.4 |
| 3 | 1:4 | 2.96 | 2.76 | 107.2 |
| | 1:8 | 1.50 | 1.38 | 108.6 |
| | 1:16 | 0.72 | 0.69 | 104.7 |

## 12 LIMITATIONS OF USE

### 12.1 Interfering Substances

Any improper handling of samples or modification of this test might influence the results.
Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 1.8 mg/mL) have no influence on the assay results.

### 12.2 Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of Progesterone in a sample.

### 12.3 High-Dose-Hook Effect

No hook effect was observed in this test.

## 13 EXPECTED NORMAL VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted with apparently healthy subjects, using the DRG Progesterone ELISA the following data were observed:

| Population | n | Mean (ng/mL) | Median (ng/mL) | 2.5th - 97.5th Percentile (ng/mL) | Range (min. - max.) (ng/mL) |
|---|---|---|---|---|---|
| **Males** | 49 | 0.36 | 0.34 | 0.05 - 0.92 | 0.05 - 0.94 |
| | | | | | |
| **Females** | | | | | |
| Follicular Phase | 35 | 0.79 | 0.76 | 0.21 - 1.72 | 0.21 - 1.80 |
| Luteal Phase | 45 | 12.89 | 13.00 | 3.78 - 24.60 | 2.90 - 27.10 |
| | | | | | |
| Postmenopausal | 28 | 0.53 | 0.59 | 0.18 - 0.83 | 0.15 - 0.84 |

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

## 14 REFERENCES


**1** Filicori m, butler jp, crowley wf jr. Neuroendocrine regulation of the corpus luteum in the human. J clin invest. 73:1638 1984.

**2** Katt ja, duncan ja, herbon l, et al. The frequency of gonadotropin releasing hormone stimulation determines the number of pituitary gonadotropin releasing hormone receptors. Endocrinology 1985; 116:2113.

**3** Csapo ai, pulkkinen mo, wiest wg: effects of lutectomy and progesterone replacement therapy in early pregnancy patients. Am j obstet gynecol 115:759, 1973.

**4** Thomas labor und diagnose


Revision date: 2023-07-18