DRG® Progesterone (serum) CLIA (CLA-4663)

CE

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1 INTRODUCTION

The **DRG Progesterone Chemiluminescence Immunoassay Kit** provides materials for the quantitative determination of Progesterone in serum and plasma.

This assay is intended for in vitro diagnostic use only.

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.

2 PRINCIPLE OF THE TEST

The DRG Progesterone CLIA Kit is a chemiluminescence immunoassay (CLIA), based on the principle of competitive binding.

The microtiter wells are coated with a polyclonal antibody directed towards an antigenic site on the Progesterone molecule. Endogenous Progesterone of a patient sample competes with a Progesterone horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off.

CE

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The amount of bound peroxidase conjugate is reverse proportional to the concentration of Progesterone in the sample. After addition of the substrate solution, the intensity of emitted light is reverse proportional to the concentration of Progesterone in the patient sample.

3 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.
- 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 luminometer.
- The luminescence substrate reagents (*Reagent A* and *Reagent B*) are sensitive to light and should be stored in the original dark bottle away from direct sunlight.
- 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 DRG International, Inc.
  The Safety Data Sheets fit the demands of: EU-Guideline 91/155 EC.

Revised 18 Feb. 2008 rm (Vers. 1.1)

4 KIT COMPONENTS

4.1 Contents of the Kit

1. ***Microtiterwells***, 12x8 (break apart) strips, 96 wells
   Wells coated with polyclonal anti-Progesterone antibody
2. ***Standard (Standard 0-6)*****,** 7 vials, 1 mL, ready to use
   Concentrations: 0 - 0.3 - 1.25 - 2.5 - 5 - 15 - 40 ng/mL
   Conversion: 1 ng/mL = 3.18 nmol/L
   contains 0.3% Proclin as a preservative
3. ***Enzyme Conjugate***, 1 vial, 25 mL, ready to use
   Progesterone conjugated to horseradish Peroxidase
   contains 0.3% Proclin as a preservative
4. ***Chemiluminescence Substrate Solution***,
   Reagent A, 1 vial, 4 mL, *Note: light sensitive!*
   Reagent B, 1 vial, 4 mL, *Note: light sensitive!*
   Reagent C, 1 vial, 6 mL
   see „Preparation of Reagents“.
5. ***Wash Solution***, 1 vial, 30 mL (**40X** concentrated)
   see „Preparation of Reagents“

Note: Additional *Standard 0* for sample dilution is available on request.

4.1.1 Equipment and material required but not provided

- A microtiter plate luminometer.
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Aqua dest.

4.2 Storage and stability of the Kit

When stored at 2-8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2-8°C. Microtiter wells must be stored at 2-8°C. 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.

4.3 Preparation of Reagents

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

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.

Chemiluminescence Substrate Solution

Mix **1 part** of the chemiluminescence ***Reagent A*** with **1 parts** of ***Reagent B*** and dilute this mixture 1:2 with ***Reagent C***. This gives the ready to use substrate solution.
The prepared substrate solution is stable for one hour. Prepare fresh before use.

If the whole plate is to be used prepare the substrate solution as follows:

Add 3 mL of each *Reagent A* and *Reagent B* into 6 mL *Reagent C*

4.4 Disposal of the Kit

The disposal of the kit must be made according to the national official regulations. Special information for this product is given in the Material Safety Data Sheets (see chapter 13).

4.5 Damaged Test Kits

In case of any severe damage to the test kit or components, DRG has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5 SPECIMEN

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

Do not use haemolytic, icteric or lipaemic specimens.

Please note: Samples containing sodium azide should not be used in the assay.

5.1 Specimen Collection

Serum:

Collect blood by venipuncture (e.g. Sarstedt Monovette # 02.1388.001), 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.

(E.g. for EDTA plasma Sarstedt Monovette – red cap - # 02.166.001;
for Heparin plasma Sarstedt Monovette – orange cap - # 02.165.001)

5.2 Specimen Storage

Specimens should be capped and may be stored for up to 24 hours at 2-8°C prior to assaying.

Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 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 *Standard 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 *Standard 0* (mix thoroughly)

b) Dilution 1:100: 10 µl dilution a) 1:10 + 90 µl *Standard 0* (mix thoroughly).

6 TEST PROCEDURE

6.1 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 standard, control or sample in order to avoid cross contamination.
- Light intensity 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.

6.2 Assay Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the holder.
2. Dispense **25 µl** of each *Standard, Control* and samples with new disposable tips into appropriate wells.
3. Dispense **200 µl** *Enzyme Conjugate* into each well.
4. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
5. Incubate for **35 minutes** at room temperature.
6. Briskly shake out the contents of the wells.
   Rinse the wells 5 times with diluted Wash Solution (400 µl per well). 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 **100 µL** of the freshly prepared Substrate Solution to each well. *(See "Preparation of Reagents.)*
8. Incubate for **10 minutes** at room temperature.
9. Read the RLU with a microtiter plate luminometer **within 20 minutes** after incubation time of substrate.

6.3 Calculation of Results

1. Calculate the average Relative Light Units (RLU) values for each set of standards, controls and patient samples.
2. Construct a standard curve by plotting the mean RLU obtained from each standard against its concentration with RLU value on the vertical(Y) axis and concentration on the horizontal (X) axis.
3. Using the mean RLU value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3.1 Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay. Below is listed a typical example of a standard curve** with the DRG Progesterone CLIA.

Standard	RLU (x10^3)	RLU/RLU_{max} (%)
Standard 0 (0 ng/mL)	3319	100
Standard 1 (0.3 ng/mL)	2393	72.1
Standard 2 (1.25 ng/mL)	1542	46.5
Standard 3 (2.5 ng/mL)	1142	34.4
Standard 4 (5 ng/mL)	808	24.4
Standard 5 (15 ng/mL)	416	12.5
Standard 6 (40 ng/mL)	242	7.3

** It is recommended to use the RLU/RLU_{max} values for comparative purposes since luminometers vary considerably between manufacturers. Results from different luminometers will show different RLU values, however, the RLU/RLU_{max} values remain consistent.

7 EXPECTED VALUES

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

In a study conducted with apparently normal healthy adults, using the DRG Progesterone CLIA the following values are observed:

Normal Women

Follicular phase: 0.2 – 1.4 ng/mL
Luteal phase: 4 – 25 ng/mL
Menopause: 0.1 – 1 ng/mL

Normal men 0.1 – 1ng/mL

8 QUALITY CONTROL

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.

The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

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 analysing 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.

After checking the above mentioned items without finding any error contact your distributor or DRG directly.

9 ASSAY CHARACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between 0 – 40 ng/mL.

9.2 Specificity of Antibodies (Cross Reactivity)

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

Steroid	Cross Reaction (%)
Progesterone	100.00
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
Cortisone	< 0.10
11-Desoxycortisol	0.10

9.3 Analytical Sensitivity

The analytical sensitivity was calculated from the mean minus two standard deviations of twenty (20) replicate analyses of *Standard 0* and was found to be 0.009 ng/mL.

9.4 Precision

9.4.1 Intra Assay Variation

The within assay variability is shown below:

Sample	n	Mean (ng/mL)	CV (%)
1	20	0,39	5,51
2	20	8,52	4,34
3	20	3,62	4,46

9.4.2 Inter Assay Variation

The between assay variability is shown below:

Sample	n	Mean (ng/mL)	CV (%)
1	12	0.44	7.40
2	12	3.13	8.97
3	12	6.44	8.52

9.5 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	40.0	19.29	20.48	94.2
	15.0	8.04	7.98	100.8
	5.0	3.14	2.98	105.4
2	40.0	21.26	20.11	105.7
	15.0	8.04	7.61	105.7
	5.0	2.87	2.61	110.2
3	40.0	17.26	20.13	85.8
	15.0	7.21	7.63	94.6
	5.0	2.60	2.63	99.0

9.6 Linearity

Sample	Dilution	Measured Conc. (ng/mL)	Expected Conc. (ng/mL)	Recovery (%)
1	None	1.00	1.00	
	1:2	0.51	0.50	102.0
	1:4	0.23	0.25	92.0
	1:8	0.12	0.13	96.0
2	None	24.75	24.75	
	1:2	11.86	12.38	95.8
	1:4	5.28	6.19	85.3
	1:8	2.88	3.09	93.1
3	None	2.06	2.06	
	1:2	0.97	1.03	94.2
	1:4	0.46	0.52	89.3
	1:8	0.24	0.26	93.2

10 LIMITATIONS OF USE

Any improper handling of samples or modification of this test might influence the results.

10.1 Interfering Substances

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.

10.2 Drug Interferences

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

10.3 High-Dose-Hook Effect

No hook effect was observed in this test.

11 LEGAL ASPECTS

11.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact DRG.

11.2 Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 10.1. Any laboratory result is only a part of the total clinical picture of a patient.

Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.

The test result itself should never be the sole determinant for deriving any therapeutic consequences.

11.3 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.

Claims submitted due to customer misinterpretation of laboratory results subject to point 10.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

12 REFERENCES

1. Filicori M, Butler JP, Crowley WF Jr. Neuroendocrine regulation of the corpus luteum in the human. J Clin Invest. 73:1638 1984.
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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