



DIAGNOSTIC AUTOMATION, INC.

23961 Craftsman Road, Suite D/E/F,
Calabasas, CA 91302

Tel: (818) 591-3030 Fax: (818) 591-8383

onestep@rapidtest.com

technicalsupport@rapidtest.com

www.rapidtest.com

IVD



See external label



2°C-8°C



Σ=96 tests

REF

Cat # 9025-16

**CHEMILUMINESCENCE
ENZYME IMMUNOASSAY (CLIA)
PROGESTERONE**

Progesterone

Cat # 9025-16

**Enzyme Immunoassay for the Quantitative Determination of
Progesterone in Human Serum or plasma**

INTRODUCTION OF CHEMILUMINESCENCE IMMUNOASSAY

Chemiluminescence Immunoassay (CLIA) detection using Microplate luminometers provides a sensitive, high throughput, and economical alternative to conventional colorimetric methodologies, such as Enzyme-linked immunosorbent assays (ELISA).

ELISA employs a label enzyme and a colorimetric substrate to produce an amplified signal for antigen, haptens or antibody quantitation. This technique has been well established and considered as the technology of choice for a wide variety of applications in diagnostics, research, food testing, process quality assurance and quality control, and environmental testing. The most commonly used ELISA is based on colorimetric reactions of chromogenic substrates, (such as TMB) and label enzymes.

Recently, a chemiluminescent immunoassay has been shown to be more sensitive than the conventional colorimetric method(s), and does not require long incubations or the addition of stopping reagents, as is the case in some colorimetric assays. Among various enzyme assays that employ light-emitting reactions, one of the most successful assays is the enhanced chemiluminescent immunoassay involving a horseradish peroxidase (HRP) labeled antibody or antigen and a mixture of chemiluminescent substrate, hydrogen peroxide, and enhancers.

The CLIA Kits are designed to detect glow-based chemiluminescent reactions. The kits provide a broader dynamic assay range, superior low-end sensitivity, and a faster protocol than the conventional colorimetric methods. The series of the kits covers Thyroid panels, such as T3, T4, TSH, Hormone panels, such as hCG, LH, FSH, and other panels. They can be used to replace conventional colorimetric ELISA that have

been widely used in many research and diagnostic applications. Furthermore, with the methodological advantages, Chemiluminescent immunoassay will play an important part in the Diagnostic and Research areas that ELISAs can not do.

The CLIA Kits have been validated on the **MPL1** and **MPL2** microplate luminometers from Berthold Detection System, **Lus2** microplate luminometer from Anthos, **Centro LB960** microplate luminometer from Berthold Technologies, and **Platelumino** from Stratec Biomedical Systems AG. We got acceptable results with all of those luminometers.

INTRODUCTION OF PROGESTERONE IMMUNOASSAY

Progesterone is a C21 steroid which is synthesized from both tissue and circulating cholesterol. Cholesterol is transformed to pregnenolone which is then converted via a combined dehydrogenase and isomerase to progesterone. The principle production sites are the adrenals and ovaries and the placenta during pregnancy. The majority of this steroid is metabolized in the liver to pregnanediol and conjugated as a glucuronide prior to excretion by the kidneys.

Progesterone exhibits a wide variety of end organ effects. The primary role of progesterone is exhibited by the reproductive organs.

In males, progesterone is a necessary intermediate for the production of corticosteroids and androgens. In females, progesterone remains relatively constant throughout the follicular phase of the menstrual cycle. The concentration then increases rapidly following ovulation and remains elevated for 4-6 days and decreases to the initial level 24 hours before the onset of menstruation. In pregnancy, placental progesterone production rises steadily to levels of 10 to 20 times those of the luteal phase peak.

Progesterone measurements are thus performed to determine ovulation as well as to characterize luteal phase defects. Monitoring of progesterone therapy and early stage pregnancy evaluations comprise the remaining uses of progesterone assays.

The Progesterone EIA kits are designed for the measurement of total progesterone in human serum or plasma.

PRINCIPLE OF THE TEST

The progesterone EIA is based on the principle of competitive binding between progesterone in the test specimen and progesterone-HRP conjugate for a constant amount of rabbit antiprogesterone. In the incubation, goat anti-rabbit IgG-coated wells are incubated with 25 µl progesterone standards, controls, patient samples, 100 µl progesterone-HRP Conjugate Reagent and 50 µl rabbit anti-progesterone reagent at room temperature (18-25°C) for 90 minutes. During the incubation, a fixed amount of HRP-labeled progesterone competes with the endogenous progesterone in the standard, sample, or quality control serum for a fixed number of binding sites of the specific progesterone antibody. Thus, the amount of progesterone peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of progesterone in the specimen increases. Unbound progesterone peroxidase conjugate is then removed and the wells washed. Next, a solution of chemiluminescent substrate is then added and read relative light units (RLU) with a Luminometer. The intensity of the emitting light is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled progesterone in the sample. By reference to a series of progesterone standards assayed in the same way, the concentration of progesterone in the unknown sample is quantified.

MATERIALS AND COMPONENTS

Materials Provided with Test Kit

1. Goat Anti-Rabbit IgG-coated microtiter wells, 96 wells.
2. Progesterone Reference Standards: 0, 0.5, 3.0, 10, 25, and 50ng/ml. Liquids, 0.5 ml each, ready to use.
3. Rabbit Anti-Progesterone Reagent, 7.0 ml.
4. Progesterone-HRP Conjugate Concentrate (11x), 1.3 ml.

5. Progesterone-HRP Conjugate Diluent, 13.0 ml
6. 50 x Wash Buffer Concentrate, 15 ml
7. Chemiluminescence Reagent A, 6.0 ml
8. Chemiluminescence Reagent B, 6.0 ml

Materials Required but not Provided

1. Distilled water.
2. Precision pipettes: 5~40 μ l, 40~200 μ l, 1.0ml
3. Disposable pipette tips.
4. Glass tube or flasks to mix Chemiluminescence Reagent A and B.
5. Microtiter well luminometer.
6. Vortex mixer or equivalent.
7. Absorbent paper.
8. Graph paper.

REAGENT PREPARATION

1. To prepare substrate solution, make an 1:1 mixing of Reagent A with Reagent B right before use. Mix gently to ensure complete mixing. Discard excess after use.
2. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 ml of Wash Buffer (50x) into distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.
3. To prepare Working Progesterone-HRP Conjugate Reagent, add 0.1 ml of Progesterone-HRP Conjugate Concentrate (11x) to 1.0 ml of Progesterone-HRP Conjugate Diluent (1:10 dilution) and mix well. The amount of conjugate diluted depends on your assay size. Discard the excess after use.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 25 μ l of standards, specimens and controls into appropriate wells.
3. Dispense 50 μ l of rabbit anti-progesterone reagent to each well.
4. Gently mix for 5 seconds
5. Dispense 100 μ l of **Working Progesterone-HRP Conjugate Reagent** into each well.
6. ***Thoroughly mix for 30 seconds. It is very important to mix them completely.***
7. Incubate at room temperature (18-25°C) for 90 minutes.
8. Rinse and flick the microwells 5 times with washing buffer(1X).
9. Dispense 100 μ l Chemiluminescence substrate solution into each well. Gently mix for 5 seconds.
10. Read wells with a chemiluminescence microwell reader 5 minutes later. (between 5 and 20 min. after dispensing the substrates).

Important Note:

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. If there are bobbles existing in the wells, the false readings will be created. Please use distilled water to remove the bobbles before adding the substrate.

CALCULATION OF RESULTS

Calculate the average read relative light units (RLU) for each set of reference standards, control, and samples.

We recommend to use a proper software to calculate the results. The best curve fitting used in the assays are 4-parameter regression or cubic spline regression. If the software is not available, construct a standard curve by plotting the mean RLU obtained for each reference standard against

PROGESTERONE concentration in Ng/ml on linear graph paper, with RLU on the vertical (y) axis and concentration on the horizontal (x) axis.

Using the mean absorbance value for each sample, determine the corresponding concentration of PROGESTERONE in ng/ml from the standard curve.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run are shown below. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. It is required that running assay together with a standard curve each time. The calculation of the sample values must be based on the particular curve, which is running at the same time.

PROGESTERONE (ng/ml)	Relative Light Units (RLU) (10 ³)
0	97.4
0.5	59.7
3	40.8
10	22.7
25	11.3
50	4.4

EXPECTED VALUES AND SENSITIVITY

Each laboratory should establish its own normal range based on the patient population. The Progesterone EIA was performed on randomly selected outpatient clinical laboratory samples. The following information is cited from reference #9.

Males: adult	0.13 – 0.97 ng/ml
Prepubertal (children)	0.70 – 0.52 ng/ml
Females: follicular phase	0.15 – 0.70 ng/ml
luteal phase	2.00 – 25.0 ng/ml
post menopause	0.06 – 1.60 ng/ml
Pregnancy:	
1st trimester	10.3 – 44.0 ng/ml
2nd trimester	19.5 – 82.5 ng/ml
3rd trimester	65.0 – 229 ng/ml

The minimum detectable concentration of the Progesterone ELISA assay as measured by 2 SD from the mean of a zero standard is estimated to be 0.2 ng/ml.

CLINICAL APPLICATION

1. Documentation of Ovulation:

Monitor the progesterone concentration during the menstrual cycle is useful in the documentation of ovulation. Progesterone concentration > 3.0 ng/ml will be a strong presumptive evidence of ovulation.

2. Normal vs. Abnormal Progesterone Levels:

Greater-than-normal levels may indicate pregnancy. High level can also indicate adrenal cancer or ovarian cancer, a molar pregnancy, or overproduction of hormones by the adrenal glands. However, levels of progesterone are higher during a multiple pregnancy than during a single pregnancy. Lower-than-normal levels may indicate amenorrhea. Abnormally low levels of progesterone can also indicate problems with ovulation. In a pregnant woman, progesterone levels fall to <5 ng/mL may indicate a threatened miscarriage.

3. Ectopic Pregnancy:

Progesterone can also be useful in ectopic pregnancy diagnosis. For values < 25 ng/ml during pregnancy, fetus viability need to be established by ultrasound. However, progesterone < 5 ng/ml in the first trimester indicates a nonviable pregnancy regardless of location of the fetus.

REFERENCES

1. Radwanska, E., Frankenberg, J., and Allen, E., Plasma progesterone levels in normal and abnormal early human pregnancy. *Fertility and Sterility*, 1978; 30, 398-402.
2. Autrere, M.B., and Benson, H., Progesterone: An overview and recent advances, *J. Par. Sci.*, 1976; 65: 783-800.
3. March, C.M., Goebelsmann, U., Nakamura, R.M., and Mishell, D.R. Jr., Roles of estradiol and progesterone in eliciting the midcycle luteinizing hormone and follicle-stimulating hormone surges, *J. Clin. Endo. Metab.*, 1979; 49, 507-513.
4. Ross, G.T., Vande Wiele, R.L., and Frantz, A.G., The Ovaries and the breasts. In: Williams, R.H., ed., *Textbook of Endocrinology*. Saunders Company, Philadelphia; 1981: 355-411.
5. Chatteraj, S.C., Endocrine function. In: Tietz, N.W., ed., *Fundamentals of Clinical Chemistry*. Saunders Company, Philadelphia; 1976: 699-823.
6. Shepard, M.K., and Senturia, Y.D., Comparison of serum progesterone and endometrial biopsy for confirmation of ovulation and evaluation of luteal function. *Fertility and Sterility*, 1977; 28: 541-548.
7. Johansson, E.D.B., and Jonasson, L.-E., Progesterone levels in amniotic fluid and plasma from women: I. Levels during normal pregnancy. *Acta Obstet. Gynec. Scand.*, 1971; 50: 339-343.
8. USA Center for Disease Control/National Institute of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.
9. Tietz, N.W. ed., *Clinical Guide to Laboratory Tests*, 3rd Edition, W.B. Saunders, Co., Philadelphia, 1995: 509-512.

Date Adopted:	Reference No.
2008-05-16	DA-Progesterone-2009



DIAGNOSTIC AUTOMATION, INC.

23961 Craftsman Road, Suite D/E/F, Calabasas, CA 91302

Tel: (818) 591-3030 Fax: (818) 591-8383

ISO 13485-2003



Revision Date: 02/17/09