



DIAGNOSTIC AUTOMATION, INC.

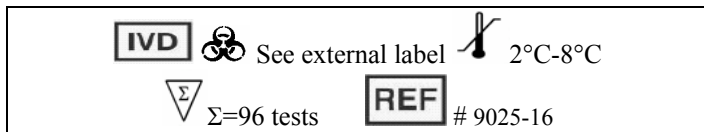
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CHEMILUMINESCENCE ENZYME IMMUNOASSAY (CLIA) PROGESTERONE

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 *MPL2* microplate luminometer 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.

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, 0.50 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. 20x Wash Buffer, 30 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: 0.025ml, 0.05ml, 0.10ml
3. Disposable pipette tips.
4. Glass tube or flasks to mix 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. Prepare the washing solution by diluting 1 part of the 20X PBS concentrate to 19 parts of distilled water.
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 µl of standards, specimens and controls into appropriate wells.
3. Dispense 100 µl of **Working Progesterone-HRP Conjugate Reagent** into each well.
4. Dispense 50 µl of rabbit anti-progesterone reagent to each well.
5. **Thoroughly mix for 30 seconds. It is very important to mix them completely.**
6. Incubate at room temperature (18-25°C) for 90 minutes.
7. Rinse and flick the microwells 5 times with washing solution.
8. Dispense 100 µl Chemiluminescence substrate solution into each well. Gently mix for 5 seconds.

- Read wells with a chemiluminescence microwell reader 5 minutes later. (between 5 and 20 min. after dispensing the substrates).

Important Note:

- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
- 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.

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