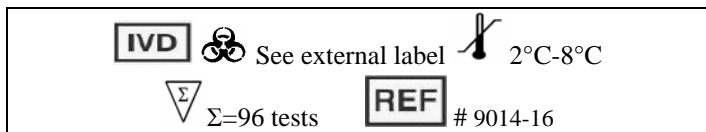




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CHEMILUMINESCENCE ENZYME IMMUNOASSAY (CLIA) PROLACTIN

INTRODUCTION OF CHEMILUMINESCENCE IMMUNOASSAY

Chemiluminescence Immunoassay (CLIA) detection using the 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, PROLACTIN, 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.

Prolactin

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.

INTRODUCTION OF Prolactin IMMUNOASSAY

Human prolactin (lactogenic hormone) is secreted from the anterior

pituitary gland in both men and woman. Human prolactin is a single chain polypeptide hormone with a molecular weight of approximately 23,000 daltons. The release and synthesis of prolactin is under neuroendocrinal control, primarily through Prolactin Releasing Factor and Prolactin Inhibiting Factor. Women normally have slightly higher basal prolactin levels than men; apparently, there is an estrogen-related rise at puberty and a corresponding decrease at menopause. The primary functions of prolactin are to initiate breast development and to maintain lactation. Prolactin also suppresses gonadal function. During pregnancy, prolactin levels increase progressively to between 10 and 20 times normal values, declining to non-pregnant levels by 3-4 weeks post-partum. Breast-feeding mothers maintain high levels of prolactin, and it may take several months for serum concentrations to return to non-pregnant levels. The determination of prolactin concentration is helpful in diagnosing hypothalamic-pituitary disorders. Microadenomas (small pituitary tumors) may cause hyperprolactinemia, which is sometimes associated with male impotence. High prolactin levels are commonly associated with galactorrhea and amenorrhea. Prolactin concentrations have been shown to be increased by estrogens, thyrotropin-releasing hormone (TRH), and several drugs affecting dopaminergic mechanism. Prolactin levels are elevated in renal disease and hypothyroidism, and in some situations of stress, exercise, and hypoglycemia. Additionally, the release of prolactin is episodic and demonstrates diurnal variation. Mildly elevated prolactin concentrations should be evaluated taking these considerations into account. Prolactin concentrations may also be increased by drugs such as chlorpromazine and reserpine, and may be lowered by bromocryptine and L-dopa.

Principle of the Test

The Prolactin Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-prolactin antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-prolactin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in the prolactin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 60 minute incubation at room temperature, the wells are washed with wash buffer to remove unbound labeled antibodies. A solution of chemiluminescent substrate is then added and read relative light units (RLU) in a Luminometers. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of prolactin in the sample. By reference to a series of Prolactin standards assayed in the same way, the concentration of Prolactin in the unknown sample is quantified.

Materials and Components

Materials provided with the test kit:

1. Antibody-coated microtiter wells. 48 wells per bag.
2. Set of Reference Standards: 0, 7.5, 25, 60, 120, and 240 ng/ml, (WHO, 1st IRP, 75/504) Lyophilized.
3. Enzyme Conjugate Reagent, 12 ml.
4. 20x Wash Buffer, 30 ml
5. Chemiluminescence Reagent A, 6.0 ml.
6. Chemiluminescence Reagent B, 6.0 ml.

Materials required but not provided:

- Precision pipettes: 40µl~200µl and 1.0 ml.
- Disposable pipette tips.
- Distilled water.
- Glass tubes or flasks to mix Chemiluminescence Reagent A and Chemiluminescence Reagent B.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Graph paper.

Specimen Collection and Preparation

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

Storage of Test Kit and Instrumentation

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (One year from the date of manufacture). Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.

Reagent Preparation

1. All reagents should be brought to room temperature (18-25°C) before use.
2. Add 1 ml of distilled water to reconstitute the lyophilized standards. Allow the reconstituted materials to stand for at least 20 minutes. Mix gently. The reconstituted standards should be stored sealed at 2-8°C
3. To prepare Chemiluminescence Substrate solution, make a 1:1 mixing of Reagent A with Reagent B right before use. Discard the excess after use.
4. Prepare the washing solution by diluting 1 part of the 20X PBS concentrate to 19 parts of distilled water.

Assay Procedure

1. Secure the desired number of coated wells in the holder.
2. Dispense 50µl of standards, specimens, and controls into appropriate wells.
3. Dispense 100µl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to have complete mixing in this step.
5. Incubate at room temperature (18-25°C) for about 60 minutes.
6. Rinse and flick the microtiter wells 5 times with washing buffer.

7. Strike the wells sharply onto absorbent paper to remove residual water droplets.
8. Dispense 100 µl Chemiluminescence substrate solution into each well. Gently mix for 5 seconds.
9. Read wells with a chemiluminescence microwell reader 15 minutes later. (between 10 and 20 min. after dispensed 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

1. Calculate the average read relative light units (RLU) for each set of reference standards, control, and samples.
2. We recommend to use a proper software to calculate the results. If the software is not available, construct a standard curve by plotting the mean RLU obtained for each reference standard against Prolactin concentration in uIU/ml on linear graph paper, with absorbance 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 of PROLACTIN in uIU/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.

PROLACTIN (ng/ml)	Relative Light Units (RLU) (10 ⁵)
0	0.1
7.5	4.0
25	14.0
60	35.4
120	56.2
240	72.5

Expected values and sensitivity

Each laboratory must establish its own normal ranges based on patient population. Based on a limited number of healthy adult blood specimens, the mean prolactin concentrations in males (N=90) and females (N=120) are estimated to be 6 and 15 ng/ml, respectively. The minimal detectable concentration of human prolactin by this assay is estimated to be 2 ng/ml.

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