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REF

Cat # 9013-16

**CHEMILUMINESCENCE
ENZYME IMMUNOASSAY (CLIA)
FOLLICLE-STIMULATING HORMONE**

FSH

Cat # 9013-16

Chemiluminescence Enzyme Immunoassay for the Quantitative Determination of FOLLICLE-STIMULATING HORMONE (FSH) in Human Serum

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.

INTRODUCTION OF FSH IMMUNOASSAY

Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH) are intimately involved in the control of the growth and reproductive activities of the gonadal tissues, which synthesize and secrete male and female sex hormones. The levels of circulating FSH and LH are controlled by these sex hormones through a negative feedback relationship. FSH is a glycoprotein secreted by the basophilic cells of the anterior pituitary. Gonadotropin-release hormone (GnRH), produced in the hypothalamus, controls the release of FSH from anterior pituitary. Like other glycoproteins, such as LH, TSH, and HCG, FSH consists of subunits designated as alpha and beta. Hormones of this type have alpha subunits that are very similar structurally, therefore the biological and immunological properties of each are dependent on the unique beta subunit.

In the female, FSH stimulates the growth and maturation of ovarian follicles by acting directly on the receptors located on the granulosa cells; follicular steroidogenesis is promoted and LH production is stimulated. The LH produced then binds to the theca cells and stimulates steroidogenesis. Increased intraovarian estradiol production occurs as follicular maturation advances, thereupon stimulating increased FSH receptor activity and FSH follicular binding. FSH, LH, and estradiol are therefore intimately related in supporting ovarian recruitment and maturation in women.

FSH levels are elevated after menopause, castration, and in premature ovarian failure. The levels of FSH may be normalized through the administration of estrogen, which demonstrate a negative feedback mechanism. Abnormal relationships between FSH and LH, and between FSH and estrogen have been linked to anorexia nervosa and polycystic ovarian disease. Although there are significant exceptions, ovarian failure is indicated when random FSH concentrations exceed 40 mIU/ml.

The growth of the seminiferous tubules and maintenance of spermatogenesis in men are regulated by FSH. However, androgens, unlike estrogen, do not lower FSH levels, therefore demonstrating a feedback relationship only with serum LH. For reasons not only fully understood, azospermic and oligospermic males usually have elevated FSH levels. Tumors of the testes generally depress serum FSH concentrations. High levels of FSH in men may be found in primary testicular failure and Klinefelter syndrome. Elevated concentrations are also present in cases of starvation, renal failure, hyperthyroidism, and cirrhosis.

Principle of the test

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

Materials Provided with Test Kit

1. Antibody-coated microtiter wells, 96 wells per bag.
2. Reference standard set, contains 0, 3.0, 10.0, 30.0, 60 and 120 mIU/ml (WHO, 2nd IRP, 78/549) human FSH, liquid, ready to use.
3. Enzyme conjugate reagent, 12.0 ml.
4. Wash Buffer Concentrate (50X), 15 ml
5. Chemiluminescence Reagent A, 6.0 ml
6. Chemiluminescence Reagent B, 6.0 ml

Materials Required but not Provided

1. Distilled water.
2. Precision pipettes: 0.04~0.2ml, 1.0ml
3. Disposable pipette tips.
4. Glass tube or flasks to mix Reagent A and B.
5. Microtiter well reader.
6. Vortex mixer or equivalent.
7. Absorbent paper.
8. Graph paper.

Reagent Preparation

1. All reagents should be allowed to reach room temperature (18-22°C) before use.
2. 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.
3. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 ml of Wash Buffer (50x) into 735 ml of distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.

Assay Procedure

1. Secure the desired number of coated wells in the holder. Make data sheet with sample identification.
2. Dispense 50µl of standard, 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 setup.
5. Incubate at room temperature (18-22°C) for 60 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the microtiter wells 5 times with washing buffer.
8. Strike the wells sharply onto absorbent paper to remove residual water droplets.
9. Dispense 100 µl 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:

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

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. 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 FSH concentration in ng/ml on linear graph paper, with RLU 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 FSH in mIU/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.

FSH (mIU/ml)	Relative Light Units (RLU) (10⁴)
0.0	0.008
3.0	1.620
10	6.680
30	23.82
60	44.47
120	76.06

Expected values and sensitivity:

It is important for each laboratory to establish the normal range limits. The following normal range should be considered as a guideline only:

Female Follicular	0~20 mIU/ml
Mid-cycle	15~30 mIU/ml
Luteal	0~20 mIU/ml
Post Menopausal	40~200 mIU/ml
Male	0~20 mIU/ml

The minimum detectable concentration of FSH by this assay is estimated to be 2.5 mIU/ml.

References

1. Marshall J.C. Clinic in Endocrinol. **Metab** 1975; 4:545
2. Cohen K.L. **Mtabolism** 1977; 26:1165
3. Rebar R.W. , Erickson G.F. and Yen S.S.C. **Fertil. Steril.**1982; 37: 35
4. Abraham G.E.. Ed. Radioassay Systems in Clinic. **Endocrinol.** Marcel Dekker, Inc, New York (1981)
5. Uotila M., Ruoslahti E and Engvall E.**J.Immunol. Methods** 1981; 42: 11

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