IVD

FOLLICLE-STIMULATING HORMONE (FSH) CHEMILUMINESCENCE IMMUNOASSAY KIT

Catalog No. CL1102-2

INTENDED USE

The Autobio Follicle-stimulating hormone (FSH) chemiluminescence immunoassay (CLIA) kit is intended for the quantitative determination of FSH concentration in human serum.

INTRODUCTION

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 grannulosa 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, there upon 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 40mIU/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

The FSH CLIA test is a solid phase two-site immunoassay. One monoclonal antibody is coated on the surface of the microtiter wells and another monoclonal antibody labeled with horseradish peroxidase is used as the tracer. The FSH molecules present in the standard solution or serum are "sandwiched" between the two antibodies. Following the formation of the coated antibody-antigen-antibody-enzyme complex, the unbound antibody-enzyme labels are removed by washing. The horseradish peroxidase activity bound in the wells is then assayed by chemiluminescence reactions. The related light unit (RLU) of the reaction is proportional to the concentration of FSH present in the sample.

MATERIALS PROVIDED

1. Antibody Coated Microtiter Plate: Microplate coated with monoclonal antibodies to Follicle-Stimulating Hormone (anti-FSH MAb) (1 plate, 48 wells/96wells)
2. Enzyme Conjugate Reagent: Horseradish peroxidase (HRP) labeled anti-FSH MAb in Stabilizing Buffer (1 vial, 6.0ml/11.0ml)
3. Reference Standards: 1, 2.5, 10, 40 and 160mIU/ml FSH in Stabilizing Buffer (5 vials, 0.5ml/ea)
4. Substrate A (1 vial, 3.5ml/6.0ml)
5. Substrate B (1 vial, 3.5ml/6.0ml)
6. PBS-T powder: PBS-Tween (1bag, 5g)

MATERIALS NOT PROVIDED

The following materials are required but not provided in the kit.

1. Precision pipettes and tips, 0.025ml, 0.1ml, 1.0ml,
2. Distilled water
3. Vortex mixer
4. Absorbent paper or paper towel
5. Graph paper
6. Luminometer

STORAGE OF TEST KIT AND INSTRUMENTATION

1. Unopened test kits should be stored at 2～8℃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. Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiration date shown, provided it is stored as prescribed above.

SPECIMEN COLLECTION AND PREPARATION

1. Blood should be drawn using standard venipuncture techniques and the serum should be separated from the red blood cells as soon as practical. Avoid grossly hemolytic, lipemic or turbid samples.
2. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with test procedures and should be avoided.
3. Specimens should be capped and may be stored up to 48 hours at 2～8℃, prior to assaying. Specimens held for a longer time can be frozen at -20℃. Thawed samples must be mixed prior to testing.

PRECAUTIONS AND WARNINGS

1. For *in vitro* diagnostic use only.
2. Handling of reagents, serum specimens should be in accordance with local safety procedures.
3. The reference satandards contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody. All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, the reference satandards and components containing animal substances should be treated as potentially infectious.
4. Avoid any skin contact with all reagents.
5. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18～25℃) before use. All reagents should be mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. Prepare Wash Solution: add 1 bag of PBS-T powder to 500ml of distilled water, and mix well with magnetic stirrer. The Wash Solution is stable at room temperature for 2 months.

IMPORTANT NOTES

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated RLU values.
2. It is recommended that no more than 32 wells be used for each assay run, if manual pipetting is used, since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of all standards and specimens, although not required, is recommended.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense 25μl of FSH standards, specimens, and controls into appropriate wells.
2. Dispense 100μl of Enzyme Conjugate Reagent to each well. Mix gently for 30 seconds.
3. Incubate for 60 minutesat 37℃.
4. At the end of the incubation, remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with washing buffer. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets. The volume of the

well is about 300μl.
5. Dispense 50μl substrate A and 50μl substrate B reagent into each well. Gently mix for 10 seconds.
6. Incubate at room temperature in the dark for 5 minutes without shaking and read the RLU values with a Luminometer.

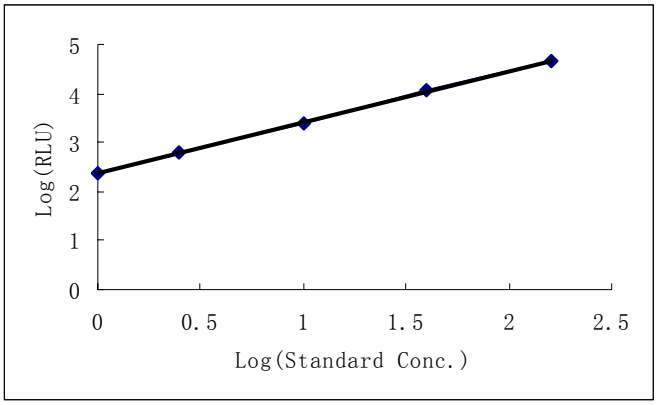
CALCULATION OF RESULTS

1. Calculate the mean value from any duplicate reagents. Where appropriate, the mean values should be used for plotting.
2. Plot the $\log_{10}$RLU for each reference standard against the common logarithm of corresponding concentration of FSH in mIU/ml on logarithmic graph paper, with RLU values on the Y-axis and and concentration on the X- axis.
3. Read the concentration for each control and sample by interpolating on the calibration curve.
4. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a linear regression logistic function curve fitting is recommended.

EXAMPLE OF STANDARD CURVE

A typical standard curve shown below is for the purpose of illustration only, and should never be used instead of the real time calibration curve.

FSH (mIU/ml)	RLU
1.0	247.40
2.5	600.24
10	2389.02
40	11571.70
160	47161.50



EXPECTED VALUES

Each laboratory must establish its own normal range based on patient population. These values are given only for guidance.

		Normal Range (mIU/ml)
Male		1.0-12.1
Female	Follicular Phase	2.5-11.4
	Ovulation Phase	3.3-21.7
	Luteal Phase	1.2-7.0
	Memopause	18.8-132.0

PERFORMANCE

A. Sensitivity

The lower detection limit is calculated from the standard curve by identifying the concentration corresponding to the mean RLU of standard diluent (based on 10 replicate analyses) plus 2 SD. Therefore, the sensitivity of the Autobio FSH CLIA kit is estimated to be not higher than 0.5mIU/ml.

B. Specificity

No interference was detected with the performance of Autobio FSH CLIA upon addition of massive amounts of the following substances to a human serum pool.

Interferents	Concentration
FSH	500mIU/ml
TSH	520 μ IU/ml
hCG	22800mIU/ml

C. Precision

a. Intra-assay Precision

Intra-assay Precision was determined by assaying 20 replicates of each control sera.

Serum	Number	Mean	SD	CV (%)
Low titer	20	3.18	0.23	6.80
High titer	20	15.02	1.05	4.60

b. Inter-assay Precision

Inter-assay Precision was determined by assaying duplicates of each control sera in 10 separate runs.

Serum	Number	Mean	SD	CV (%)
Low titer	10	3.6	0.3	6.90
High titer	10	17.1	1.1	4.70

D. High Dose Hook Effect

In this FSH assay, patient samples spiked to FSH levels up to 20,000mIU/m do not demonstrate aparadoxical decrease in the RLUs (high dose hook effect).

E. Accuracy

For 180 samples in the range of 1mIU/ml to 160mIU/ml, the relationship between the Autobio FSH CLIA kit and Beckman access 2® assay is described by the equation below:

Reference	Number of Specimens	Least Square Regression Analysis	Correlation Coefficient
Beckman access 2®	180	y = 0.9029x + 0.8192	0.9502

LIMITATIONS

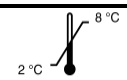
1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert and with adherence to good laboratory practice.
2. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference thus anomalous values may be observed. Additional information may be required for diagnosis.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

QUALITY CONTROL

Good laboratory practice requires that quality control specimens be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges. Controls containing sodium azide should not be used.

SYMBOLS

LOT	**BATCH CODE**
	USE BY
	MANUFACTURER
Σ	**CONTAINS SUFFICIENT FOR <n> TESTS**
IVD	***IN VITRO* DIAGNOSTIC MEDICAL DEVICE**

	**TEMPERATURE LIMITATION**
	**CATALOGUE NUMBER**
	**CONSULT INSTRUCTIONS FOR USE**

REFERENCES

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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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