FSH EIA 96 TEST
IMMUNOASSAY TEST KIT Catalog Number: 6107325

CE

Enzyme Immunoassay for the Quantitative Determination of Follicle-Stimulation Hormone (FSH) Concentration in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2 to 8°C.

INTENDED USE

For the quantitative determination of follicle-stimulation hormone (FSH) concentration in human serum.

INTRODUCTION

Follicle-Stimulation 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 the 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 are dependent on the unique beta subunits.

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 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 Quantitative Test is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes a mouse monoclonal anti-α-FSH antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-β-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 45 minutes incubation at room temperature, the wells are washed with water to remove unbound-labeled antibodies. A solution of TMB Reagent is added and incubated at room temperature for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of FSH is directly proportional to the color intensity of the test sample.

REAGENTS

Materials provided with the kit:

- Mouse monoclonal anti-α-FSH antibody coated microtiter plate with 96 wells.
- Enzyme Conjugate Reagent, 13 ml.
- FSH reference standards, containing 0, 5, 15, 50, 100, and 200 mIU/ml (WHO, 2nd IRP 78/549) human FSH, lyophilized, 1 set.
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 11 ml.

Materials required but not provided:

- Precision pipettes, 50 µl, 100 µl and 1.0 ml.
- Distilled water.
- Disposable pipette tips.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- A microtiter plate reader at 450 nm wavelength, with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater.
- 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 AND INSTRUMENTATION

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. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

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REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) before use.
2. Reconstitute each lyophilized standard with 1.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. Reconstituted standards will be stable for up to 30 days when stored sealed at 2-8°C.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 50 µl of standard, specimens, and controls into appropriate wells.
3. Dispense 100 µl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to have a complete mixing in this setup.
5. Incubate at room temperature (18-25°C) for 45 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the microtiter wells 5 times with ***distilled or deionized water***. *(Please do not use tap water.)*
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100 µl TMB Reagent into each well. Gently mix for 10 seconds.
10. Incubate at room temperature in the dark for 20 minutes.
11. Stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix for 30 seconds. *It is important to make sure that all the blue color changes to yellow color completely.*
13. Read the optical density at 450 nm with a microtiter plate reader ***within 15 minutes***.

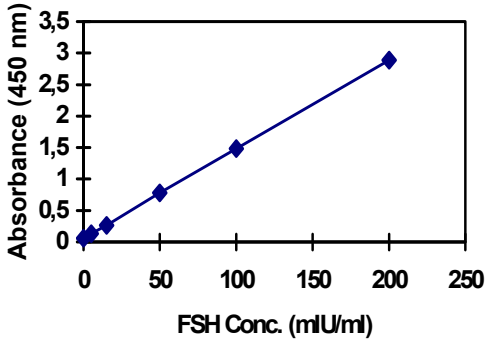
CALCULATION OF RESULTS

1. Calculate the mean absorbance value (A_{450}) for each set of reference standards, specimens, controls and patient samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in mIU/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or 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 with optical density readings at 450 nm shown in the Y-axis against FSH concentrations shown in the X-axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve in each experiment.

FSH Standards (mIU/ml)	Absorbance (450 nm)
0	0.058
5	0.133
15	0.265
50	0.782
100	1.483
200	2.885



EXPECTED VALUES AND SENSITIVITY

Based on random selected outpatient clinical laboratory samples, the mean FSH values in males (N=100) and females (N=150) are 11 and 12 mIU/ml, respectively. The mean FSH values in post-menopausal (N=60) and pregnant females (N=60) are 94 and 1.0 mIU/ml, respectively. The minimum detectable concentration of FSH by this assay is estimated to be 2.5 mIU/ml.

LIMITATIONS OF THE PROCEDURE

1. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
2. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

REFERENCES

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QUALITY SYSTEM CERTIFIED
ISO 9001 ISO 13485

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