FOLLICLE-STIMULATING HORMONE (FSH)

ENZYME IMMUNOASSAY TEST KIT
Catalog Number: 10001

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

Intended use

For the quantitative determination of follicle-stimulating hormone (FSH) concentrations 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, 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

The 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 to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 2N HCl, 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.

Materials and components

Materials provided with the test kits:

- Antibody-coated microtiter wells, 96 wells per bag.
- Reference standard set, contains 0, 5, 20, 50, 100 and 200 MIU/mL (WHO, 2nd IRP, HMG) human FSH, in liquid form (ready to use) or lyophilized form.
- Enzyme conjugate reagent, 12 mL.
- TMB Substrate, 12 mL.
- Stop Solution, 12 mL.
- Wash Buffer Concentrate(50X),15mL

Materials required but not provided:

- Precision pipettes: 0.04~0.2mL and 1.0 mL.
- Disposable pipette tips.
- Distilled water.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Graph paper.
- Microtiter well reader.

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 kits 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 expiring date shown, provided it is stored as prescribed above. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

Reagent preparation

1. All reagent should be brought to room temperature(18-22°C) before use.
2. If reference standards are lyophilized, reconstitute each standard with 0.5 mL distilled water. Allow the reconstituted Material to stand for at least 20 minutes. Reconstituted standards should be sealed and stored at 2-8°C.
3. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 mL of Wash Buffer (50x) into distilled water to prepare 750 mL of washing buffer (1x). Mix well before use.

Assay procedures

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**µLof 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 (18-22°C) for 60 minutes.
6. Remove the incubation mixture by flicking plate content into a waste container.
7. Rinse and flick the microtiter wells 5 times with washing buffer(1X).
8. Strike the wells sharply onto absorbent paper or paper Towels to remove all residual water droplets.
9. Dispense**100**µL of TMB solution into each well.Gently mix for 5 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 optical density at 450nm with a microtiter reader Within 15 minutes.

Important Note

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

Calculation of results

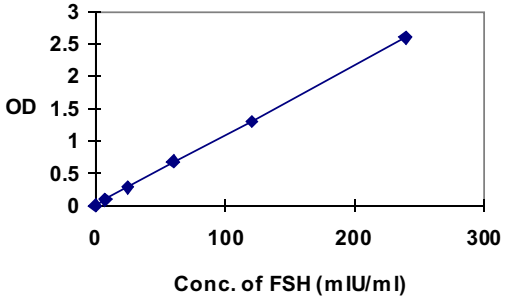
Calculate the mean absorbance value (A_{450}) for each set of reference standards, specimens, controls and patient samples. Constructed a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in mIU/mL on graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of FSH in mIU/ mL from the standard curve.

Example of standard curve

Results of typical standard run with optical density reading at 450nm shown in the Y- axis against FSH concentrations shown in the X-axis.

FSH (mIU/mL)	Absorbance (450nm)
0	0.007
5.0	0.095
20.0	0.286
50.0	0.669
100.0	1.307
200.0	2.584

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.



Expected values and sensitivity

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.

Performance characteristics

1. Accuracy: Comparison between Our Kits and commercial available Kits provide the following data
N = 124
Correlation Coefficient = 0.980
Slope = 1.01
Intercept = 0.34
Mean (Our) = 28.8
Mean (DPC) = 26.0

2.Precision
1].Intra-Assay:

Concentrations	Replicates	Mean	S.D.	% CV
Leve l	20	9.17	0.54	5.93
Level l	20	21.04	1.24	5.90
LevelIII	20	50.62	2.16	4.27

2].Inter-Assay:

Concentrations	Replicates	Mean	S.D.	% CV
Level	20	8.71	0.65	7.50
Level l	20	20.09	1.82	9.04
LevelIII	20	55.42	4.29	7.75

3.Linearity
Two patient sera were serially diluted with 0 mIU/mL standard In a linearity study. The average recovery was 101.0 %.

Sample A			
Dilution	Expected	Observed	% Recov.
Undiluted	112.54	112.54	
2x	56.27	56.29	100.0
4x	28.14	28.58	101.6
8x	14.07	15.08	107.2
16x	7.03	7.45	105.9
Average Recovery: 103.7 %			

Sample B			
Dilution	Expected	Observed	% Recov.
Undiluted	98.78	98.78	
2x	49.39	49.41	100.0
4x	24.69	23.31	94.4
8x	12.35	12.03	97.4
16x	6.17	6.23	101.0
Average Recovery: 98.2 %			

4.Recovery
Various patient serum samples of known FSH levels were mixed and assayed in duplicate. The average recovery was 98.7 %.

Expected Concentration	Observed Concentration	% Recovery
5.03	5.09	101.1
17.41	17.47	100.3
29.76	28.55	95.9
34.15	33.05	96.8
39.78	41.82	105.1
52.83	49.25	93.2
Average Recovery: 98.7 %		

5.Sensitivity
The minimum detectable concentration of this assay is Estimated to be 2.0 mIU/mL.

6.Cross-reactivity
The following human materials were tested for Crossreactivity of the assay:

Antigens	Concentration	Equivalent FSH	%Cross-Reactivity
HCG	500,000 mIU/mL	0.0 mIU/mL	0.0
LH	500 mIU/mL	0.0 mIU/mL	0.0
TSH	500 IU/mL	0.0 mIU/mL	0.0
Prolactin	500 ng/mL	0.5 mIU/mL	0.1

7.Hook Effect
No hook effect was observed up to 3,000 mIU/mL FSH in this assay.

Limitations of the Procedure

There are some limitation of the assay. We should let our customers know about that.

1)As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

2)Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot guarantee to eliminate all the effects of that.

3)The wash procedure (steps 6-8) is critical. Insufficient washing will result in poor precision and falsely elevated absorbance. The use of tap water for washing could result in a higher background absorbance.

References

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