POINTE SCIENTIFIC
A MedTest Company

Follicle-Stimulation Hormone (FSH) Enzyme Immunoassay Test Kit

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

For In Vitro Diagnostic Use

Store at 2 to 8°C.

Intended Use

For the quantitative determination of FSH concentration in human serum. The assay is useful in the diagnosis and treatment of pituitary and gonadal disorders.

Explanation of the Test

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.[1,2]

FSH is a glycoprotein secreted by the basophil cells of the anterior pituitary. Gonadotropin-releasing 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 in structure; therefore the biological and immunological properties of each hormone are dependent on the unique beta subunit.[3,4,5]

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.[6,7,9]

FSH levels are elevated after menopause, castration, and in premature ovarian failure. The levels of FSH may be normalized through the administration of estrogens, 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.[8]

The growth of the seminiferous tubules and maintenance of spermatogenesis in men are regulated by FSH. However, androgens, unlike estrogens, do not lower FSH levels, therefore demonstrating a feedback relationship only with serum LH.[10,11,12]

For reasons not fully understood, azospermic and oligospermic males usually have elevated FSH levels. Tumors of the testes generally depress serum FSH concentrations, but levels of LH are elevated, as determined by radioimmmunoassay. It has been postulated that the apparent LH increase may be caused by cross-reactivity with hCG-like substances secreted by tumors of the testes.[11, 12]

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.[1,3]

Principle of the Assay

The FSH EIA Test is based on the principle of a solid phase enzyme-linked immunosorbent assay.[13,14] The assay system utilizes mouse monoclonal anti-alpha FSH for solid phase (microtiter wells) immobilization, and mouse monoclonal anti-beta FSH in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in the FSH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 45 minute incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of Tetramethylbenzidine (TMB) is added and incubated for 20 minutes at room temperature, resulting in the development of a blue color. The color development is stopped with the addition of 1N HCl, and the resulting yellow color is measured spectrophotometrically at 450 nm. The concentration of Follicle-Stimulating Hormone is directly proportional to the color intensity of the test sample.

Reagents and Materials Provided

1. *Antibody-Coated Wells*
   Microtiter wells coated with mouse monoclonal anti-α-FSH
2. *Enzyme Conjugate Reagent*
   Contains mouse monoclonal anti-β-FSH conjugated to horseradish peroxidase
3. *Reference Standard Set*
   Contains 0, 5, 15, 50, 100, and 200 mIU/mL (WHO, 2nd IRP, 78/549) human FSH in bovine serum with preservatives. Lyophilized. See instructions for reconstitution under Reagent Preparation.
4. *TMB Reagent (One-Step)*
   Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution.
5. *Stop Solution (1N HCl)*
   Contains diluted hydrochloric acid

Reagents and Materials

Materials Required But Not Provided

1. Distilled or deionized waters
2. Precision pipettes: 0.05, 0.1, 0.2, and 1 ml
3. Disposable pipette tips
4. Microtiter well reader capable of reading absorbance at 450nm
5. Vortex mixer or equivalent
6. Absorbent paper
7. Linear graph paper
8. QC material (e.g., BioRad LyphoCheck Controls)

Follicle-Stimulation Hormone (FSH) Enzyme Immunoassay Test Kit

WARNINGS AND PRECAUTIONS

1. **CAUTION:** This kit contains human material. The source material used for manufacture of this kit tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling and disposal should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.[21]
2. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
3. Do not use the reagent when it becomes cloudy or contamination is suspected.
4. Do not use the reagent if the vial is damaged.
5. Replace caps on reagents immediately. Do not switch caps.
6. Each well can be used only once.
7. Do not pipette reagents by mouth.
8. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
9. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
10. For in vitro diagnostic use.

STORAGE CONDITIONS

1. Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. The opened and used reagents are stable until the expiration date if stored properly at 2-8°C.
3. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

INSTRUMENTATION

A microtiter well reader with a bandwidth of 10nm or less and an optical density range of 0 to 2 OD or greater at 450nm wavelength is acceptable for absorbance measurement.

SPECIMEN COLLECTION AND PREPARATION

1. 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. Avoid grossly hemolytic, lipemic, or turbid samples.
2. Specimens should be capped and may be stored for up to 48 hours at 2-8°C prior to assaying. Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Reconstitute each lyophilized standard with 1.0 mL dH_20. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be stored sealed at 2-8°C, and are stable at 2-8°C for at least 30 days.

PROCEDURAL NOTES

1. Manual Pipetting: It is recommended that no more than 32 wells are used for each assay run. Pipetting of all standards, samples, and controls should be completed within 3 minutes. A multi-channel pipette is recommended.
2. Automated Pipetting: A full plate of 96 wells may be used in each assay run. However, it is recommended that pipetting of all standards, samples, and controls be completed within 3 minutes.
3. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
4. It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

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.
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 dionized 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 100µl of TMB Reagent 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 100µl of Stop Solution to each well.
12. Gently mix for 30 seconds. *Ensure that all of the blue color changes completely to yellow.*
13. Read absorbance at 450nm with a microtiter plate reader ***within 15 minutes***.

CALCULATION OF RESULTS

1. Calculate the average absorbance value (A_{450}) for each set of reference standards, controls and samples.

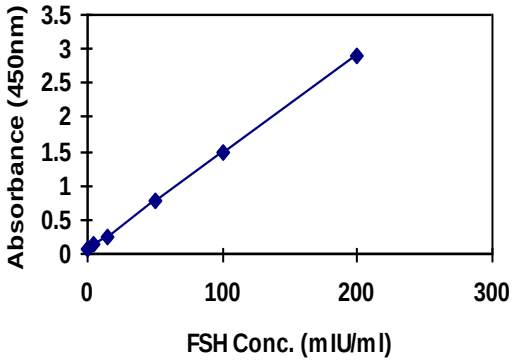
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in mIU/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.

3. Using the mean absorbance value for each sample, determine the corresponding concentration of FSH in mIU/ml from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. Any diluted samples must be further corrected by the appropriate dilution factor.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with absorbency readings at 450nm 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.

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



* The absorbance (450nm) values can be varied due to incubation at different room temperature in different laboratories.

EXPECTED VALUES

Each laboratory should establish its own normal range based on patient population. Differences in assay technique and the use of various standards may affect results. The results provided below are cited from a major commercial kit package insert.

Sample	N	Range (mIU/mL)	Mean (mIU/mL)
Male	144	1.5-11.5	4.1
Normally Menstruating Females			
Follicular Phase	111	3.2 - 10.0	5.8
Mid-Cycle Peak	11	7.5 - 20.0	12.3
Luteal Phase	89	1.3 - 11.0	3.8
Post Menopausal Female	24	36.0 - 138.0	70.5

Performance Characteristics

1. Accuracy

A statistical study using 136 patient samples demonstrated good correlation of results with a commercially available kit as shown below. Comparison between this kit and Serono's Serozyme kit provides the following data:

N = 136
Correlation coefficient = 0.99
Slope = 1.05
Intercept = -0.55
Mean = 18.8mIU/ml
Serono Mean = 18.4mIU/ml

2. Sensitivity

The minimal detectable concentration of human FSH by this assay is estimated to be 1.5 mIU/ml.

3. Precision

a. Intra-Assay Precision

Within-run precision was determined by replicate determinations of three different control sera in one assay. Within-assay variability is shown below:

Serum Sample	1	2	3
Number of Replicates	26	26	26
Mean FSH (mIU/ml)	4.9	13.8	30.8
Standard Deviation	0.2	0.5	1.1
Coefficient of Variation (%)	4.8%	3.5%	3.7%

b. Inter-Assay Precision

Between-run precision was determined by replicate measurements of three different control sera over a series of individually calibrated assays. Between-assay variability is shown below:

Serum Sample	1	2	3
Number of Replicates	30	30	30
Mean FSH (mIU/ml)	5.1	12.6	30.0
Standard Deviation	0.3	0.5	1.6
Coefficient of Variation (%)	4.9%	3.6%	5.4%

4. Recovery and Linearity Studies

a. Recovery

Various patient serum samples of known FSH levels were mixed and assayed in duplicate. The average recovery was 98.0%.

	Expected Concentration (mIU/ml)	Observed Concentration (mIU/ml)	% Recovery
1.	1.01	0.85	84.2
	2.20	1.80	81.8
	4.5	4.3	95.6
	9.1	9.2	101.1
	17.8	19.4	109.0
	36.9	36.1	97.8
	58.9	61.4	104.2
2.	0.85	0.81	95.3
	2.17	1.95	89.9
	4.2	4.1	97.6
	8.9	9.7	109.0
	18.3	17.9	97.8
	35.0	34.8	99.4
	56.4	61.7	109.4

PERFORMANCE CHARACTERISTICS

4. Recovery and Linearity Studies

b. Linearity

Two patient samples were serially diluted with zero mIU/ml standards in a linearity study. The average recovery was 103.9%.

#	Dilution	Expected Conc. (mIU/ml)	Observed Conc. (mIU/ml)	% Recovery
1.	Undiluted	58.1	58.1	100.0
	1:2	29.1	32.5	116.3
	1:4	14.5	15.7	108.3
	1:8	7.3	7.5	102.7
	1:16	3.6	3.5	97.2
	1:32	1.8	1.6	88.9
2.	Undiluted	56.8	56.8	100.0
	1:2	28.4	32.4	114.1
	1:4	14.2	14.9	104.9
	1:8	7.1	8.1	114.1
	1:16	3.6	3.8	105.6
	1:32	1.8	1.7	94.4

5. Specificity

The following hormones were tested for cross-reactivity in the assay:

Hormone Tested	Concentration	% Cross Reactivity
HCG	1,000 mIU/ml	0
(WHO 1st IRP 75/539)	5,000 mIU/ml	0
	10,000 mIU/ml	0
	50,000 mIU/ml	0
	100,000 mIU/ml	0
TSH	25 μIU/ml	0
(WHO 2nd IRP 50/558)	50 μIU/ml	0
	100 μIU/ml	0
LH	50 mIU/ml	0
(WHO 1st IRP 68/40)	100 mIU/ml	0
	500 mIU/ml	0
Prolactin	50 ng/ml	0
(WHO 1st IRP 75/504)	100 ng/ml	0
	500 ng/ml	0
Growth Hormone	50 ng/ml	0
(WHO 1st IRP 66/217)	100 ng/ml	0
	200 ng/ml	0

6. Hook Effect

No hook effect is observed in this assay at FSH concentrations up to 1,500 mIU/mL.

QUALITY CONTROL

Good laboratory practice requires that quality control specimens (controls) be run with each calibration curve to verify assay performance. Controls containing sodium azide cannot be used. To ensure proper performance, control material should be assayed repeatedly to establish mean values and acceptable ranges.

STANDARDIZATION

The FSH Reference Standards are calibrated against the World Health Organization's Second International Reference Preparation of Follicle-Stimulating Hormone for Immunoassay, (WHO 2nd IRP-78/549).

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
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.
3. The serum samples used in this assay must not contain any additives; EDTA has been found to interfere with the test procedure.
4. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
5. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

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