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BXE0631A

96 TESTS

STORE AT 2-8°C

FOR IN- VITRO DIAGNOSTICS USE ONLY

FSH

FOLLICLE STIMULATING HORMONE ELISA

Intended Use:

The Quantitative Determination of Follicle Stimulating Hormone Concentration in Human Serum by a Microplate Immunoenzymometric assay (IEMA/ELISA).

Summary And Explanation Of The Test:

Follicle Stimulating hormone (FSH) is a glycoprotein consisting of two subunits with an approximate molecular mass of 35,500 daltons. The a-subunit is similar to other pituitary hormones [luteinizing stimulating hormone (LH), thyroid stimulating hormone (TSH) and chorionic gonadotropin (CG)] while the β-subunit is unique. The β-subunit confers the biological activity to the molecule. Stimulation by gonadotropin-releasing hormone (GnRH) causes release of FSH, as well as LH, from the pituitary and is transported by the blood to their sites of action, the testes or ovary.

In men, FSH acts on the Sertoli cells of the testis, stimulating the synthesis of inhibin, which appears to specifically inhibit further FSH secretion, and androgen-binding protein. Thus, it indirectly supports spermatogenesis.

In women, FSH acts on the granulosa cells of the ovary, stimulating steroidogensis. All ovulatory menstrual cycles have a characteristic pattern of FSH, as well as LH, secretion. The menstrual cycle is divided into a follicular phase and a luteal phase by the midcycle surge of the gonadotropins (LH and FSH). As the follicular phase progresses, FSH concentration decreases. Near the time ovulation occur, about midcycle, FSH peaks (lesser in magnitude than LH) to its highest level.

The clinical usefulness of the measurement of Follicle Stimulating hormone (FSH) in ascertaining the homeostasis of fertility regulation via the hypothalamic - pituitary - gonadal axis has been well established (1,2).

In this method, FSH calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of FSH) are added and the reactants mixed. Reaction between the various FSH antibodies and native FSH forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-Follicle Stimulating hormone antibody bound conjugate is separated from the unbound enzyme-follicle stimulating hormone conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known follicle stimulating hormone levels permits construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with follicle stimulating hormone concentration.

Principle:

Immunoenzymometric assay:

The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-FSH antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or stearic hindrance, to form a soluble

sandwich complex. The interaction is illustrated by the following equation:

$$Enz_{Ab_{(p)}} + Ag_{(p)} + {}^{Bt}Ab_{(m)} \underset{k_d}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab_{(p)}\,Ag_{(p)}\,{}^{Bt}Ab_{(m)}$$

$^{Bt}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)

$Ag_{(p)}$ = Native Antigen (Variable Quantity)

$Enz_{Ab_{(p)}}$ = Enzyme labeled Antibody (Excess Quantity)

$Enz_{Ab_{(p)}}Ag_{(p)}{}^{Bt}Ab_{(m)}$ = Antigen Antibodies Sandwich Complex

k_a = Rate Constant of Association

k_{-d} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:

$$Enz_{Ab_{(p)}}Ag_{FSH}{}^{Bt}Ab_{(m)} + Streptavidin_{C.W.} \Rightarrow \text{Immobilized complex}$$

$Streptavidin_{C.W.}$ = Streptavidin immobolized on well

Immobilized complex = sandwich complex bound to the solid surface

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

Reagents:

Materials Provided: Store at 2-8°C

(FSH) Kit Contents;	Volume
FSH Calibrators, 6 level concentrations as **mentioned on the label.**	6 x 1.0ml
FSH Enzyme Reagent	1x13ml
Streptavidin coated Microplate.	96 Wells
Wash Solution Concentrate.	1x 20ml
Substrate A	1x 7ml
Substrate B	1x 7ml
Stop Solution.	1x8ml
Product Insert	1

Note 1: Do not use reagents beyond the kit expiration date.

Note 2: Opened reagents are stable for sixty (60) days when stored at 2-8°C.

Note 3: Above reagents are for a single 96-well microplate

Required But Not Provided:

1. Pipette capable of delivering 50µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.300ml volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials

Precautions: For In Vitro Diagnostic Use

Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition,1988.

Specimen Collection and Preparation:

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells. Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml of the specimen is required.

Reagent Preparation:

Wash Buffer

Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store at room temperature 20-27°C for up to 60 days.

Working substrate Solution: Prepare 1+1 mixture of Substrate A and Substrate B. Use immediately after preparation. Prepare enough quantities for the assay run.

Test Procedure:

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-27°C).

1. Format the microplate wells for each serum reference, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C**
2. Pipette 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of FSH-Enzyme Reagent solution to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 300µl of wash buffer (see Reagent Preparation Section) decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of Working Substrate Solution to all wells. **Always add reagents in the same order to minimize reaction time differences between wells**

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds). **Always add reagents in the same order to minimize reaction time differences between wells.**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

Quality Control:

Each laboratory should assay controls at levels of a low, normal, and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents.

Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established

performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

Results:

A dose response curve is used to ascertain the concentration of follicle stimulating hormone in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding FSH concentration in mIU/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of FSH for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in mIU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.214) intersects the dose response curve at (43.2 mIU/ml) FSH concentration (See Figure 1).

Sample ID	Well Number	Abs (A)	Mean Abs (B)	Value (mIU/ml)
CAL A	A1	0.001	0.001	0
	B1	0.001		
CAL B	C1	0.146	0.139	5
	D1	0.133		
CAL C	E1	0.276	0.277	10
	F1	0.278		
CAL D	G1	0.680	0.689	25
	H1	0.698		
CAL E	A2	1.444	1.399	50
	B2	1.354		
CAL F	C2	2.471	2.412	100
	D2	2.354		
Ctrl 1	E2	0.162	0.157	5.6
	F2	0.152		
Ctrl 2	G2	0.545	0.546	19.9
	H2	0.547		
Patient	A3	1.173	1.214	43.2
	B3	1.255		

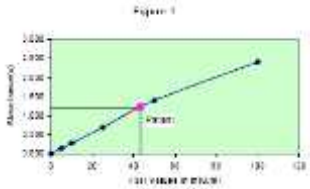
EXAMPLE 1

Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction.
*The data presented in Figure 1 are for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

Q.C. Parameters:

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator F should be > 1.3. 2. Four out of six quality control pools should be within the established ranges.



Limitations Of Procedure:

A. Assay Performance

1. It is important that the time of reaction in each well is held constant for reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
4. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time-deviation during reaction.
5. Plate readers measure vertically. Do not touch the bottom of the wells.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.

B. Interpretation

1. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
2. FSH is suppressed by estrogen but in woman taking oral contraceptives the level may be low or normal. Excessive dieting and weight loss may lead to low gonadotropin concentrations.
3. Follicle Stimulating hormones is dependent upon diverse factors other than pituitary homeostasis. Thus, the determination alone is not sufficient to asses clinical status

Expected Ranges Of Values:

A study of an apparent normal adult population was undertaken to determine expected values for the FSH ELISA Microplate Test System. The expected values are presented in Table 1.

TABLE I
Expected Values for the FSH IEMA Test System (in mIU/ml (2nd IRP 78/549)

	Women
Follicular phase	3.0 -- 12.0
Midcycle	8.0 -- 22.0
Luteal phase	2.0 -- 12.0
Postmenopausal	35.0 -- 151.0
	Men
	1.0 -- 14.0

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

Performance Characteristics:

A. Precision

The within and between assay precision of the FSH ELISA Microplate Procedure were determined by analyses on three different levels of control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 3 and Table 4.

TABLE 2
Within Assay Precision (Values in mIU/ml)

Sample	N	X	σ	C.V.
Level 1	16	5.9	0.25	4.3%
Level 2	16	16.0	0.68	4.3%
Level 3	16	41.3	1.18	2.9%

TABLE 3
Between Assay Precision* (Values in mIU/ml)

Sample	N	X	σ	C.V.
Level 1	10	5.9	0.41	6.9%
Level 2	10	16.0	0.48	3.0%
Level 3	10	40.9	1.48	3.6%

*As measured in ten experiments in duplicate.

B. Accuracy

This FSH ELISA Microplate Procedure was compared with a reference radioimmunoassay. Biological specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 106. The least square regression equation and the correlation coefficient were computed for the FSH ELISA in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This Method	17.4	y = 0.98(x) – 1.7	0.978
Reference	19.5		

Only slight amounts of bias between the ELISA FSH procedure and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

C. Sensitivity

The Follicle Stimulating Hormone procedure has a sensitivity of 0.04 mIU. This is equivalent to a sample containing 0.8 mIU/ml FSH concentration.

D. Specificity

The cross-reactivity of the FSH ELISA method to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of Follicle Stimulating Hormone needed to produce the same absorbance.

Substance	Cross Reactivity	Concentration
Follitropin (FSH)	1.0000	–
Lutropin Hormone (hLH)	< 0.0001	1000ng/ml
Chorionic Gonadotropin (hCG)	< 0.0001	1000ng/ml
Thyrotropin (TSH)	< 0.0001	1000ng/ml

Instruments and Application:

Fortress Diagnostics' immunoassay products are designed to work in both manual and automated lab environments and are compatible with any open-ended instrumentation, including chemistry analyzers, microplate readers and microplate washers. There may or may not be an application developed for your particular instrument please contact info@fortressdiagnostics.com
Fortress offers several instruments, including Plate Reader/Plate Washer. Please enquire by email.

Reference:

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