BIOGENIX
INC. PVT. LTD.

FSH ELISA

Enzyme Immunoassay for Quantitative Determination of Follicle-Stimulating Hormone in Human Serum

Instructions for use

1. SYMBOL LEGEND

IVD	In vitro diagnostic medical device	CE	EC Declaration of conformity
REF	Catalogue number	LOT	Batch code
	Use by		Manufacturer
	Date of manufacture		Consult operating instructions
	Temperature limitation		Biological risks
H2O	Deionized or distilled water	CONJ	Conjugate
MP	Coated microplate (96 wells)	SUB	Substrate
WASH P 20X	Wash solution, 20X concentrated	STOP	Stop solution
CAL	Calibrators	OD	Optical density
CONTROL	Control		Contains sufficient for <n> tests
RCNS	Reconstitute with specified volume of liquid	TRIAL 5000X	Trial

2. INTENDED USE

FSH kit is provided for the **quantitative determination of follicle-stimulating hormone (FSH) in human serum**.

FSH is a glycoprotein with a molecular mass of about 30 000 Da, that consists of two subunits – alpha and beta. FSH is secreted by frontal lobe of pituitary gland.

Together with LH and testosterone, FSH is necessary for spermatogenesis in spermatic ducts of testicles. In pubescent females FSH induces follicle growth and maturation in ovaries. Increased FSH levels are observed in patients with different forms of hypogonadism (primary ovary or testicle insufficiency, polycystic ovaries, menopause etc), renal insufficiency, cirrhosis and also as a result of castration. As a rule, decreased FSH levels are observed in the case of testicle malignancies.

FSH measurement is useful for the diagnosis of menopause, exact determination of ovulation time and for endocrine therapy monitoring.

3. PRINCIPLE OF TEST

FSH kit is a “sandwich” type of solid-phase enzyme immunoassay, based on two monoclonal antibodies that are specific for different epitopes of FSH β-subunit. One of these antibodies is conjugated with horseradish peroxidase (HRP); the other is coated onto the inner surface of microwells. FSH molecules from the serum sample bind to both immobilized antibody and anti-FSH-peroxidase conjugate. Then the wells are washed with wash solution to remove any material not bound on the inner surface of the wells. Quantity of the bound conjugate is directly proportional to FSH level in the sample.

During the incubation with TMB solution the colour is developing. The intensity of the colour is directly proportional to FSH concentration in specimens. FSH concentration in the patient sample is read from a standard curve that is processed in each assay.

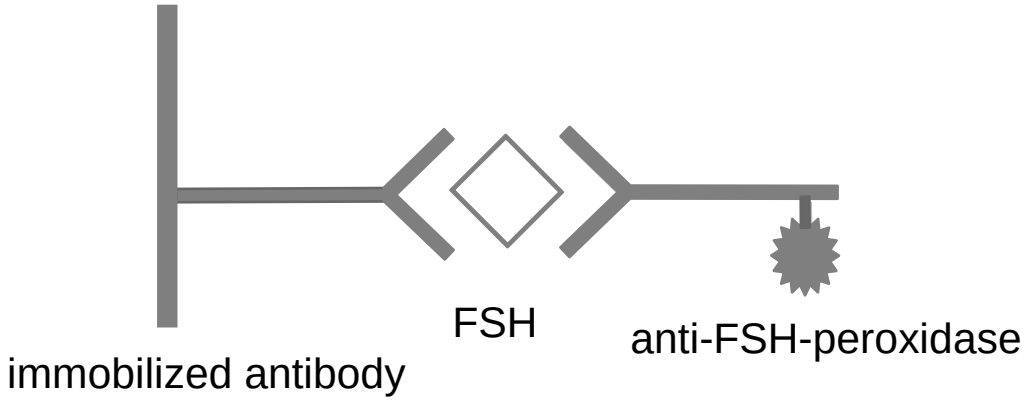

Fig. 1. Assay scheme

4. STORAGE AND STABILITY OF THE KIT

The expiration date of the kit is printed on the box label; the expiration date for each component is printed on the respective label.

FSH kit should be stored at +2…+8 °C upon receipt, preferably in the original kit box, until the expiration date. Storage at +25 °C is allowed but for no more than 5 days.

Shelf life of the kit is 18 months.

After initial opening the kit is stable until the expiration date if stored at +2…+8 °C.

If used in several separate experiments, after initial opening kit contents should be stored as follows:

- unused strips: in a firmly closed resealable zipper bag at +2…+8 °C until the expiration date;
- vials with conjugate, calibrators and control (ready-to-use): at +2…+8 °C until the expiration date; vials with calibrators and control (reconstituted): at +2…+8 °C for no more than 1 month after opening;
- vials with concentrated Trial, concentrated wash solution and stop solution: at +2…+8 °C until the expiration date;

- vial with substrate: at +2…+8 °C until the expiration date, protected from light;
- wash solution and Trial solution prepared for use: at room temperature (+18…+25 °C) for no more than 5 days or at +2…+8 °C for no more than 4 weeks, in a firmly closed bottle;

Damaged Test Kits

In case of any severe damage of the test kit or components, it has to be informed in writing, during one week after receiving the kit. Usage of severely damaged single components for a test run is not recommended.

5. SAMPLE COLLECTION AND STORAGE

Collect blood by venipuncture. After clotting, the serum is separated by centrifugation.

Do not use plasma, haemolyzed (bright red) or lipemic (milky) serum samples as well as samples containing sodium azide as preservative.

Store serum samples at +2…+8 °C for no more than 5 days. Aliquot and freeze samples for longer storage (–20 °C and lower). Avoid repeated freezing.

6. EXPECTED VALUES

Serum samples collected between 9 and 11 a.m. from apparently healthy people at the age of 19–65, were assayed with **FSH kit**. The results are given in the table on the next page. These limits should be considered as guidelines only.

Category	№	Mean (mIU/mL)	Range (mIU/mL)
Females			
Normally menstruating (19–35 years old)	120		
Follicular phase		4.6	1.8-11.3
Midcycle peak		7.9	4.9-20.4
Luteal phase		3.3	1.1-9.5
Postmenopausal (49–65 years old)	15	68.4	31.0-130
Males (21–39 years old)	40	3.9	1.0-11.8

It is highly recommended for each laboratory to determine its own reference range of FSH concentrations.

7. QUALITY CONTROL

It is recommended to use control samples according to the state and federal regulations. The use of control samples is advised to assure the day to day validity of results.

8. REAGENT PREPARATION

Allow all the reagents to reach room (+18…+25 °C) temperature, and then thoroughly stir.

MP Keep **microplate** at room temperature for at least 30 minutes before opening the bag. Place required number of strips onto strip holder. Place unused strips into the resealable zipper bag and reseal duly.

CAL **CONTROL** **Calibrators and Control**

Liquid calibrators and control are ready to use.

Prepare lyophilized calibrators and control as follows. Gently

tap on the vial caps to knock off all the dry matter. Open the vials and carefully place the caps upside down on the clean dry surface. Add 0.5 mL of distilled or deionized water to each vial with lyophilized calibrators and control, close vials with the corresponding caps and leave for 10 min at room temperature without stirring. Then stir gently avoiding foaming, until the dry matter is completely dissolved. Leave for another 10 minutes at room temperature stirring gently periodically. Make sure that no dry matter is left on the caps and walls of the vials.

WASH P Prepare required volume of **wash solution** by dilution of the concentrate 20-fold with distilled or deionized water. For example:

5 mL of **WASH P** **20X** + 95 mL of water.

Mix thoroughly, avoid foaming.

SUB Protect **substrate** from direct light.

9. SAMPLE PREPARATION

Allow samples to reach room temperature. Stir samples gently in order to ensure homogeneity.

10. PERFORMANCE CHARACTERISTICS OF THE ASSAY

10.1. Calibration - Traceability

FSH kit was calibrated against the WHO 2nd International Reference Preparation 78/549.

10.2. Specificity

According to Product specification provided by the supplier, cross-reactivity of both monoclonal antibodies with hCG is less than 0.1%, with TSH is less than 3.5% and with LH does not exceed 1.5%.

10.3. Analytical Sensitivity

Analytical sensitivity of **FSH kit**, i.e. concentration that can be distinguished from zero calibrator, is 0.25 mIU/mL. It is defined as mean OD of 10 replicates of calibrator 0 plus two standard deviations.

10.4. Measurement Range

FSH kit was validated for measurement of FSH concentration within the concentration diapason (without dilution) 0.25-100 mIU/mL.

10.5. Hook Effect

Hook effect is determined by spiking calibrators matrix with antigen. No **high-dose hook effect** was observed for FSH concentrations up to 50 000 mIU/mL.

10.6. Intra- and Inter-Assay Variation

For **intra-assay CV determination** 8 serum samples were assayed in 9 replicates each. The results are shown below.

Sample	Mean FSH concentration, mIU/mL	Intra-assay CV	
		SD	CV, %
HS 1	**3.0**	**0.23**	**7.7**
HS 2	**2.7**	**0.1**	**3.7**
HS 3	**5.5**	**0.1**	**1.8**
HS 4	**5.8**	**0.1**	**1.7**
HS 5	**9.7**	**0.2**	**2.1**
HS 6	**56.6**	**1.0**	**1.8**
HS 7	**84.1**	**1.4**	**1.7**
HS 8	**3.0**	**0.23**	**7.7**

For **inter-assay CV determination** 8 serum samples were assayed 3 times by different operators with 1-week interval. Each specimen was assayed in 9 replicates. The results are shown below.

Sample	Mean FSH concentration, mIU/mL			Inter-assay CV	
	1 assay	2 assay	3 assay	SD	CV, %
HS 1	5.2	5.8	4.9	0.46	8.6
HS 2	6.2	5.9	6.0	0.15	2.5
HS 3	8.0	7.3	8.2	0.47	6.0
HS 4	10.6	11.2	10.8	0.31	2.8
HS 5	15.5	15.9	14.9	0.50	3.3
HS 6	25.3	24.9	25.5	0.31	1.2
HS 7	59.5	57.9	59.9	1.06	1.8
HS 8	90.3	95.6	88.9	3.53	3.9

11. LIMITATION OF THE METHOD

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. To state a diagnosis, the physician is supposed to consider all the available clinical and laboratory findings.

12. SAFETY PRECAUTIONS

- **This kit is for in vitro diagnostic use only.** Operator should follow the manual closely in order to ensure reliable data. The manual is valid for the present kit only, within the listed composition. Any substitution of kit components is not allowed by CE regulations.
- Do not use kit or its components after the expiration date indicated on the label. Take into account stability period for reconstituted reagents.
- Do not mix or use together reagents from different lots of the kit except substrate, stop solution and wash solution.
- Do not use substrate, stop solution and wash solution supplied by other vendors.
- Use only “P”-labeled wash solution.
- Note that stop solution is 1N HCl solution. Avoid contacts with skin and mucosa. In case of contact rinse affected area thoroughly with plenty of water and seek medical advice.

- Take into account the following common procedural notes:
  - always pipette reagents into wells immediately after washing procedure;
  - avoid contamination of the solutions;
  - in case of partial use of the kit, dispense only required volume of the reagent into the tray;
  - do not pour unused reagents back into the original vials;
  - avoid exposure to direct sunlight during incubations;
  - always pipette reagents in the same order to minimize reaction time differences between wells; the total dispensing time for the calibrators, control and samples must not exceed 15 min;
  - the incubation temperature for all the immunological reactions must be kept at +37 °C;
  - do not touch the bottom of the wells;
  - calibrators should be measured in each separate assay. It is also recommended to measure each time FSH concentration in the control.
- TMB solution should be colourless. Light colouring of solution is admissible. Avoid direct exposure of substrate to sunlight.
-  Source materials of human origin used for kit components preparation were tested and found negative for HBsAg, anti-HIV and anti-HCV antibodies. However, none of known laboratory test guarantees absence of these viral agents. Therefore, all kit components and patient's samples should be handled as potentially hazardous.
-  After usage strips, calibrators, control, specimens and all consumables which contacted with specimens during handling, storage or assay (tubes, vials, gloves, pipette tips etc.) should be collected separately and sterilized by autoclaving. Instead of autoclaving pipette tips may be sterilized by disinfectant treatment. After sterilization all components and expendable materials may be utilized as non-dangerous garbage. Other components of the kit should be discarded into conventional garbage.

-  During manual washing procedure do not discard the contents of the wells directly to drainage. Use a container with disinfectant solution.
-  As the kit contains potentially hazardous material, the following precautions should be taken:
  - do not smoke, eat or drink while performing the assay;
  - always use protective gloves;
  - never pipette material by mouth;
  - in case of spilling, wipe up the spills promptly and wash affected area thoroughly using decontaminant.
- GLP including all general and individual regulations should be applied for the kit usage.

 As the kit contains irritant (CONJ, CAL, CONTROL), the following precautions should be observed:

- P261 - Avoid breathing spray;
- P272 - Contaminated work clothing should not be allowed out of the workplace;
- P280 - Wear protective gloves/protective clothing/eye protection;
- P302+P352 - IF ON SKIN: Wash with plenty of soap and water;
- P333+P313 - If skin irritation or rash occurs: Get medical advice/attention;
- P363 - Wash contaminated clothing before reuse;
- P501 - Dispose of contents/container in accordance with national regulation.

Precautionary statements according to Regulation EC № 1272/2008.

13. MATERIAL PROVIDED

13.1. Material Provided

MP	**Microplate:** 12 breakable 8-well strips (total 96 wells) coated with anti-FSH monoclonal antibodies	1 pcs
CONJ	**Conjugate:** solution contains anti-FSH monoclonal antibodies conjugated with HRP	14 mL, ready to use
0–5 CAL	**FSH calibrators:** protein-based solution or lyophilized preparations containing known FSH concentrations – 0; 2; 5; 25; 50; 100 mIU/mL. The concentrations of calibrators may be different for schemes with or without shaking. For exact FSH concentrations, see vial labels.	6 vials, 0.5 mL each; ready to use or lyophilized preparations
CONTROL	**FSH control:** protein-based solution or lyophilized preparation containing known FSH concentration. The range of FSH concentration may be different for schemes with or without shaking. For exact range of FSH concentration see vial label.	0.5 mL, ready to use or lyophilized preparation
SUB	**Substrate (TMB solution):** 3,3',5,5'-tetramethylbenzidine solution in citrate buffer containing hydrogen peroxide	14 mL, ready to use
WASH P 20X	**Wash solution P, 20X concentrated:** surfactant in buffered saline, sufficient for preparation of 560 mL of solution	2x14 mL, concentrated
STOP	**Stop solution** 1N HCl solution	14 mL, ready to use

13.2. Equipment and Materials Required but not provided

- 1-channel calibrated variable precision pipettes, with disposable tips;
- 8-channel calibrated variable precision pipette, with disposable tips;
- microplate incubator/shaker (+37 °C, shaking speed 500–800 rpm);
- manual or automatic equipment for rinsing wells;
- calibrated microplate reader (450 nm);
- vortex tube mixer;
- deionized or distilled water;
- graduated beaker and cylinder of appropriate volume;
- latex or plastic gloves;
- trays for pipetting reagents with 8-channel pipette;
- disinfectant;
- absorbent material (for manual wash).

13.3. Test Procedure

FSH kit is designed for 96 tests. This is sufficient for the **quantitative assay** of 40 unknowns, 6 calibrators, 1 control and 1 blank (OD of TMB solution) in duplicates, provided that all the strips are used simultaneously.

13.3.1. Assay Procedure

13.3.1.1. Protocol with shaking

(See assay scheme, section 13.5)

A. Pipette**:**

- **100 µL** of **conjugate** **CONJ** into each well **except wells A1-A2 (blank);**

B. Pipette**:**

- **50 µL** of calibrators **CAL**, control **CONTROL** and patient's samples in duplicates**;**

except wells A1-A2.

Note: total time of dispensing must not exceed 15 minutes, otherwise the test result may be unreliable, because the time of incubation will substantially vary for different samples.

C. Incubate for **60 minutes at +37 °C while shaking (500–800 rpm)**.

D. Wash **5 times**, as described in section 13.3.2.

E. Pipette **100 µL** of **substrate** **SUB** into each well (including blank); incubate strips **at room temperature** (+18…+25 °C) **in the dark for 15-30 minutes**, depending on the colour intensity, or **10 minutes while shaking (500-800 rpm) at +37 °C**.

F. Pipette **100 µL** of **stop solution** **STOP** into each well (including blank) in the same sequence and at the same speed as used for dispensing TMB substrate. Shake for 1–2 min at room temperature.

G. Read OD at **450 nm within** 20 min.

13.3.1.2. Protocol without shaking

(See assay scheme, section 13.6)

A. Pipette:

- 100 μL of conjugate CONJ into each well except wells A1-A2 (blank);

B. Pipette:

- 50 μL of calibrators CAL, control CONTROL and patient's samples in duplicates;

except wells A1-A2.

Note: total time of dispensing must not exceed 15 minutes, otherwise the test result may be unreliable, because the time of incubation will substantially vary for different samples.

C. Incubate for 120 minutes at +37 °C (pre-shake for 1-2 minutes at room temperature).

D. Wash 5 times, as described in section 13.3.2.

E. Pipette 100 μL of substrate SUB into each well (including blank); incubate strips at room temperature (+18…+25 °C) in the dark for 15-30 minutes, depending on the colour intensity.

F. Pipette 100 μL of stop solution STOP into each well (including blank) in the same sequence and at the same speed as used for dispensing TMB substrate. Shake for 1–2 min at room temperature.

G. Read OD at 450 nm within 20 min.

13.3.2. Wash Procedure

It is advisable to use an automatic microplate washer set at 5 wash cycles and a volume of 300 µL of wash solution per well per cycle.

If an automatic washer is not available, the wash procedure can be carried out manually as follows:

- remove the contents of the wells into a container with disinfectant;
- dispense 300 µL of wash solution, prepared according to section 8, into each well, shake the plate carefully for 5 – 10 sec and remove the contents of the wells; repeat 5 times;
- strike the wells sharply on absorbent material to remove any liquid residue.

13.4. Data Processing

If the reader cannot be adjusted to zero using the substrate blank in wells A1-A2, subtract mean OD value of wells A1-A2 from all OD values before further calculations.

Example:

OD (Cal 5) measured = 2.28 and OD (blank) = 0.06;

OD (Cal 5) calculated = 2.28 – 0.06 = 2.22

13.4.1. Data Reliability (for OD Measured at 450 nm)

The data should meet the following criteria:

- average blank OD (in wells A1-A2) ≤0.100;
- average OD of Cal 5 ≥1.0 (after blank subtraction).
- control’s concentration must fall within the acceptability ranges that is shown on the vial label.

If the data obtained do not meet the criteria, the results are considered unreliable and the test should be repeated.

Specialized software for quantitative determination is recommended. Mean OD values of the calibrators at 450 nm versus their respective FSH concentrations using 4PL or 5PL fit (see typical standard curve, fig. 2). Calculate concentration of FSH in samples using standard curve.

Any extrapolation of the standard curve to FSH above the nominal value of the calibrator 5 (approximately 100 mIU/mL) is forbidden.

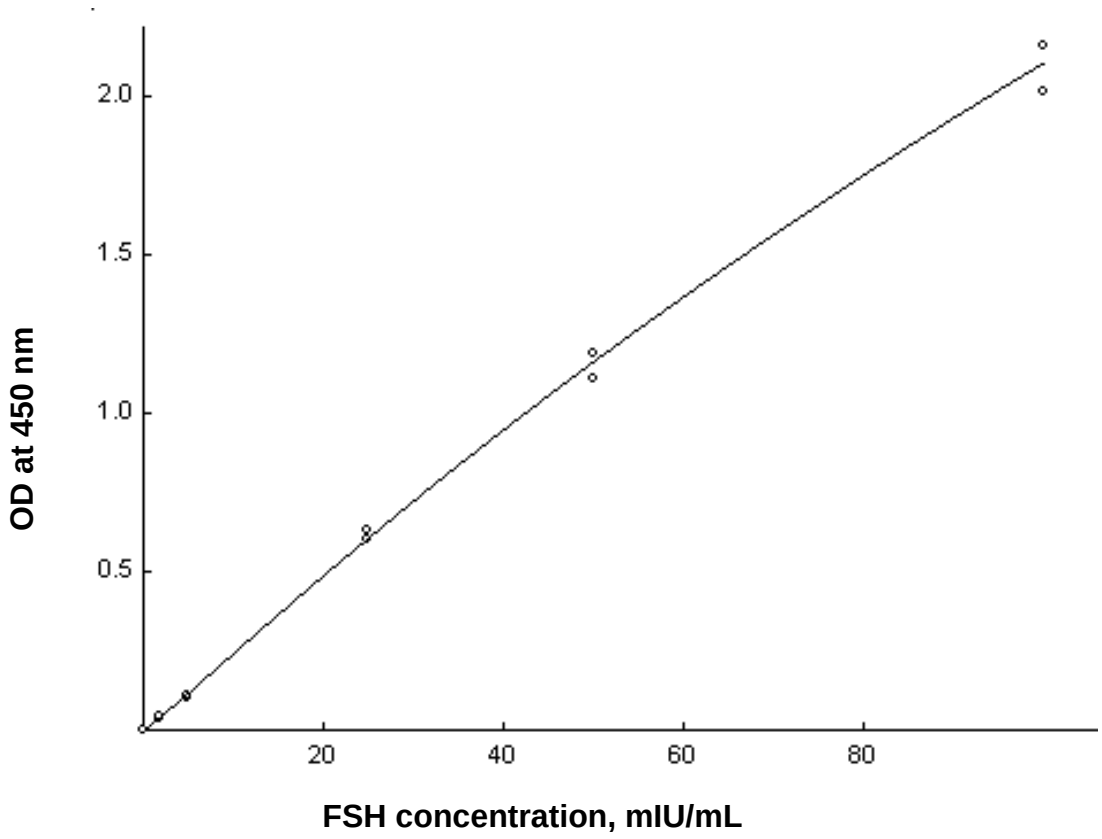

Fig. 2. Example of typical standard curve.

Do not use for evaluation of real assay data!

13.5. Assay scheme with shaking

Wells / Reagents	«Blank»	**CAL** **CONTROL**	Samples
CONJ	–	100 µL	100 µL
CAL **CONTROL**	–	50 µL	–
Samples	–	–	50 µL
Incubation No.1	60 min, +37 °C, 500–800 rpm		
WASH P (diluted)	5 x 300 µL		
SUB	100 µL	100 µL	100 µL
Incubation No.2	15–30 min, +18…+25 °C, in the dark		
	10 min, +37 °C, 500–800 rpm		
STOP	100 µL	100 µL	100 µL
Stirring	1–2 min, +18…+25 °C		
OD measuring	450 nm		
Calculations	Corresponding software		

13.6. Assay scheme without shaking

Wells / Reagents	«Blank»	**CAL** **CONTROL**	Samples
CONJ	–	100 µL	100 µL
CAL **CONTROL**	–	50 µL	–
Samples	–	–	50 µL
Incubation No.1	120 min, +37 °C (pre-shake for 1-2 minutes at room temperature)		
WASH P (diluted)	5 x 300 µL		
SUB	100 µL	100 µL	100 µL
Incubation No.2	15–30 min, +18…+25 °C, in the dark		
STOP	100 µL	100 µL	100 µL
Stirring	1–2 min, +18…+25 °C		
OD measuring	450 nm		
Calculations	Corresponding software		

BIOGENIX INC. PVT. LTD.
Factory: B - 19/A, S.I.L Ancillary Estate, Amausi Industrial Area, Nadarganj, Kanpur Road, Lucknow - 226008 (U.P.), India
Email: biogenix2007@yahoo.com, info@biogenixinc.com
Website: www.biogenixinc.com
Customer care no: +919140971443