

## FSH ELISA Kit

96 Tests Kit

Enzyme Immunoassay for the  
Quantitative Determination of  
Follicle Stimulating Hormone (FSH)  
Concentration in Human Serum/Plasma  
(For In Vitro Diagnostic Use Only)  
Catalogue No. PT-FSH-96

**PISHTAZ TEB DIAGNOSTICS**

### 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 granulosa cells; follicular steroidogenesis is promoted and LH production is stimulated. The LH 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. 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.

### Test Principle

The FSH Quantitative Test Kit is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes an 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 with the solid phase antibodies, after incubation and washing, the enzyme conjugate will be added, resulting in the sandwich formation of FSH between solid phase and conjugated antibodies. After second wash step a solution of TMB is added and incubated for 15 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.

## Materials provided with the kit

1. Anti-FSH coated microplate. 96 wells per bag.
2. Anti-FSH-HRP conjugate: 6 ml, ready to use solution.
3. Standards set including 0, 5, 10, 25, 50, 100 IU/L of FSH calibrated against WHO 83/575 1<sup>st</sup> IRP.
4. Serum control: 1 ml, known serum concentration (the exact value printed on the label of the serum control vial).
5. TMB reagent: 12 ml, ready to use solution.
6. Wash solution: 50 ml, concentrated (20X).
7. Stop solution: 12 ml.
8. Cardboard sealer

## Materials required but not provided

- Precision pipettes: 50 µl and 100 µl
- Disposable pipette tips.
- Distilled water.
- Absorbent paper or paper towel.
- Graph paper.
- Microtiter well reader.

## General Information

1. Do not mix kit reagents from different batch/lot numbers. All kit components must be used only in their original kit.
2. All reagents obtained from human sources are negative for HBs Ag, HCV and HIV. To prevent risk of contamination, use personal protective equipments like gloves, lab coats, etc. and avoid direct contact with reagents.

## Storage Conditions

1. Kit should be stored at 2-8 °C upon receipt and when it is not in use.
2. Keep Un-used wells in their sealed bag with desiccants.
3. Do not use expired date reagents.

## Specimen Collection and Preparation

The kit is for use with serum or plasma. Serum or plasma should be prepared from a whole blood specimen obtained by approved aseptic technique. If testing cannot be done within an hour after sample collection, refrigerate (maximum 48 hours) the specimen immediately and let it return to room temperature before testing. If prolong storage is required, samples should be stored at -20 °C (maximum 30 days). Avoid freeze-thaw of specimen during storage.

## Reagents Preparation

1. All reagents should be allowed to reach room temperature (22-28 °C) before use.
2. Working wash solution: dilute concentrated wash solution 1:20 with distilled water before use.

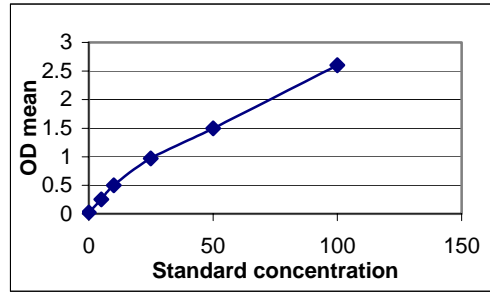
## Test Procedure

1. Secure the desired number of coated wells in the holder and keep the remaining with desiccants in tightly closed special bag.
2. Dispense 50 µl of standard, serum control and specimen in appropriate wells.
3. Cover the microtiter wells with cardboard sealer firmly. Leave wells for 30 minutes at room temperature (22-28 °C).
4. Remove the sealer and take out wells contents by flicking the microplate into a waste container.

Rinse and flick the microtiter wells 5 times (each time with 300 µl of working wash solution).

5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
6. Add 50 µl of Anti-FSH-HRP conjugate into the wells.
7. Seal the plate with cardboard sealer again.

- Leave wells for 30 minutes at room temperature (22-28 °C).
8. Repeat step 4
  9. Dispense 100 µl of chromogen/ substrate (TMB) into the microplate wells.
  10. Incubate the microplate wells at room temperature and dark for 15 minutes, to develop color.
  11. Stop the reaction by adding 100 µl of stop solution to the microplate wells.
  12. Measure absorbance at 450 nm by ELISA reader. (Use 630 nm filter as reference filter if it's available).



Note: All absorbances shown in above curve and table are for the purpose of illustration only, and should not be used to calculate unknowns. Users should obtain their own data and standard curves.

### Result Calculation

1. Calculate mean absorbance value of standards and samples at 450 nm. (Use 630 nm filter as reference filter if it's available).
2. Construct a point to point standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in IU/L on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Use the mean absorbance value for each sample, determine the corresponding concentration of FSH in IU/L from the standard curve.

### Expected Values

It is important for each laboratory to establish the normal range limits. The following normal range should be considered as a guideline only:

	Mean (IU/L)	Reference value (IU/L)
Adult female:		
Follicular phase	5.2	1.5-9.7
Mid-cycle	14	8-20
Luteal phase	4.1	1.1-7.2
Menopause	70	30-110
Adult male (< 60 y/o)	6	2-10

### Example of Standard curve

Standards IU/L	OD
0	0.02
5	0.25
10	0.50
25	0.97
50	1.50
100	2.60

### Performance Characteristics

#### 1. Minimum Detection Limit

Based on mean absorbance of zero standard + 3SD the minimum detectable concentration of FSH by this assay is estimated to be 1 IU/L.

#### 2. Test Precision

Four serum samples with different concentrations of FSH were repeatedly tested. Results are shown in tables 1 and 2:

Table no. 1 (Intra-assay)

No.	No. of tests performed	Means IU/L	SD IU/L	CV%
1	24	7	0.14	2.0
2	24	11	0.32	2.9
3	24	32	1.10	3.4
4	24	85	2.30	2.7

Table No. 2 (Inter-assay)

No.	No. of tests* performed	Means IU/L	SD IU/L	CV%
1	10	10	0.45	4.5
2	10	24	0.95	4
3	10	35	1.2	3.4
4	10	92	2.4	2.6

\*Each test has been run in duplicate

### 3. Test Recovery

The certain amount of hFSH was added into 4 different serums with known concentration of hFSH and then their recovery were determined. The results shown below:

Table no. 3: Test recovery

No.	hFSH level IU/L	hFSH added IU/L	Exp. IU/L	Obs. IU/L	Rec. (%)
1	2	5	3.5	3.6	103
1	2	25	13.5	12.5	93
1	2	100	56	58	104
2	7	5	6	6.5	108
2	7	25	16	15	106
2	7	100	53.5	51	95
3	14	5	9.5	9	95
3	14	25	19.5	21	108
3	14	100	57	55	96
4	45	5	25	24	96
4	45	25	35	38	109
4	45	100	72.5	69	95

Exp.: Expected, Obs.: Observed,  
 Rec.: Recovery

### 4. Test Linearity

To verify test linearity, four different serum samples with known hFSH concentrations were diluted sequentially by zero standard. Then the serums were tested by FSH ELISA test. The results and serum recovery were determined considering dilution factor.

Table no. 4: Test linearity

No.	hFSH undiluted specimen	Recovery (%)			
		1:2	1:4	1:8	1:16
1	20	97	98	97	100
2	39	96	103	99	97
3	47	95	101	97	99
4	80	100	97	96	97

### 5. Test Specificity

To determine cross reaction of FSH ELISA kit with closely related kits, the following hormones were tested: hLH, hTSH and hCG. Results are shown in the following table:

Table no. 5 (Specificity test result, Cross reaction)

Analyte name	Concentration (IU/L)	Presumptive concentration hFSH
hLH (IU/L)	500	<1
	250	<1
	100	<1
	50	<1
hTSH (mIU/L)	500	<1
	250	<1
	100	<1
	50	<1
hCG (IU/L)	200000	<1
	100000	<1
	10000	<1
	1000	<1




### 6. Hook effect

To rule out possible hook effect occurrence, the hFSH assay was done on serums with high concentration of hFSH (up to 2500IU/L) and no "hook effect" was seen.

**Reference**

- Wide, L., Loraine, Ed. J. A., Bell, E. T. (1976) Human pituitary gonadotropins: hormone assays and their clinical application, pp 87-140, Churchill Livingstone, Edinburgh, London and New York

**FSH Test Procedure**
**Step 1**




	Standard	Serum Control	Sample
<b>Reagent</b>			
Standard	50 µl	None	None
Serum Control	None	50 µl	None
Sample	None	None	50 µl

Cover the microplate wells with cardboard sealer tightly and incubate them for 30 minutes at room temperature.

**Step 2**

Remove plate cover and discard contents of the wells. Wash the microplate wells for 5 times according to test manual.




**Step 3**

Anti FSH-HRP conjugate	50 µl	50 µl	50 µl
			

Cover the microplate wells again with cardboard sealer and incubate them for 30 minutes at room temperature.




**Step 4**

Remove the microplate wells cover and remove contents by flicking of the wells into a waste container. Rinse and flick the microtiter wells 5 times according to test manual.

TMB	100 µl	100 µl	100 µl
			

Incubate wells for 15 minutes at room temperature in dark.

**Step 5**

Stop Solution	100 µl	100 µl	100 µl
			

Read absorbance at 450 nm (Use 630 nm filter as reference filter if it's available).