

**REF**

GD7045 00

# FSH

Enzyme-immunoassay for the quantitative  
determination of follicle stimulating hormone  
in human serum

**IVD**

## INDICATION

Follicle Stimulating hormone (FSH) is a glycoprotein consisting of two subunits with an approximate molecular mass of 35,500 daltons. The  $\alpha$ -subunit is similar to other pituitary hormones [luteinizing stimulating hormone (LH), thyroid stimulating hormone (TSH) and chorionic gonadotropin (hCG)] while the  $\beta$ -subunit is unique. The  $\beta$ -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 steroidogenesis. 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.

## PRINCIPLE OF THE ASSAY

The present test is a one-step immunoassay, based on principle of "sandwich" method. The assay system utilizes a high affinity and specificity monoclonal antibody (enzyme conjugated and immobilized) directed against a distinct antigenic determinant on the intact FSH molecule. The test sample is allowed to react simultaneously with the two antibodies, resulting in the FSH molecules being sandwiched between the one immobilized on microwell plates and the other conjugated to horseradish peroxidase (HRP). After incubation, the wells are washed with a washing solution to remove unbound labelled antibody. A solution of Chromogen/Substrate is added and incubated, resulting in the development of a blue color. The color development is stopped by the addition of the Stop Solution, changing the color to yellow. The concentration of FSH in the sample is directly proportional to the color intensity, measured spectrophotometrically at 450 nm.

## KIT CONTENT

- 1. Reagent A – Microplate**  
12x8 strips.  
8 wells breakable strips, coated with anti FSH monoclonal antibodies. The strips are assembled on a plastic frame and contained in a sealed bag with desiccant. Bring the strips to room temperature before use, to prevent any moisture formation inside the bag.
- 2. Reagent B – Enzymatic Tracer**  
1 vial of 12 ml.  
Ready to use solution containing monoclonal anti FSH antibody, conjugated with Horseradish peroxidase (HRP), and 0.1% ProClin 300 and 0.004% Gentamycin sulphate as preservatives.
- 3. Reagent C – Washing Solution 25x**  
1 vial of 50 ml.  
Concentrated solution to be diluted 1:25 with distilled water. It contains a detergent in Phosphate buffer.
- 4. Reagent D/E – Chromogen/Substrate**  
1 vial of 12 ml.  
Ready to use solution containing Tetramethylbenzidine (TMB) and H<sub>2</sub>O<sub>2</sub> in Citric acid buffer.  
**Avoid any skin contact and light exposure.**
- 5. Reagent F – Stop Solution**  
1 vial of 15 ml.  
Ready to use solution containing Sulphuric acid 0.2 M.  
**Avoid any skin contact.**
- 6. FSH Standards:**  
6 vials of 0.5 ml each (S<sub>0</sub> - 2.0 ml).  
Ready to use liquids containing FSH in protein-based buffer and 0.1% ProClin 300 and 0.004% Gentamycin sulphate as preservatives.  
Approximately FSH concentrations are the following:  
S<sub>0</sub>: 0 mIU/ml, S<sub>1</sub>: 5 mIU/ml, S<sub>2</sub>: 10 mIU/ml,  
S<sub>3</sub>: 25 mIU/ml, S<sub>4</sub>: 50 mIU/ml, S<sub>5</sub>: 100 mIU/ml.  
(Calibrated against WHO 3<sup>rd</sup> IRP 94/632)  
Actual concentrations to be used for calculation are stated on the labels of the vials.
- 7. FSH Control:**  
1 vial of 0.5 ml.  
Ready to use human serum based liquid containing a defined quantity of FSH and 0.1% ProClin 300, 0.004% Gentamycin sulphate as preservatives.  
Refer to the vial label for acceptable range.
- 8. Cardboard sealers:**  
2 cardboard sealers to be used to cover the plate during the incubations.
- 9. Package insert:** instruction for use GD7045 00 it/ing.

## MICROBIOLOGICAL STATE AND CLEANING GRADE

1. All the materials of human origin resulted negative to HbsAg, HIV 1&2 and HCV FDA approved tests. Anyhow, as no test can guarantee the absolute absence of infective agents, handle reagents as potentially infected, especially standards, controls and samples. All objects come in direct contact with samples and all residuals of the assay should be treated or eliminated as potentially infected. Best procedures for inactivation are treatments with autoclave at 121°C for 30 minutes or with sodium hypochlorite at a final concentration of 2.5 % for 24 hours.
2. Avoid any contact with skin and mucous membrane, in particular for Chromogen/Substrate and Stop Solutions.
3. Use protective disposable talk-free gloves.
4. Avoid contaminating reagents when taking them from the vials. We recommend to use automatic pipettes with disposable tips. When dispensing reagents, do not touch with tips the wall of wells in order to avoid cross-contaminations.
5. For the washing step, follow carefully the indications reported in "WASHING INSTRUCTION".
6. Avoid the substrate/chromogen to come in contact with oxidizing agents or metallic surfaces; avoid intense light exposure during incubation or reagent preparation.

## STORAGE AND STABILITY OF THE KIT

1. The kit has to be stored at 2-8 °C and used before the expiry date stated on the label.
2. Unused strips have to be placed in the bag containing the desiccant and firmly sealed before restore at 2-8 °C. After opening the strips are stable up to the expiry date stated on the label.
3. All other reagents can be repeatedly used up to exhaustion if stored at 2-8 °C, provided that they are handled carefully to avoid any environment contamination. Under these conditions the reagents are stable up to the expiry date stated on the labels.

## AUXILIARY MATERIALS

- Semi automatic pipettes of 25, 100 and 150 µl
- Vortex mixer and absorbent paper
- Chronometer
- Ultrapure Elisa grade water
- Thermoshaker at 37 (± 0.5) °C or thermostatic incubator at 37 (± 0.5) °C
- Photometric reader of microplates or microstrips, linear up to at least 2 OD and supplied with filter of 450 nm.
- Automatic microplates washing device or manual apparatus capable of aspirating and dispensing volumes of 300 µl.

## SAMPLES

Serum only may be used. The kit is not calibrated for the determination of FSH in plasma, saliva or other specimens of human or animal origin. The blood should be collected in plain redtop venipuncture tube without additives and gel barrier. Separate serum as soon as possible to avoid any hemolysis. Samples can be stored at 2-8 °C for a short time (max three days). For longer storage the specimen should be frozen. Avoid repeated freezing and thawing. Highly lipemic, hemolysed, preserved by sodium azide or microbiologically contaminated samples should not be used in the assay.

## REAGENTS PREPARATION

- **WASHING SOLUTION:** dilute 1:25 with distilled or ELISA grade water (e.g.: 20 ml of Reagent C + 480 ml of distilled water) and mix carefully before use. The diluted washing solution can be stored for 3 days at 2-8 °C. The concentrated solution may present a sediment that can be dissolved at 35-39 °C and shaking. It is recommended to store washing solution at room temperature for immediate use.

## WASHING INSTRUCTION

A good washing procedure is essential to obtain correct and precise analytical results.

We therefore recommend to use a good quality ELISA microplate washer, maintained at a good level of washing mechanical performances.

Generally, 5 automatic washing cycles of 0.3 ml/well are sufficient to avoid false positive reactions and remove high background. Anyhow we recommend to calibrate the washing system on the kit itself so to match the declared analytical performances.

In case of manual washing, we suggest to perform 5 washing cycles, dispensing and aspirating 0.3 ml/well per cycle.

In any case the liquid washed out from the plates must be inactivated with a sodium hypochlorite solution at a final concentration of 2.5%, before being thrown away or autoclaved, as it must be considered as potentially infected.

## ASSAY PROCEDURE

1. At least one hour before use, bring all reagents, standards, control and samples to room temperature (18-30 °C), mixing them carefully on vortex.
2. Do not mix reagents from different lots.
3. We recommend to distribute standards, control and samples in duplicate.
4. Distribution and incubation times must be the same for all wells in the same analysis.
5. Avoid long interruptions between each step of the assay procedure.
6. It is suggested to eliminate the excess of washing solution from the microplate after washing by blotting it gently on an absorbent paper pad.
7. The colour developed in the last incubation is stable for a maximum of 20 minutes. Otherwise, in case of reading after 10-15 min after dispensing stop solution, immediately place the strips **in the dark**.
8. The "blinking" of the instrument is to be carried out in the blank reagent well (well A1).

### ASSAY SCHEME

- Put the desired number of microstrips into the frame.
- If suggested concentration in the sample exceeds 100 mIU/ml, dilute this sample accordingly, using only Standard 0 mIU/ml (the use of any other reagent may lead to false results). The results obtained should be multiplied by the dilution factor.
- Follow the scheme:

	Microplate wells coated with anti-FSH antibody			
	REAGENTS	Blank	Standard, Control	Sample
Immunological reaction	Standard, Control	-	25 µl	-
	Sample	-	-	25 µl
	Enzymatic Tracer (Reagent B)	-	100 µl	100 µl
	- Cover the strips with cardboard sealer - Incubate on a <b>thermoshaker</b> (approximately 500-800 rpm) <b>30 minutes at 37 (±0.5) °C</b> or on a <b>thermostatic incubator 60 minutes at 37 (±0.5) °C</b>			
Washing	- Peel out the cardboard sealer and aspirate the reaction solution from all wells - Rinse 5 times with 300 µl of diluted washing solution, carefully aspirating off the remaining liquid			
Colorimetric reaction	Reagent D/E (Chromogen-Substrate)	100 µl	100 µl	100 µl
	- Cover the strips with cardboard sealer - Incubate <b>20-30 minutes at room temperature</b> (22-28 °C), avoiding light exposure			
	Reagent F (Stop Solution)	150 µl	150 µl	150 µl
	- Gently mix for 5-10 seconds - Read the absorbance of each well against Blank at 450 nm			

### CALCULATION OF RESULTS

- Calculate the mean of the absorbance values for each point of the standard curve, control and of each sample.
- Subtract the absorbance value of the Blank from the mean absorbance values of standards, control and samples.
- Draw a calibration curve on a linear graph paper with the mean optical densities on the Y-axis and the standards concentrations on the X-axis.
- Interpolate the values of the samples on the standard curve to obtain the corresponding values of concentration expressed in mIU/ml.

### VALIDITY OF THE TEST

For the test to be valid the following criteria must be met:

- Blank OD 450 nm:  $\leq 0.1$
- Standard 5 (100 mIU/ml) OD 450 nm:  $\geq 1.3$
- Calculated value of Control should be within the established range stated on the label.

### EXPECTED VALUES

Group		Value, mIU/ml
Women	follicular phase	< 10
	midcycle	5-16
	luteal phase	< 10
	postmenopausal	2-150
Men		1.4 - 14

It is strongly recommended that each laboratory should determine its own normal and abnormal values according to the examined population.

### Note:

- The results obtained with this kit should never be used as the sole basis for clinical diagnosis. Any laboratory results is only a part of the total clinical picture of the patient.
- Some individuals may have heterophilic antibodies to mouse or other animal proteins that can possibly interfere with the test. Therefore, the result from any patient who have received preparation of mouse antibodies for diagnosis or therapy should be interpreted with caution.
- The present test is not intended for newborn screening.

### ANALYTICAL PERFORMANCES

#### Assay range

The range of the assay is 0 – 100 mIU/ml.

#### Analytical Sensitivity

The lower detection limit is calculated from the standard curve determining the resulting concentration of the mean OD of 10 replicates of Standard 0 mIU/ml plus 2 SD. The sensitivity of the test is 0.3 mIU/ml.

#### Precision

##### a. Intra Assay Variation

Sample	n	Mean, mIU/ml	SD	%CV
1	32	0.82	0.065	7.9
2	32	54.40	1.850	3.4

##### b. Inter Assay Variation

Sample	Mean, mIU/ml	SD	%CV
1	0.82	0.065	7.9
2	54.36	2.58	4.7

**Specificity**

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

Substance	Cross-reactivity (%)
FSH	100
hCG	0.001
TSH	0.0000
LH	0.02

**Recovery**

Spiked samples were prepared by adding defined amounts of FSH to patient serum sample. The results are tabulated below:

Added (mIU/ml)	Measured (mIU/ml)	Expected (mIU/ml)	Recovery (%)
-	10.8	-	-
100	51.6	55.4	96.1
50	27.2	30.4	93.2
0	5.9	5.4	109

**Linearity**

Patient serum sample was diluted with Standard 0 mIU/ml. The results are tabulated below:

Sample	Measured (mIU/ml)	Expected (mIU/ml)	Recovery (%)
Undiluted	100	-	-
1:2	48.3	50	96.5
1:4	23.6	25	94.2
1:8	12.2	12.5	97.3

**Accuracy**

The present kit was compared with a Chemiluminescent microparticle immunoassay as a reference test. 942 specimens (values ranged from 0.1 to 150 mIU/ml) were tested. The following linear regression curve was calculated:

$$y = 1.056x - 0.532 \text{ mIU/ml} \quad r = 0.96$$

**Hook Effect**

No "hook effect" was observed in this test.

**PRECAUTIONS IN USE**

The reagents are not considered harmful according to the 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.

The use of laboratory reagents according to good laboratory practice is recommended.

**Waste Management**

Please refer to local legal requirements.

**REFERENCES**

- Harris, G.W. and Naftolin., The Hypothalamus and Control of Ovulation, Brit. Med. Bullet., 26, 1-9 (1970).
- Knobil, E., The Neuroendocrine Control of the Menstrual Cycle, Rec. Prog. Horm. Res., 36, 52-88 (1980).
- Jeffcoate, S.L., Clinics in Endocrinol. Metab., 4, 521-543 (1975).
- Pierce, J.G. and Parsons, T.F., Glycoprotein hormones: Structure and Function, Annual Rev. Biochem., 50, 465-495 (1981).
- Ross, G.T., VandeWeile, R.L., and Frantz, A.G. Chapter 7 in "The Ovaries and the Breasts" in "Textbook of Endocrinology" (R. H. Williams, Ed.), W.B. Saunders Co. (1981).
- Engvall, E., Methods in Endocrinology, Volume 70, Van Vunakis, H. and Langone, J.J. (eds.), Academic Press, New York, 419-492 (1980).
- Uotila, M. Ruoslahti, E. and Engvall, E., J. Immunol. Methods, 42, 11-15 (1981).