Human FSH ELISA Kit

For the quantitative determination of human follicle stimulating hormone (FSH) concentrations in serum

Catalogue Number: EL10013

96 tests

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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S7.5 (11/13-05) FSH

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INTENDED USE

This Human FSH ELISA Kit is to be used for the *in vitro* quantitative determination of human follicle stimulating hormone (FSH) concentrations in serum. This kit is intended FOR LABORATORY RESEARCH USE ONLY and is not for use in diagnostic or therapeutic procedures.

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 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 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 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. 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.

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. 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 radioimmunoassay. 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. 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.

PRINCIPLES OF THE ASSAY

This FSH enzyme linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for FSH. Standards or samples are then added to the microtiter plate wells and FSH if present, will bind to the antibody pre-coated on the wells. In order to quantitate the amount of FSH present in the sample, a standardized preparation of horseradish peroxidase (HRP) conjugated monoclonal antibody specific for FSH is added to each well to "sandwich" the FSH immobilized on the plate. The microtiter plate undergoes incubation, and then the wells are thoroughly washed to remove all unbound components. Next, a TMB (3,3',5,5' Tetramethyl-benzidene) substrate solution is added to each well. This enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain FSH and enzyme-conjugated antibody will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm.

In order to measure the concentration of FSH in the sample this Human FSH ELISA Kit includes a set of calibration standards (6 standards). The calibration standards are assayed at the same time as the samples and allow the operator to produce a standard curve of Optical Density (O.D.) versus FSH concentration (mIU/mL). The concentration of FSH in the samples is then determined by comparing the O.D. of the samples to the standard curve.

REAGENTS PROVIDED

All reagents provided are stored at 2-8°C. Refer to expiration date on the label.

	96 tests

1. **MICROTITER PLATE** (Part EL13-1) ***96 wells***
   Pre-coated with anti-human FSH monoclonal antibody.
2. **CONJUGATE** (Part EL13-2) ***12 mL***
   Anti-FSH monoclonal antibody conjugated to horseradish peroxidase (HRP) with preservative. *Ready to use.*
3. **STANDARD – 200 mIU/mL** (Part EL13-3) ***1 vial***
   Lyophilized human FSH in a buffered protein base with preservative that will contain 200mIU/mL after reconstitution.
4. **STANDARD – 100 mIU/mL** (Part EL13-4) ***1 vial***
   Lyophilized human FSH in a buffered protein base with preservative that will contain 100 mIU/mL after reconstitution.
5. **STANDARD - 50 mIU/mL** (Part EL13-5) ***1 vial***
   Lyophilized human FSH in a buffered protein base with preservative that will contain 50 mIU/mL after reconstitution.
6. **STANDARD – 25 mIU/mL** (Part EL13-6) ***1 vial***
   Lyophilized human FSH in a buffered protein base with preservative that will contain 25mIU/mL after reconstitution.
7. **STANDARD – 5 mIU/mL** (Part EL13-7) ***1 vial***
   Lyophilized human FSH in a buffered protein base with preservative that will contain 5mIU/mL after reconstitution
8. **STANDARD – 0 mIU/mL** (Part EL13-8) ***1 vial***
   Lyophilized buffered protein base with preservative that will contain 0 mIU/mL after reconstitution.
9. **SUBSTRATE A** (Part EL13-9) ***10 mL***
   Buffered solution with H_2O_2.
10. **SUBSTRATE B** (Part 30007) ***10 mL***
    Buffered solution with TMB.
11. **STOP SOLUTION** (Part 30008) ***14 mL***
    2N H_2SO_4. Caution: Caustic Material!

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100μL and 50-200μL for running the assay.
2. Pipettes: 1 mL, 5 mL 10 mL, and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Incubator (37°C).
7. Microtiter plate reader (450nm ± 2nm)
8. Automatic microtiter plate washer or squirt bottle.
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water.
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standards, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Human serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
8. All samples should be deposed of in a manner that will inactivate human viruses.
   Solid Waste: Autoclave 60 min. at 121°C.
   Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate viruses before disposal.
9. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
10. The Substrate B contains 20% acetone, keep reagent away from sources of heat or flame.

SAMPLE PREPARATION

COLLECTION, HANDLING AND STORAGE

Serum: Blood should be drawn using standard venipuncture techniques and serum separated from blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) and serum extracted. This kit is for use with serum samples without additives only.

- Avoid grossly hemolytic, lipidic or turbid samples.
- Serum samples to be used within 24-48 hours may be stored at 2-8°C otherwise samples must be stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- When performing the assay slowly bring samples to room temperature.
- It is recommended that all samples be assayed in duplicate.
- DO NOT USE HEAT-TREATED SPECIMENS.

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

1. **FSH Standards:** Reconstitute each FSH Standard vial with **0.6 mL** of distilled or deionized water. Allow each solution to sit for at least 15 minutes with gentle agitation. The FSH standard stock solutions are stable at 4°C for 3 months. Avoid freeze-thaw cycles.

2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table below for correct amounts of Substrate Solution to prepare.

Wells Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
16 wells	1.5	1.5	3.0
32 wells	3.0	3.0	6.0
48 wells	4.0	4.0	8.0
64 wells	5.0	5.0	10.0
80 wells	6.0	6.0	12.0
96 wells	7.0	7.0	14.0

ASSAY PROCEDURE

1. Prepare all FSH Standards before starting assay procedure (see Preparation Reagents). *It is recommended that all Standards and Samples be added in duplicate to the Microtiter Plate.*

2. First secure the desired number of coated wells in the holder, and then add 50 μL of Standards or samples to the appropriate well of the antibody pre-coated Microtiter Plate.

3. Add 100μL of Conjugate into each well. Mix the content by gently tapping the ELISA plate 10-20 times at the side. Cover and incubate for **1 hour at 37°C water bath**.

4. Prepare Substrate Solution no more than 15 minutes before end of incubation (see Preparation of Reagents).

5. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with distilled or de-ionized water then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

   Automated Washing: Aspirate all wells, then wash plates **FIVE times** using distilled or de-ionized water. Always adjust your washer to apirate as much liquid as possible and set fill volume at 350 μL/well/wash (range: 350-400μL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for soaking time of 10 seconds or shaking time of 5 seconds between washes.*

6. Add 100μL Substrate Solution to each well. Cover and incubate for **15 minutes at 37°C.**

7. Add 100μL of Stop Solution to each well. Mix well.

8. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 30 minutes.

CALCULATION OF RESULTS

This standard curve is used to determine the amount of follicle stimulating hormone (FSH) in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the six standard concentrations on the vertical (Y) axis versus the corresponding FSH concentration (mIU/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D. value for each standard and sample. All O.D. values are subtracted by the mean value of the zero-standard (0 mIU/mL) before result interpretation. Construct the standard curve using graph paper or statistical software.

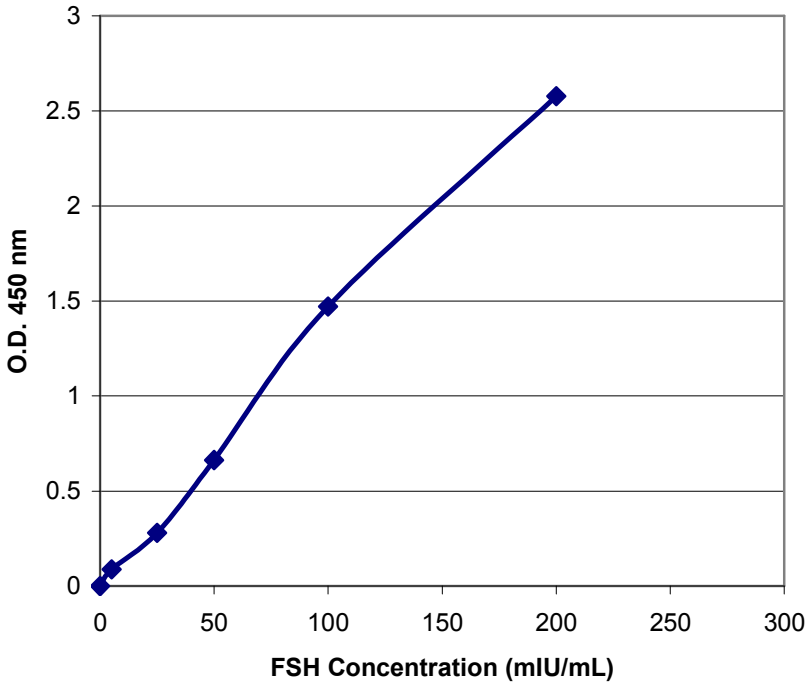
2. To determine the amount of FSH in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding FSH concentration.

TYPICAL DATA

Results of a typical standard run of FSH ELISA are shown. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain their own standard curve.

EXAMPLE
Results of a typical standard run are shown below:

FSH (mIU/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted
0	0.005, 0.010	0.007	0
5	0.097, 0.093	0.095	0.088
25	0.287, 0.286	0.287	0.280
50	0.681, 0.658	0.670	0.663
100	1.500, 1.454	1.477	1.470
200	2.663, 2.505	2.584	2.577



PERFORMANCE CHARACTERISTICS

1. **SENSITIVITY**
   The minimal detectable concentration of FSH by this assay is estimated to be 1.5 mIU/mL.

2. **SPECIFICITY**
   This kit exhibits no detectable cross-reaction with LH, HCG, TSH, and Prolactin. hGH can be detected in this assay.

3. **CALIBRATION**
   This immunoassay is calibrated against NIBSC/WHO, 3rd IS, HMG.

4. **HOOK EFFECT**
   In this assay, no hook effect is observed up to 5000 mIU/mL.

5. **EXPECTED NORMAL VALUES**
   Each laboratory must establish its own normal range based on patient population. The results provided below are based on random selected out-patient clinical laboratory samples

Sample	N	Range (mIU/mL)	Mean (mIU/mL)
Male	100	1.0-30	11.0
Female	150	1.0-40	12.0
Post Menopausal Female	60	45-180	94.0
Pregnant Female	60	0-15	1.0

CITATIONS

1. Q Xu et al. Br J Cancer. Isolation of tumour stem-like cells from benign tumours. Jul 21, 2009; 101(2): 303–311.

2. Akyol S, Cınar SA, Purisa S, Aydinli K. Relationship between lymphocytes, IL2 and the hormones E2, LH, PRG and FSH in menopausal and postmenopausal women. Am J Reprod Immunol. 2011 Oct;66(4):304-9.