

Instruction Manual No. M-200

Follicle Stimulating Hormone (FSH)

**For Quantitative Determination of FSH
In Human Serum**

For In Vitro Research Use Only

**Follicle Stimulating Hormone (FSH)
ELISA KIT Cat. No. 0200, 96 Tests**

Kit Components	Cat #
Anti-FSH coated strips, 96 wells,	201
FSH Std. A or Sample Diluent, 11 ml (0 mIU/ml)	202
FSH Std. B, 0.75 ml; (5 mIU/ ml)	203
FSH Std. C, 0.75 ml, (10 mIU/ ml)	204
FSH Std. D, 0.75 ml; (20 mIU/ ml)	205
FSH Std. E, 0.75 ml; (50 mIU/ ml)	206
FSH Std. F, 0.75 ml; (100 mIU/ ml)	207
All standards are calibrated to WHO STD 2 nd IRP HMG	
Anti-hFSH-HRP conjugate, 11 ml	208
HRP substrate Soln. A, 11 ml	200SA
TMB substrate Soln. B, 11 ml;	200SB
Stop solution, 10 ml,	T-10
Complete Instruction Manual,	M-200

Introduction

FSH and human luteinizing hormone (LH) are glycoprotein hormones with mol. wt. Of approx. 30 kDa. Each hormone is composed of 2 polypeptide chains, alpha and beta subunits. LH, FHS, TSH, and HCG share the same alpha subunits. The beta subunit structure differs among these hormones and determines specificity and biological action.

FSH and LH are secreted by the basophilic cells of the anterior pituitary in response to the Gonadotropin-releasing hormone (GnRH) produced by the hypothalamus. In both males and females, FSH and LH control the development and maintenance of the gonadal tissue, which synthesize and secrete steroid hormones. In females, FSH controls the developing ovarian follicles and, in males, FSH maintaining spermatogenesis in the testes with the aid of LH and testosterone. LH promotes secretion of estrogen and progesterone by the ovary and of testosterone by the testes. LH also triggers ovulation. These steroid hormones control the circulating levels of LH and FSH by a negative feedback effects on the hypothalamus. The roles of FSH and LH are thus interrelated and mutually potentiating and for this reason are routinely performed concurrently in the differential diagnosis of hypothalamic, pituitary or gonadal dysfunction. Additionally, the hormone levels are used to assess the menstrual cycle for ovulation timing and monitoring of ovulation induction, determination of menopause and for monitoring endocrine therapy.

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

Based on sixteen replicates of the zero standard, the minimum FSH concentration detectable using this assay is 0.9 mIU/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

2. PRECISION

Intra-assay precision:

Three serum samples (mean FSH concentrations: 13, 34, and 129 mIU/ml) were run in sixteen replicates in an assay. The samples showed good intra-assay precision with %CV of 6.2, 3.2, and 5.0, respectively.

Inter-assay precision:

Three serum samples were run in duplicate in sixteen independent assays. The samples showed good inter-assay precision (7-10 % CV). The actual values were: mean 13 mIU/ml, SD 0.93 mIU/ml, %CV 7; mean 33 mIU/ml, SD 2.41 mIU/ml, %CV 7.2; mean 126 mIU/ml, SD 15.1 mIU/ml, %CV 6.6, respectively.

3. RECOVERY

A known amount of FSH (50 and 100 mIU/ml) was added to two patient sera (with original FSH concentrations of 14 and 32 mIU/ml) and final FSH concentrations measured. The assay showed excellent mean recoveries of about 92.5% (range 91-97%).

4. LINEARITY

Three different patient samples (with original FSH concentrations of 18, 34, and 129 mIU/ml) were diluted (1:2, 1:5, and 1:10) with the zero standard and their final FSH values determined. The samples showed excellent mean recoveries of about 104% (range 93-118%).

5. HIGH DOSE HOOK EFFECT

FSH concentrations of up to 50000 mIU/ml did not show any hook effect

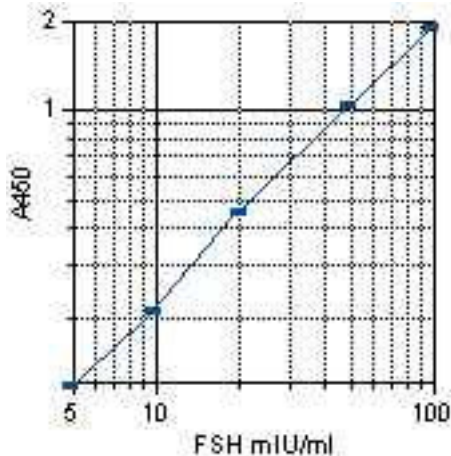
6. SPECIFICITY

The specificity of FSH kit was determined by measuring interference from high concentrations of hLH (up to 200 mIU/ml), hTSH (up to 50 uIU/ml), and HCG (up to 10000 mIU/ml). These hormones had a minimal interference in the FSH assay (0.01% or less)

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	A450 Net Abs.	Calculated Conc. (mIU/ml)
A1, A2	Std. A (0 mIU/ml)	0.009	
B1, B2	Std. B (5.0 mIU/ml)	0.122	
C1, C2	Std. C (10 mIU/ml)	0.218	
D1, D2	Std. D (20 mIU/ml)	0.468	
E1, E2	Std. E (50 mIU/ml)	1.055	
F1, F2	Std. F (100 mIU/ml)	1.947	
F1, F2	Sample 1	0.0340	15.0

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical standard assay curve (do not use this for calculating sample values)

PRINCIPLE OF THE TEST

FSH ELISA kit is based on simultaneous binding of human FSH from samples to two antibodies, one immobilized on microtiter well plates, and other conjugated to the enzyme horseradish peroxidase. After a washing step, chromogenic substrate is added and color developed. The enzymatic reaction (color) is directly proportional to the amount of FSH present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm. and FSH concentrations in samples and control are read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (20-100 μ l) and Multichannel pipet with disposable plastic tips. Reagent troughs, Plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The FSH ELISA test is intended for *in vitro research use* only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions. The Controls Serum has been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site. TMB (substrate), H₂SO₄ (stop solution), and Proclin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates). All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum.. If sera cannot be immediately assayed, these could be stored at -20°C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4°C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions.

Once opened/used standards are stable for two month at 2-8°C. The unused portions of the standards should be frozen in suitable aliquots for long-term use. Repeated freezing and thawing is not recommended.

TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. Dispense 200-300 µl of wash buffer to all wells. Mix for 5 seconds and discard or aspirate the solution. The step should be done just before adding the samples, do not allow the wells to dry at any time during the assay.

1. Label or mark the microtiter well strips to be used on the plate.
2. Pipet 50 µl of standards, control, and serum samples into appropriate wells in *duplicate*.
3. Pipet 100 µl of Ab-enzyme conjugate into each well.. Mix gently
4. Cover the plate and incubate for **60 minutes** at room temperature.
5. Aspirate and wash the wells 3-5 times with 300 µl of running water or distilled water. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
6. Pipet 100 µl of TMB substrate Soln A followed by 100 µl of Soln B at timed intervals into each well. Mix gently. Alternatively, required volume of soln A and B can be mixed in a separate tube and add 200 µl/well (blue color develops).
7. Cover the plate and incubate for **30 minutes** at room temperature.
8. Stop the reaction by adding 50 µl of stopping solution to all wells at the same timed intervals as in step 7 (color turns yellow). Mix gently.
9. Measure the absorbance at 450 nm using an ELISA reader. Color is stable for at least 30 min after stopping.

DILUTION OF SAMPLES

Serum samples do not usually require dilution. However, if dilution is desired, the zero standard (standard A) must be used and the results obtained should be multiplied by the appropriate dilution factor.

LIMITATIONS

1. This kit is for in vitro research use only. The FSH values should be used in an adjunct to other data available.
2. FSH values have been reported to be affected by estrogen and certain drug therapies and specimens from such patient should be interpreted with caution.
3. Due to extremely high concentration of HCG in pregnant women, measurement of similar hormones such as FSH and LH may yield falsely elevated results.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standard from the mean absorbance values of standards, control, and samples. Draw the standard curve on log-log graph paper by plotting net absorbance values of standards against appropriate FSH concentrations. Read off the FSH concentrations of the control and patient samples.

EXPECTED VALUES

1. The differences in assay techniques and a variety standard preparation used, it is advisable for each lab to establish their own normal values.
2. Plasma levels of FSH and LH in women vary with the menstrual cycle. FSH levels rise slightly and then decline progressively during the early follicular phase, whereas as LH levels are relatively stable. An abrupt rise in LH at midcycle, initiated by increasing estrogen secretion by the developing follicle and accompanied by FSH rise, triggers ovulation. Both hormone levels decline during the luteal phase. Levels of FSH and LH in males are similar to those in females during follicular phase. FSH and LH increase in response to age-related decrease in gonadal functions in both sexes. In women, this occurs at menopause, and in men a gradual increase is seen during the sixth to eight decade.

Female Follicular mid-Cycle	5-20 mIU/ml
Luteal	15-35 mIU/ml
Post Menopausal	5-20 mIU/ml
Male	40-120 mIU/ml