ELISA kits available from ADI (see details at the web site)

#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	**#1200**	Human Albumin (Urinary)
#1750	Human IgG (total)	**#1760**	Human IgM
#1800	Human IgE	**#1810**	Human Ferritin
#1210	Human Transferrin (Tf)	**#0020**	Beta-2 microglobulin
#1600	Human Growth Hormone (GH)		

#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	**#1830**	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	**#1510**	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		

#0030	Human Insulin	**#0040**	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Follicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	#**0410**	HCG-free beta

#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	**#1110**	Human Free T4 (fT4)
#1650	Human free triiodothyronine (fT3)	**#1700**	Human T3 (total)

#1850	Human Cortisol	**#1860**	Human Progesterone
#1865	Human Pregnolone	**#1875**	Human Aldosterone
#1880	Human Testosterone	**#1885**	Human free Testosterone
#1910	Human Androstenedione	**#1920**	Human Estradiol
#1925	Human Estrone	**#1940**	Dihydrotestosterone (DHT)
#1950	Human DHEA-sulphate (DHEA-S)		
#3400	Human serum Neopterin		

#3000	Human Rheumatoid Factors IgM (RF)
#3100	Human anti-dsDNA
#3200	Anti-Nuclear Antibodies (ANA)

Instruction Manual No. M-200

Follicle Stimulating Hormone (FSH)

ELISA KIT Cat. No. 0200, 96 Tests

For Quantitative Determination of FSH In Human Serum

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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

Kit Components	Cat #
Anti-FSH monoclonal antibody coated strips, 96 wells,	201
FSH **Std. A** , (0 IU/L), **2 ml**	202A
FSH **Std. B**, (5 IU/L), 0.50 ml	202B
FSH **Std. C**, (10 IU/L), 0.50 ml	202C
FSH **Std. D**, (25 IU/L), 0.50 ml	202D
FSH **Std. E**, (50 IU/L), 0.50 ml	202E
FSH **Std. F**; (100 IU/L), 0.50 ml	202F
FSH **Low & High controls**, 0.5 ml/vial, exact values printed on vial, #LC-200, #HC-200	
Anti-hFSH-**HRP conjugate (51X)**, 300 µl	203
HRP **substrate Solution** (ready-to-use) ; 16 ml	TMB-200
Assay Buffer 25 ml	204
Wash buffer (10X), 50 ml (dilute 1:10 with distilled water, **50 ml stock to 450 ml dH2O**)	W B - 1 0
Stop solution, 6 ml,	T-10
Complete Instruction Manual,	M-200

Intended Use:

ADI's Follicle Stimulating Hormone (FSH) for Quantitative Determination of FSH In Human Serum. **For In Vitro Research Use Only (RUO).**

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 1 IU/L. 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: 7.41, 48.57, and 138.12 IU/L) were run in sixteen replicates in an assay. The samples showed good intra-assay precision with %CV of 5.8, 3.5, and .3.4 respectively.

Inter-assay precision: Three serum samples were run ten times over a period of 4 weeks.

Sample	Mean	SD	CV%
1	7.11	0.24	3.4
2	44.31	2.01	4.5
3	120.63	7.74	7.7

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 IU/L did not show any hook effect

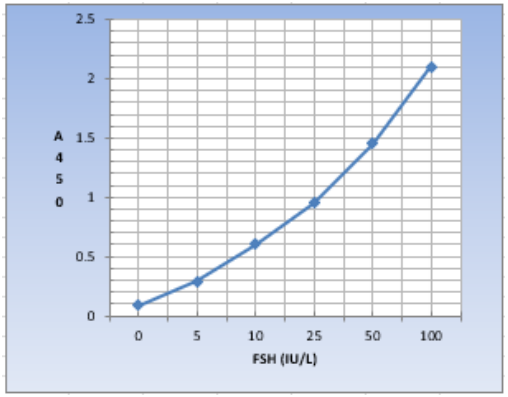
6. SPECIFICITY

The specificity of FSH kit was determined by measuring interference from high concentrations of hLH (up to 200 IU/L), hTSH (up to 50 uIU/ml), and HCG (up to 10000 IU/L). 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. (IU/L)
A1, A2	**Std. A** (0 IU/L)	0.072	
B1, B2	**Std. B** (5.0 IU/L)	0.214	
C1, C2	**Std. C** (10 IU/L)	0.342	
D1, D2	**Std. D** (25 IU/L)	0.602	
E1, E2	**Std. E** (50 IU/L)	1.397	
F1, F2	**Std. F** (100 IU/L)	2.372	
F1, F2	**Sample 1**	0.266	7.2

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 std curve (Plot linear graph Draw the best curve through the points. Do not use this for calculating sample values).

Performance Characteristics

A total of 90 sera were tested by this ELISA and a reference ELISA kit.

Correlation	Slope	Intercept
0.97	0.95	0.37

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 µl) and Multichannel pipet with disposable plastic tips. Reagent troughs, Plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The Alpha Diagnostic International 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 Prolcin-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^{O}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^{O}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.

Preparation of the reagents:

Dilute wash buffer (1:10) with distilled water (50 ml stock in 450 ml. Store at **4°C**.
Prepare 1X solution **HRP conjugate**. **Dilute 40 µl stock conjugate in 2 ml of assay buffer**. (**240 µl in 12 ml** for complete 96-well plate).

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8ºC 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. **Dilute wash buffer (1:10) (50 ml stock to 450 ml of distilled or deionized water). Dilute stock conjugate in assay buffer**.

1. Label or mark the microtiter well strips to be used on the plate.
2. Pipet **25 µl** of standards, control, and serum samples into appropriate wells in *duplicate*.
3. Pipet **100 µl** of assay buffer into each well**.**. Mix gently
4. Cover the plate and incubate for **30 minutes** on plate shaker (200 rpm) at room temperature.
5. Aspirate and wash the wells **3 times with 300 µl of 1 X wash buffer**. 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 working HRP conjugate into each well**.**. Mix gently Cover the plate and **incubate on plate shaker for 30 minutes** at room temperature.
7. Aspirate and wash the wells **3 times with 300 µl of 1 X wash buffer**
8. Dispense **100 ul TMB substrate per well.** Mix gently.
9. Cover the plate and incubate for **15-20 minutes** on plate shaker at room temperature. Note: The incubation time at this step can be changed within a few minutes so as so to produce the maximum color (after adding the stop solution to about 2.5-3.00 or within the readable range of the ELISA reader. It will not impact the sample.
10. 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.
11. 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.

LIMITATATIONS

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

Check FSH standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. To construct the standard curve, plot the absorbance for the FSH standards (vertical axis) versus the FSH standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or 3. Sensitivity unknown sample.

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.

Species Cross reactivity

The antibodies (anti-human FSH) used this kit has not been tested for potential cross-reactive with FSH from species like monkey, mouse, and rat etc. The kit may work in other species.