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FSH ELISA Kit

Name	FSH ELISA Kit
INTENDED USE	The FSH SA ELISA Kit is intended for the quantitative measurement of FSH in human serum.
SUMMARY AND EXPLANATION	Follicle-Stimulating Hormone (FSH) is a glygoprotein produced by the anterior pituitary gland. 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 follicular growth, prepares ovarian follicles for action by LH and enhances the LH induced release of estrogen. FSH levels are elevated after menopause, castration and in premature ovarian failure. Although there are significant exceptions ovarian failure is indicated when random FSH concentrations exceed 40 mIU/ml. In the male, FSH stimulates seminiferous tubule and testicular growth and is involved in the early stages of spermatogenesis. Oligospermic males usually have elevated FSH levels. Tumors of the testes generally depress serum FSH concentrations, but levels of LH are elevated. 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.
PRINCIPLE OF THE TEST	The FSH ELISA kit is a solid phase assay using streptavidin/biotin method. The samples and Anti-FSH/Anti-Biotin conjugate are added to the wells coated with Streptavidin. FSH in the patient’s serum forms a sandwich between specific antibodies labeled with biotin and HRP. Unbound protein and HRP conjugate are washed off by

wash buffer. Upon the addition of the substrate, the intensity of color is proportional to the concentration of FSH in the samples. A standard curve is prepared relating color intensity to the concentration of the FSH.

MATERIALS PROVIDED	96 Tests
Microwells coated with Streptavidin	12x8x1
FSH Standard: 6 vials (ready to use)	0.5ml
FSH Enzyme Conjugate: 1 bottle (ready to use)	12 ml
TMB Substrate: 1 bottle (ready to use)	12ml
Stop Solution: 1 bottle (ready to use)	12ml
20X concentrate: 1 bottle	25ml

MATERIALS NOT PROVIDED

Distilled or deionized water
Precision pipettes
Disposable pipette tips
ELISA reader capable of reading absorbance at 450nm
Absorbance paper or paper towel
Graph paper

STORAGE AND STABILITY

1. Store the kit at 2 - 8 C.
Keep microwells sealed in a dry bag with desiccants.
The reagents are stable until expiration of the kit.
Do not expose reagents to heat, sun, or strong light.

WARNINGS AND PRECAUTIONS

Potential biohazardous materials:
The Standard contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and

	Biomedical Laboratories." 1984 Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed. It is recommended that standards, control and serum samples be run in duplicate Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.
SPECIMEN COLLECTION HANDLING	Collect blood specimens and separate the serum immediately. Specimens may be stored refrigerated at (2-8 C) for 5 days. If storage time exceeds 5 days, store frozen at (-20 C) for up to one month. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well. Do not use grossly lipemic specimens.
REAGENTS PREPARATION	20X Buffer: Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25C).
ASSAY PROCEDURE	Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use. Place the desired number of coated strips into the holder Pipette 50 l of FSH standards, control and patient’s sera in to selected wells. Add 100 l of enzyme conjugate to all wells. Cover the plate and incubate for 60 minutes at room temperature (18-26 C). Remove liquid from all wells. wells three times with 300 l of 1X wash buffer. Blot on absorbent paper towels.

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Add 100 l of TMB substrate to all wells.

Incubate for 15 minutes at room temperature.

Add 50 l of stop solution to all wells. Shake the plate gently to mix the solution.

Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

CALCULATION OF RESULTS

The standard curve is constructed as follows:

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. See example of the standard attached.

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 unknown sample.

Example of a standard Curve

	Conc. mIU/mL	OD 450 nm
Std 1	0	0.09
Std 2	5	0.20
Std 3	10	0.32
Std 4	25	0.69
Std 5	50	1.31
Std 6	100	2.46

LIMITATIONS OF THE TEST

The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient’s history, physical findings and other diagnostic procedures.

Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

EXPECTED VALUES

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values may be used as initial guideline ranges only:

Classification	(mIU/ml)
Male	2.0-15

Female	
Follicular/Luteal phase	2.0-10
Mid-cycle	2.0-20
Pregnant	Less than 2.0
Postmenopausal	Greater than 15

PERFORMANCE CHARACTERISTICS

Correlation with a Reference ELISA kit:

A total of 89 sera were tested by this ELISA and a reference ELISA kit. Results were as follows:

Correlation	Slope	Intercept
0.97	0.95	0.37

2. Precision

Intra-Assay

Serum	No. of Replicates	Mean mIU/ml	Standard Deviation	Coefficient of Variation (%)
1	16	9.6	0.6	6.3
2	16	21.8	1.22	5.6
3	16	49.6	3.3	6.7

Inter-assay

Serum	No. of Replicates	Mean mIU/ml	Standard Deviation	Coefficient of Variation (%)
1	16	9.2	0.63	6.8
2	16	20.9	1.33	6.4
3	16	50.5	3.12	6.2

Sensitivity

The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 20 times in the same run.

Serum	No. of Replicates	Mean mIU/ml	Standard Deviation	Mean + 2SD (Sensitivity)
Zero	20	0.102	0.122	0.353

Standard				

Recovery

Known quantities of FSH were added to a serum that contained a low concentration of FSH.

Expected Value(mIU/ml)	Recovered (mIU/ml)	Percentage of Recovery
49	54	110
89	87	98

Linearity

Two different patient samples were diluted with the “0” calibrator to 1:2, 1:4 and 1:8. FSH values were assayed and results were corrected with the dilution factor. The results of these dilution tests are as follows:

Serum	Original Value (mIU/ml)	Percentage of Recovery	Percentage of Recovery	Percentage of Recovery
1 2	94.7 29	1:2	1:4	1:8
1 2	94.7 29	112 95	115 93	96 103

REFERENCES:

Qiu Q; Kuo A; Todd H; Dias JA; Gould JE; Overstreet JW; Lasley BL. Enzyme immunoassay method for total urinary follicle-stimulating hormone (FSH) beta subunit and its application for measurement of total urinary FSH. Fertil Steril 1998; 69(2):278-85.

Ulloa-Aguirre A; Timossi C. Structure-function relationship of follicle-stimulating hormone and its receptor. Hum Reprod Update 1998; 4(3): 260-83.

Desai MP; Khatkhatay MI; Sankolli GM; Joshi UM. Importance of selection of separation system in the development of enzyme immunoassay: an experience with follicle stimulating hormone (FSH) assay. J Immunoassay, 12(1):83-98 1991

Nordin BE; Morris HA; Need AG; Horowitz M; Robertson WG. Relationship between plasma calcium fractions, other

bone-related variables, and serum follicle-stimulating hormone levels in premenopausal, perimenopausal, and postmenopausal women. Am J Obstet Gynecol 1990;163(1 Pt 1):140-5.

Rose MP. Follicle stimulating hormone international standards and reference preparations for the calibration of immunoassays and bioassays. Clin Chim Acta 1998; 273(2):103-17.

Popovic V; Micic D; Damjanovic S; Calovic L; Rolovic Z; Mijovic A; Petakov M; Manojlovic D; Micic J. Further evidence for differential regulation of follicle-stimulating hormone (FSH) and luteinizing hormone (LH): increased FSH and decreased LH levels in a patient with familial pure gonadal dysgenesis. Postgrad Med J 1992; 68(805):925-7.