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Follicle Stimulating Hormone [FSH] (human) ELISA Kit

(Catalog # K7425-100, 100 assays; Store at 2-8^{0}C)

I. Introduction:

Follicle Stimulating Hormone (FSH) is a glygoprotein produced by the anterior pituitary gland. 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. BioVision's human FSH kit is a solid phase sandwich ELISA Kit. The samples, and anti-FSH-HRP conjugate are added to the wells coated with monoclonal antibody to FSH beta subunit. FSH in the sample binds to anti-hFSH MAb on the well and the anti-FSH-HRP second antibody then binds to FSH. 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.

II. Application:

Quantitative protein detection, establishing normal range etc.

III. Specificity:

Human FSH.

IV. Sample Type:

- Serum

V. Kit Contents:

Components	K7425-100	Part No.
Plate coated with FSH MAb	12 stripsx8 wells	K7425-100-1
FSH Standard	6 x 0.5 ml	K7425-100-2.x
FSH Enzyme Conjugate	12 ml	K7425-100-3
Wash Concentrate (20X)	25 ml	K7425-100-4
TMB Substrate	12 ml	K7425-100-5
Stop Solution	12 ml	K7425-100-6

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips.

VII. Storage Conditions and Reagent Preparation:

Store kit at 2-8^{o}C. Keep microwells sealed in a dry bag with desiccants. Spin tubes briefly to bring down all components to the bottom of tubes. Reagents are stable until the expiration of the kit. Do not expose reagent to heat, sun, or strong light.

- **Wash Concentrate:** 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 (18-26°C).

VIII. Warning & 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, 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.
- This test kit is USA FDA exempt product.
- Do not pipette by mouth.
- 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.

IX. Sample Preparation and Storage:

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

X. Assay Protocol:

Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use. Check hFSH standard value on each standard vial. This value miFSHt vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.

1. Place the desired number of coated strips into the holder
2. Pipet 50 µl of FSH standards, control and sample into designated wells.
3. Add 100 µl of enzyme conjugate to all wells.
4. Cover the plate and incubate for 60 min. at room temperature (18-26° C).
5. Remove liquid from all wells & wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
6. Add 100 µl of TMB substrate to all wells & incubate for 15 min. at room temperature.
7. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.

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

XI. Calculation: Construct the standard curve, plot the absorbance for the FSH standards (vertical axis) versus the FSH standard concentrations (horizontal axis). 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:

Standard	OD (450 nm)	Part No.
Standard 1 (0 mIU/ml)	0.013	K7425-100-2.1
Standard 2 (5 mIU /ml)	0.240	K7425-100-2.2
Standard 3 (10 mIU /ml)	0.510	K7425-100-2.3
Standard 4 (25 mIU /ml)	1.107	K7425-100-2.4
Standard 5 (50 mIU /ml)	1.669	K7425-100-2.5
Standard 6 (100 mIU /ml)	2.379	K7425-100-2.6

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	Normal Range (mIU/ml)
Males	2.0-15
Females	
Follicle/Luteal phase	2.0-10
Mid-cycle	2.0-20
Pregnant	Less than 2
Postmenopausal	Greater than 15

Performance Characteristics

Correlation with a reference ELISA kit: A total of 89 sera were tested by this ELISA & a reference ELISA kit. Results are as follows:

Correlation	Slope	Intercept
0.97	0.95	0.37

Precision

Intra-Assay

Sample	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

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

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

Recovery: known quantities of hFSH were added to a serum that contained a low conc. of FSH.

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

Linearity: Two different 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		
		1:2	1:4	1:8
1	94.7	112	115	96
2	29	95	93	103

XII. RELATED PRODUCTS:

LH (human) ELISA Kit (K7426)
Prolactin (human) ELISA Kit (K4687)
FSH, human (4781)
Chorionic Gonadotropin (hCG) (human) ELISA Kit (K7424)
Prolactin (mouse/rat) ELISA Kit (K4688)

FOR RESEARCH USE ONLY! Not to be used on humans.