

CALDON BIOTECH INC.
Follicle Stimulating
Hormone (FSH)
ELISA

Catalog No. FS046F
 (96 tests)

INTENDED USE

The FSH ELISA kit is used for the quantitative measurement of FSH in human serum or plasma.

SUMMARY AND EXPLANATION

Follicle-Stimulating Hormone (FSH) is a glycoprotein 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 is a solid phase direct sandwich ELISA method. The samples and diluted anti-FSH-HRP conjugate are added to the wells coated with Mab to FSH beta subunit. FSH in the patient's serum binds to anti-FSH 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.

MATERIALS PROVIDED

1. Microwells coated with FSH MAb (12x8x1 wells). 96 wells.
2. FSH Standard: 6 vials (0.7 mL each). Ready to use.

3. Enzyme Conjugate: 1 bottle (12 mL). Ready to use
4. TMB Substrate: 1 bottle (12 mL). Ready to use.
5. Stop Solution: 8 mL. Ready to use.
6. 10X Wash Concentrate: 1 bottle (50 mL).

STORAGE AND STABILITY

1. Store the kit at 2 - 8 °C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.

WARNINGS AND PRECAUTIONS

Potential biohazardous materials:

1. The calibrator and controls 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
2. This test kit is designed for in vitro diagnostic use only.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that standards, control and serum samples be run in duplicate.
6. 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

1. Collect blood specimens and separate the serum immediately.
2. 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.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.

- Do not use grossly lipemic specimens.

REAGENTS PREPARATION

10X Wash Buffer Concentrate: To prepare working wash buffer, add the contents of the bottle to 450 ml of distilled water. Store at room temperature.

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
- Pipet 50 µL of FSH standards, control and patient's sera.
- Add 100 µL of enzyme conjugate to all wells.
- Cover the plate and incubate for 30 minutes at room temperature (18-26 °C).
- Remove liquid from all wells. Fill wells with working wash buffer. Wash three times. Blot on absorbent paper towels.
- Add 100 µL of TMB substrate to all wells.
- Incubate for 10 minutes at room temperature.
- Add 50 µL of stop solution to all wells. Shake the plate gently to mix the solution.
- Read absorbance on ELISA Reader at 450 nm within 20 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.

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)
<i>Males</i>	2.0-15
<i>Female</i>	
Follicular/Luteal	2.0-10
Phase Mid-cycle	2.0-20
Pregnant	Less than 2.0
Postmenopausal	Greater than 15

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.

PERFORMANCE CHARACTERISTICS

1. 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
0.92	0.85	0.3

2. Precision

Intra-Assay

Serum	No. of Replicates	Mean IU/mL	Standard Deviation	Coefficient of Variation%
High	16	42	2.9	6.9
Normal	16	19	1.4	7.3
Low	16	8	0.6	7.5

Inter-assay

Serum	No. of Replicates	Mean IU/mL	Standard Deviation	Coefficient of Variation%
High	10	39	3.1	7.9
Normal	10	21	1.7	8.1
Low	10	7	0.8	11.4

3. 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 IU/mL	Standard Deviation	Mean + 2SD (Sensitivity)
Zero Standard	20	1.5	0.3	2.1 mIU/mL

4. 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
5.5	5.3	96
16.4	16.9	103
30.7	28.6	93

5. 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		
		1:2	1:4	1:8
1	44	97	102	94
2	29	95	93	103

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