

2. Precision

Serum	No. of Replicates	Mean mIU/ml	Standard Deviation	Coefficient of Variation (%)
1	16	0.12	0.014	11.6
2	16	0.363	0.023	6.3
3	16	0.769	0.04	5.2

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 mIU/ml	Standard Deviation	Mean + 2SD (Sensitivity)
Zero Standard	20	0.003	0.0014	0.0058 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)	% of Recovery
2.5	2.3	92
10	11	110
20	19.2	96

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	2	96	101	93
2	5	94	97	102

REFERENCES

- Offie P Soldin; Eve G. Hoffman A. Waring, Steven J. Soldin. Pediatric refrance intervals for FSH, LH, Estradiol, T3, Cortisol and growth hormone on the DPC Immulonite 1000. Clinica Chemica Acta 355 (2005) 205-210.
- 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;
- 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.

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Cat#: FS551F (96 Tests)

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Ultra Sensitive Follicle Stimulating Hormone (FSH) lumELISA

Catalog No. : FS551F (96 Tests)

INTENDED USE

The Calbiotech, Inc. (CBI) FSH lumELISA (Chemiluminescence Enzyme Linked Immunosorbent Assay) 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.

In children, abnormalities in concentration of FSH can be aid in the diagnosis of pituitary disorders, and may be indicative of problems in the reproductive system of both genders, infertility problems, early and delay puberty.

PRINCIPLE OF THE TEST

The FSH is a solid phase direct sandwich LUMELISA 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 enzyme activities are proportional to the concentration of FSH in the samples. A standard curve is prepared relating light units to the concentration of the FSH.

MATERIALS PROVIDED	96 Tests
Microwell coated with FSH MAb	6x8x2 (96)
FSH Standard: 7 vials (ready to use)	0.2 ml
Enzyme Conjugate, 20X: 1 vial	0.7 ml
Assay Diluent, 1 bottle (ready to use)	12 ml
Luminol Substrate, 3X: 1 bottle	4 ml
Luminol Buffer: 1 bottle	8 ml
Wash Concentrate, 20X: 1 bottle	25 ml

MATERIALS NOT PROVIDED

- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- Microplate luminometer
- Absorbance paper or paper towel
- Graph paper

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.
4. Do not expose reagents to heat, sun, or strong light.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials:
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 USA FDA exempt product.
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.
5. Do not use grossly lipemic specimens.

REAGENTS PREPARATION

1. 20X Enzyme Conjugate: Prepare 1X working dilution at 1:20 with assay diluent as needed, e.g. 0.1 ml of the stock conjugate in 1.9 ml of assay diluent is sufficient for 20 wells. The diluted conjugate has to be used the same day.
2. 20X Wash Buffer Concentrate: Prepare 1X wash buffer by adding the contents of the bottle to 475 ml of distilled water. Store 1X wash buffer at room temperature.
3. 3X Luminol Substrate: Prepare 1X Substrate solution by adding 1 part of Luminol to 2 parts Luminol buffer as needed.

ASSAY PROCEDURE

Prior to assay, allow reagents to stand at room temperature.
Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder
2. Add 25 µl of FSH standards, control and patient's sera into selected wells.
3. Add 100 µl of 1X Enzyme Conjugate to all wells.
4. Cover the plate and incubate for 60 minutes at room temperature (18-26° C) with shaking.
5. Remove liquid from all wells. Wash wells five times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
6. Add 100 µl of 1X Luminol substrate to all wells.
7. Read the relative light units in each well using Luminometer (0.2-1 second integration time) within 5 min of substrate addition.

Note: Loss of sensitivity in low range standards may be observed if the wait time is more than 10 minutes after adding the substrate

CALCULATION OF RESULTS

The standard curve is constructed as follows:

1. 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.
2. To construct the standard curve, plot the RLU (Relative Light Units) for each FSH standard point (vertical axis) versus the FSH standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the concentration for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Example of Standard Curve

	RLU	Conc. mIU/mL
Std 1	7883	0
Std 2	33396	0.05
Std 3	130666	0.2
Std 4	760970	1
Std 5	1541193	2
Std 6	8045890	10
Std 7	32608776	40

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:

	Male (mIU/ ml)	Female (mIU/ ml)
Adult	1.48-14.26	Follicular phase: 1.37-9.90
		Mid-cycle : 6.17-17.20
		Luteal phase : 1.09-9.20
		Post menopausal : 14.9-124.30
Children		
2 weeks	1.22-5.19	2.09-30.45
1-18 months	0.19-2.97	1.14-14.35
19 months – 7 y	0.25-1.92	0.70-3.39
8-9 y	0.30-1.67	0.28-5.64
10-11 y	0.20-5.79	0.68-7.26
12-14 y	0.23-10.3	1.02-9.24
15-18 y	0.81-8.18	0.33-10.54

LIMITATIONS OF THE TEST

1. 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.
2. 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
0.95	0.91	0.2