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Product Information

Follicle Stimulating Hormone (FSH) ELISA

Catalog Number **SE120057**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

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.

The Follicle Stimulating Hormone (FSH) ELISA Kit is intended for the quantitative measurement of FSH in human serum.

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 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.

Components

MATERIALS PROVIDED	96 Tests
Microwells coated with Streptavidin	12 x 8 x 1
FSH Standard: 6 vials (ready to use)	0.5 mL
FSH Enzyme Conjugate: 1 bottle (ready to use)	12 mL
TMB Substrate: 1 bottle (ready to use)	12 mL
Stop Solution: 1 bottle (ready to use)	12 mL
20x Wash concentrate: 1 bottle	25 mL

Reagents and Equipment Required but Not Provided.

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

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

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.

Reagent preparation
Prepare 1× Wash buffer by adding the contents of the bottle (25 mL, 20×) to 475 ml of distilled or deionized water. Store at room temperature (18–26 °C).

Storage/Stability
Store the kit at 2–8 °C.

Procedure
Notes: 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, controls, and serum samples be run in duplicate

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

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.

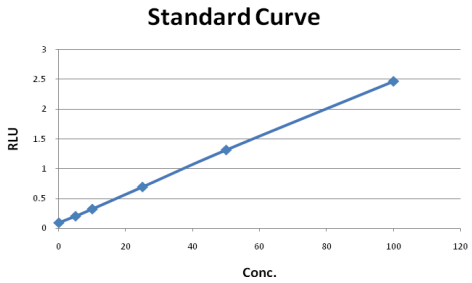
Bring all specimens and kit reagents to room temperature (18–26 °C) and gently mix.

1. Place the desired number of coated strips into the holder.
2. Pipette 50 µL of FSH standards, control, and sera into selected wells.
3. Add 100 µL of Enzyme Conjugate to all wells.
4. Cover the plate and incubate for 60 minutes at room temperature (18–26 °C).
5. Remove liquid from all wells. Wash wells 3 times with 300 µL of 1× Wash buffer. Blot on absorbent paper towels.
6. Add 100 µL of TMB Substrate to all wells.
7. Incubate for 15 minutes at room temperature.
8. Add 50 µL of Stop Solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the Stop Solution.

Results
Calculations
The standard curve is constructed as follows:
1. Check FSH standard value on each standard vial. This value might vary from lot to lot.
2. 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.
3. 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

	Concentration 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



Note: 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.

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

References

1. Qiu, Q. et al., Enzyme immunoassay method for total urinary follicle-stimulating hormone (FSH) beta subunit and its application for measurement of total urinary FSH. Fertil. Steril., **69**(2), 278-85 (1998).
2. Ulloa-Aguirre, A., and Timossi, C., Structure-function relationship of follicle-stimulating hormone and its receptor. Hum. Reprod. Update, **4**(3), 260-83 (1998).
3. Desai, M.P. et al., 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).
4. Nordin, B.E. et al., 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., **163**(1 Pt 1), 140-5 (1990).
5. Rose, M.P., Follicle stimulating hormone international standards and reference preparations for the calibration of immunoassays and bioassays. Clin. Chim. Acta, **273**(2), 103-17 (1998).
6. Popovic, V, et al., 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., **68**(805), 925-7 (1992).

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