ORIGENE

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Product Information

Follicle Stimulating FSH ELISA kit

Streptavidin Format

Catalog Number: EA100841
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use
The FSH ELISA Kit is intended for the quantitative measurement of FSH in human serum.

Background
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.

Components

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

Materials and Equipment Required but Not Provided

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

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.

Reagent Preparation

20X Wash 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 (18-26°C).

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C).
- 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.
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

1. Place the desired number of coated strips into the holder
2. Pipette 50 µl of FSH standards, control and patient's sera in to 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 three times with 300 µl of 1X 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 stopping solution.

Calculation of Results

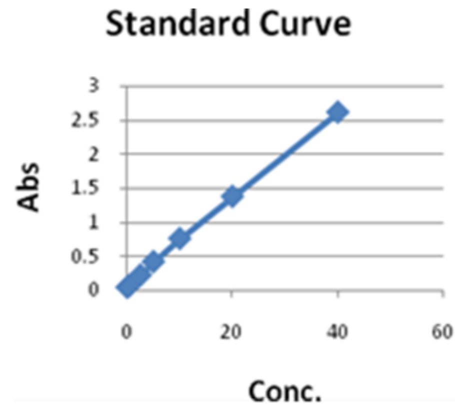
The standard curve is constructed as follows:

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 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

	Conc. mIU/mL	OD450 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



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)
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

References

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6. 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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