Follicle Stimulating Hormone (FSH)

ENZYME IMMUNOASSAY TEST KIT

Enzyme Linked Immunosorbent Assay (ELISA) for Quantitative Determination of Follicle Stimulating Hormone (FSH) in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

FSH Sandwich ELISA test is intended for the quantitative determination of Follicle Stimulating Hormone (FSH) in human serum. For In Vitro Diagnostic Use only.

INTRODUCTION

Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) are intimately involved in the control of the growth and reproductive activities of the gonadal tissues, which synthesize and secrete male and female sex hormones. The levels of circulating FSH and LH are controlled by these sex hormones through a negative feedback relationship.

FSH is a glycoprotein secreted by the basophilic cells of the anterior pituitary. Gonadotropin-release hormone (GnRH), produced in the hypothalamus, controls the release of FSH from anterior pituitary. 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 the growth and maturation of ovarian follicles by acting directly on the receptors located on the grannulosa cells; follicular steroidogenesis is promoted and LH production is stimulated. The growth of the seminiferous tubules and maintenance of spermatogenesis in men are regulated by FSH. Tumors of the testes generally depress serum FSH concentrations. 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 ASSAY

FSH Quantitative Test Kit is a sandwich-based enzyme-linked immunosorbent assay. The test employs polyclonal anti-FSH antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-FSH antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in the FSH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound labeled antibodies. A solution of TMB is added resulting in the development of a blue color. The color development is stopped by addition of stop solution and absorbance is determined for each well using an ELISA reader. The concentration of FSH is directly proportional to the color intensity of the test sample.

MATERIALS AND COMPONENTS

Materials provided with the test kits:

- Coated Microwells: Microwells coated with anti- FSH antibody.
- FSH Enzyme Conjugate. Ready to use.
- TMB Substrate. Ready to use
- Stop Solution. Ready to use
- FSH Standard set of 6 standards labeled as A to F in liquid form. Ready to use. For standard Concentrations refer vial label.
- Wash Buffer Concentrate (20X)
- Control set
- Pack Insert
- Plate Sealers
- Protocol Sheet
- Microwell holder

Materials required but not provided

- Precision pipettes: 10-100µl, 20-200µl, 100-1000µl
- Disposable pipette tips
- Distilled water
- Disposable Gloves
- ELISA reader
- ELISA washer

STORAGE AND STABILITY

1. **FSH** kit is stable at 2-8°C upto expiry date printed on the label.
2. Coated microwells should be used within one month upon opening the pouch provided that once opened, the pouch must be resealed to protect from moisture. If the colour of the dessicant has changed from blue to pink at the time of opening the pouch, another coated microwells pouch should be used.
3. Diluted Wash Buffer is stable for upto one week when stored at 2-8°C.

SPECIMEN COLLECTION

1. Collect Blood specimen by venipuncture according to the standard procedure.
2. Only serum should be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However, specimens can be stored up to 48 hours at 2-8°C.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.
7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
8. Specimen should be free from particulate matter and microbial contamination.

PRECAUTIONS

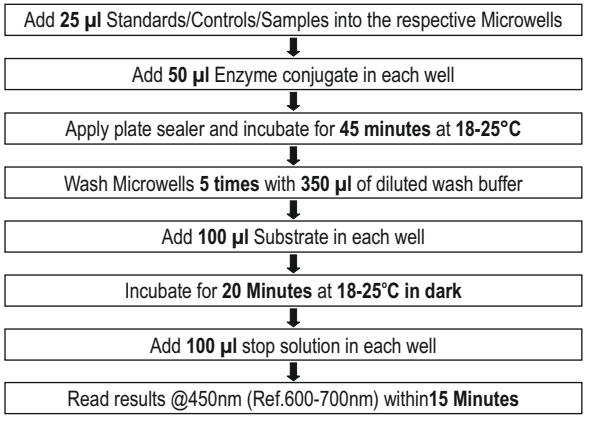
1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not eat, drink or smoke in the area where testing is done.
4. Use protective clothing and wear gloves when handling samples.
5. Use absorbent sheet to cover the working area.
6. Immediately clean up any spills with sodium hypochlorite.
7. All specimens, standards and controls should be considered potentially infectious and discarded appropriately.
8. Neutralize acid containing waste before adding hypochlorite.
9. Do not use kit after the expiry date.
10. Do not mix components of one kit with another.
11. Always use new tip for each specimen and reagent.
12. Do not allow liquid from one well to mix with other wells.
13. Do not let the strips dry in between the steps.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. Dilute Wash Buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.

TEST PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense **25 µl** of standards, controls and sera into the appropriate wells.
2. Dispense **50 µl** of Enzyme Conjugate into each well. Incubate at room temperature (18-25°C), for **45 minutes**.
3. After incubation, empty the microtitre wells and wash the plate **5 times** with **350 µl** of diluted wash buffer. Strike the microtitre plate sharply onto the absorbent paper towel to remove all residual droplets.
4. Dispense **100 µl** of TMB Substrate into each well. Incubate at room temperature(18-25°C) in the dark, for **20 minutes**.
5. Stop the reaction by adding **100 µl** of Stop Solution to each well. Gently mix for 10 seconds until the blue color completely changes to yellow.
6. Read the optical density at 450/630 nm with a microtiter plate reader within **15 minutes**.



CALCULATION OF RESULTS

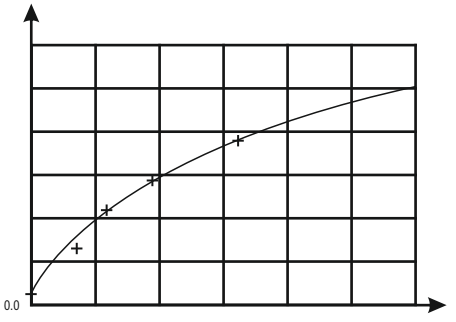
Construct a standard curve by plotting the absorbance obtained from each reference standards against its concentrations in mIU/ml on the graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the absorbance values for each specimen to determine the corresponding concentration of FSH in mIU/ml from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

Example of Standard curve

Results of a typical standard run with optical density reading at 450nm (ref 600-700nm) shown in the Y axis against FSH concentrations shown in the X axis.

Suggest: Use 4-Parameter Standard curve to calculate sample values.

FSH Values (mIU/ml)	Absorbance (450nm)
A	0.013
B	0.149
C	0.493
D	1.076
E	1.721
F	2.191



This standard curve is for the purpose of illustration only and should not be used to calculate samples. Each user should obtain his or her own standard curve and data.

Expected values and sensitivity

Female Follicular	0~20 mIU/ml
Mid-cycle	15~30mIU/ml
Luteal	0~20 mIU/ml
Post Menopausal	40~200 mIU/ml
Male	0~20mIU/ml

The minimum detectable concentration of FSH by this assay is estimated to be 2.5 mIU/ml.

PERFORMANCE CHARACTERISTICS

A) Internal Evaluation:

1. Accuracy: In an internal study **FSH** was evaluated against commercially available licensed kit with 90 random clinical samples & **FSH** has demonstrated >98% clinical correlation with the commercially available licensed kit.
   Precision: **FSH** was evaluated with licensed external Quality controls for Precision Studies & following is the data:

Controls	No. of testings	Mean Control values with **FSH**	Coefficient of Variation (CV)
Level 1	10	8.32	4.87
Level 2	10	20.22	4.48
Level 3	10	45.96	5.42

B) External Evaluation:

FSH ELISA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **FSH** ELISA has demonstrated 97% correlation with the reference method.
*Data file: Orchid Biomedical Systems (P) Ltd.

IMPORTANT NOTE

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended to use the multi channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of standards, Controls and samples is not mandatory but may provide information on reproducibility & application errors.

LIMITATIONS OF THE ASSAY

1. As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. The activity of the enzyme used is temperature-dependent and the OD values may vary. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the OD values. Corresponding variations apply also to the incubation times. However, the standards are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.
3. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
4. Insufficient washing (e.g., less than 5 wash cycles, too small wash buffer volumes, or shortened reaction times) can lead to incorrect OD values.

BIBLIOGRAPHY

1. Marshall J.C. **Clinic in Endocrinol. Metab** 1975; 4:545
2. Cohen K.L. **Metabolism** 1977; 26:1165
3. Rebar R.W. , Erickson G.F. and Yen S.S.C. **Fertil. Steril.**1982; 37: 35
4. Abraham G.E.. Ed. Radioassay Systems in Clinic. **Endocrinol.** Marcel Dekker, Inc, New York (1981)
5. Uotila M., Ruoslahti E and Engvall E.**J.Immunol. Methods** 1981; 42: 11.
6. Data on file: Orchid Biomedical Systems (P) Ltd.

SYMBOL KEYS

Temperature Limitation	Consult Instructions for use
Manufacturer	*In vitro* Diagnostic Medical Device
Use by	Catalogue Number
Date of Manufacture	Batch Number / Lot Number
This side up	Contains sufficient for <n> tests
Do not reuse	

Orchid Biomedical Systems (P) Ltd.*

M 46-47, Phase III B, Verna Industrial Estate, Verna, Goa - 403 722, INDIA.
Email id: sales@orchidbiomedical.com

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