Bovine FSH

PRODUCT PROFILE AND INSTRUCTIONS

INTENDED USE
The FSH ELISA Test is an immunoassay designed for the quantitative determination of follicle-stimulating hormone concentrations in Bovine serum. The test is designed for professional use only and should be employed by a trained/skilled professional. The assay is designed to measure circulating levels of FSH in Bovine and related species.

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 the 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 LH produced then binds to the theca cells and stimulates steroidogenesis. Increased intraovarian Estradiol production occurs as follicular maturation advances, thereupon stimulating increased FSH receptor activity and FSH follicular binding. FSH, LH, and Estradiol are therefore intimately related in supporting ovarian recruitment and maturation of the ovum in female.

TEST PRINCIPLE
The FSH ELISA Test Kit is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a rabbit polyclonal anti-Bovine FSH antibody for solid phase (microtiter wells) immobilization and a goat anti-FSH antibody in coupled to enzyme (horseradish peroxides) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in FSH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 3-hour incubation period, the wells are washed with buffer to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 2N HCl, and the absorbency is measured spectrophotometrically at 450nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled FSH in the sample. By reference to a series of FSH standards assayed in the same way, the concentration of FSH in the unknown sample is quantified.

MATERIALS PROVIDED
1. Antibody-coated microtiter wells, 96-well plate
2. Reference Standard, 0.8ml
   (0,1.0, 2.5, 5 10, 25 ng/mL)
3. Enzyme Conjugate Reagent, 12 mL
4. TMB Color Reagent, 12 mL
5. 20X Wash buffer, 20 mL
6. Stop solution (2N HCl), 6mL
7. Instructions

MATERIALS REQUIRED, BUT NOT PROVIDED
1. Precision pipettes: 50uL, 100uL, 200uL, and 1.0mL
2. Disposable pipette tips
3. Distilled water
4. Glass tubes or flasks to prepare TMB Solution
5. Vortex mixer or equivalent
6. Absorbent paper of paper towel
7. Graph paper

SPECIMEN COLLECTION AND PREPARATION
Serum should be prepared from a whole blood specimen obtained by acceptable techniques. This kit is for use with serum samples and not for whole blood.

STORAGE OF TEST KIT AND INSTRUMENTATION
Unopened test kits should be stored at 2-8^{O}C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as prescribed above. A microtiter plate reader with a bandwidth of 10nm or less, with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at a 450nm wavelength is acceptable for use in absorbency measurement
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REAGENT PREPARATION
1. All reagents should be brought to room temperature (18-25^{O}C) before use.
2. To prepare the wash buffer add one part of the reagent buffer to 19 parts of distilled water. Prepare desired amount and excess solution can be stored (refrigerate) and is stable for one week.

ASSAY PROCEDURE

One must follow accurately these steps to ensure correct results. Use clean pipettes and sterile, disposable tips:

1. Secure the desired number of coated wells in the holder.
2. Dispense 100ul of standards, specimens, and controls into appropriate wells.
3. Dispense 100ul of Enzyme Conjugate into each well. Shake for 30 seconds. It is very important to shake very well at this step.
4. Incubate at 37^{O}C for 3 hours.
5. Remove the incubation mixture by dumping plate contents into a waste container.
6. Rinse and dump the microtiter wells five (5) times with diluted wash buffer.
7. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
8. Dispense 100ul of TMB solution into each well. Gently mix for 10 seconds.
9. Incubate at room temperature for 20 minutes, in the dark.
10. Stop reaction by adding 50ul of 2N HCl to each well.
11. Gently mix for 30 seconds. It is important to observe a color change from blue to yellow.
12. Read optical density at 450nm with a microtiter well reader.

Important note: The wash steps are very critical and insufficient washing will result in poor precision and falsely elevated absorbency readings.

CALCULATION OF RESULTS

Calculate the mean absorbency value (A450) for each set of reference standards, specimens, controls and patient samples. Construct a standard curve by plotting the mean absorbency obtained from each reference standard against its concentration in ng/ml on graph paper, with absorbency values on the vertical or Y axis, and concentrations on the horizontal or X axis. Use the mean absorbency values for each specimen to determine the corresponding concentration of FSH in ng/ml from the standard curve.

Limitations & Warranty

The present ELISA is designed for helping the scientist to analyze test samples only. There are no warranties, expressed, implied or otherwise indicated, which extend beyond this description of this product. Endocrine Technologies, Inc. is not liable for property or laboratory damage, personal injury, or test samples loss, or economic loss caused by this product. Warranty is limited to replacement of similar ELISA Kit damaged during shipment or leaking solutions within 30 days, with written explanation and return of the ELISA product. The analyst should establish the standard curve and a small number of samples before proceeding to analyze a large number of samples.

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Revised February 2010

QUALTI CONTROL DATA

Examples of ETI Bovine FSH Standard Curve:

A typical FSH ELISA standard curve run as a quality control of each lot is given below:

Bovine FSH concentBovineion ng/mL	Absorbency 450nm
0 ng/ml	0.06
1.0 ng/ml	0.573
2.5 ng/ml	1.657
10 ng/ml	2.165
25. ng/ml	2.65
50 ng/ml	2.91

EXPECTED VALUES : SENSITIVITY AND SPECIFICITY

The minimum detectable concentBovineion of FSH by this assay is estimated to be 0.5ng /ml.

Sensitivity and specificity:

The sensitivity of the assay is 0.5 ng/mL and each laboBovineory should establish its own base levels based on the species and physiological situation.

In ETI Bovine/mouse FSH ELISA assay, the following materials have the following cross-rectivity:
Bovine/Mouse FSH 100%, TSH < 0.08%, HCG < 0.01%, LH < 0.05% GH <0.001%.

Good LaboBovineory practice requires that quality control specimens be run with each standard curve to establish assay performance characteristics such as recovery, linearity, precision and specificity.

LIMITATIONS OF THE TEST

1. The present Endocrine's ELISA system designed here is for estimation of FSH levels in serum/plasma samples only.
2. The wells should be adequately washed to obtain reproducible results. The washing step is extremely important and should be followed according to the instructions..
3. The assay should be performed by trained and skilled professional only.