FSH (Rodent) ELISA Kit

Catalog Number KA2330

96 assays

Version: 09

Intended for research use only

Table of Contents

Introduction

Intended Use

The FSH (Rodent) ELISA Kit is an immunoassay designed for the quantitative determination of follicle-stimulating hormone concentrations in 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 Rodent and related species and don't use for other species.

Background

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, hCG, and 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.

Principle of the Assay

The FSH (Rodent) ELISA Kit is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a rabbit polyclonal anti-rat FSH antibody for solid phase (microtiter wells) immobilization and a goat anti-rat 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 at 37ºC, 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 2 N HCl, and the absorbency is measured spectrophotometrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present and is directly related to

the amount of 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.

General Information

Materials Supplied

List of component

Component	Amount
Antibody-coated 96-well plate	96 wells/plate
Enzyme Conjugate Reagent	12 mL
TMB Color Reagent	12 mL
20X Wash buffer	20 mL
Stop solution (2 N HCl)	6 mL
Standard/Sample Diluent	20 mL
Lyophilized Standards (0, 1.0, 2.5, 5.0, 10, 25 ng/mL), QC1 (~2.0 ng/mL) and QC2 (~10-15 ng/mL). Reconstitute in 1 mL using Standard/Sample Diluent.	1 set

Storage Instruction

Unopened test kits should be stored at 4-8ºC. The microtiter plate should always be kept in a sealed bag with desiccants to minimize exposure to damp air at room temperature. Opened test kits will remain stable until the expiration date shown, provided it is stored as prescribed above. Do not leave any reagents at room temperature more than 3 hours.

Materials Required but Not Supplied

- ✓ Precision pipettes: 50 µL, 100 µL, 200 µL, and 1.0 mL
- ✓ Disposable pipette tips
- ✓ Distilled water
- ✓ Glass tubes or flasks to prepare TMB Solution
- ✓ Vortex mixer or equivalent
- ✓ Absorbent paper of paper towel
- ✓ Graph paper
- ✓ A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at a 450 nm wavelength is acceptable for use in absorbency measurement.

Precautions for Use

For Research Use Only, Not for Diagnostic Purposes.

Please read the protocol carefully before beginning this assay.

✓ Limitation of the test

- The present ELISA system designed here is for estimation of FSH levels in serum/plasma samples only.
- The wells should be adequately washed to obtain reproducible results. The washing step is extremely important and should be followed according to the instructions.
- Trained and skilled professional only should perform the assay.
- The present ELISA is designed for helping the scientist to analyze test samples from rodent species only. There are no warranties, expressed, implied or otherwise indicated, which extend beyond this description of this product. Abnova is not liable for property or laboratory damage, personal injury, or test samples loss, or economic loss caused by this product. The analyst should establish the standard curve and a small number of samples before proceeding to analyze a large number of samples.

Assay Protocol

Reagent Preparation

- ✓ All reagents should be brought to room temperature (18-25ºC) before use.
- ✓ To prepare the wash buffer added one part of the reagent buffer to 19 parts of distilled water. Prepare desired amount and excess solution can be stored (refrigerated) and is stable for one week.
- ✓ Lyophilized standards should be diluted 1 mL using Standard/Sample Diluent and these samples can be stored at -20ºC for long term.

Sample 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.

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 100 µL of standards, specimens, and controls into appropriate wells.
3. Dispense 100 µL of Enzyme Conjugate into each well. Shake for 30 seconds. It is very important to shake the plate at this step.
4. Incubate at 37ºC 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 100 µL 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 50 µL of 2 N 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 450 nm 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.

Data Analysis

Calculation of Results

Calculate the mean absorbency value (A450) for each set of reference standards, specimens, controls and 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.

Resources

References

1. Knobil, E. The neuroendocrine control of the menstrual cycle, Rec. Prog. Horm. Res. 36:52-88; 1980
2. Harris, G.W. and Naftolinf. The hypothalamus and control of ovulation. Brit. Med. Bullet. 26: 1-9; 1970
3. Shome, B. and Parlow, A.F. J. Clin. Endocrinol. Metabl. 39:199-205; 1974
4. Uotila, M.; Ruoslahti, E. and Engvall, E. J. Immunol. Methods. 42: 11-15; 1981
5. Goodman MF: Probl Vet Med. 1992, 4(3):433-44. Review.
6. Nishiyama T, et al.: J Am Anim Hosp Assoc. 1999, 35(4): 348-52
7. Chiba K, et al: Endocr J.1997, 44 (2): 205-18
8. Murphy BD, et al: J Repod Fertil Suppl.1993;47:181-8
9. Valtonen M, et al: J Reprod Fertil Suppl.1993;47:133-7

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H