Rodent FSH

PRODUCT PROFILE AND INSTRUCTIONS

INTENDED USE

The FSH CLIA Test is an immunoassay designed for the quantitative determination of follicle-stimulating hormone concentrations in serum of Rodents. 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.

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, 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.

TEST PRINCIPLE

The FSH CLIA Test 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 37C, the wells are washed with buffer to remove unbound labeled antibodies. A solution of CLIA reagent is added and incubated for 2-5 minutes, resulting light emission of light and is measured in a CLIA Reader. The intensity of the light formed is proportional to the amount of enzyme present and is directly 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. Enzyme Conjugate Reagent, 12 mL
3. CLIA Reagent A and B, 6 mL each.
4. 20X Wash buffer, 20 mL
5. Reference Standard, 0.8ml
   (0, 1.0, 2.5, 5.0, 10, 25 ng/mL) QC Level 1 (2-5ng/ml) and
   QC Level 2 (10-15 ng/mL)
6. 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 CLIA Reagent
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

Note of Caution: Immediately after receiving the kit all standards, if not used, should be kept at -20^{O}C. Unopened test kits should be stored at 2-8^{O}C. 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 for more than 3 hours.

A micro titer plate CLIA Reader is required.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25^{O}C) before use.
2. 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.
3. CLIA Reagent preparation: Mix 1:1 ratio of desired amount just 5 minutes before the use and store in a dark Area. Don't make it too much in advance.

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 enzyme conjugate.
3. Dispense 10ul standards, specimens, and controls into appropriate wells into each well. Shake for 30 seconds. It is very important to shake the plate 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 CLIA Reagent ready to use solution into each well. Gently mix for 10 seconds.
9. Incubate at room temperature for 2-5 minutes, in the dark.
10. Read the light intensity development in a 96 well CLIA 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 light intensity (RLU) 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.

Sensitivity: The estimated sensitivity of the assay in this system is about 100pg/ml

LIMITATIONS OF THE TEST

1. The present Endocrine's CLIA assay 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. Trained and skilled professional only should perform the assay.

Limitations & Warranty

The present CLIA assay 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 test Kit damaged during shipment or leaking solutions within 30 days, with written explanation and return of the CLIA product. The analyst should establish the standard curve and a small number of samples before proceeding to analyze a large number of samples.

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

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