Leinco Technologies, Inc.

PRODUCT DATA SHEET

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Human Follicle Stimulating Hormone (FSH) MICRO-ELISA Test Kit

Prod. No.: T109
Pkg. Size: 96 Tests

Description

The MICRO-ELISA FOLLICLE STIMULATING HORMONE test is a solid phase sandwich-type enzyme immunoassay (ELISA) Diagnostic Kit for the *in vitro* quantitative determination of follicle stimulating hormone concentration in human serum.

Summary and Explanation of the Test

Human follicle stimulating hormone (FSH) is a product of the pituitary and has a molecular weight of approximately 30,000 daltons. Control of synthesis and secretion is provided by the hypothalamic product, gonadotrophin releasing hormone (GnRH). GnRH acts to stimulate the production and release of both FSH and luteinizing hormone (LH) by the pituitary. FSH, like the other pituitary glycoproteins, thyroid stimulating hormone (TSH), luteinizing hormone (LH) and human chorionic gonadotropin (HCG), is composed of two subunits designated alpha and beta. The alpha chain is common to all of these hormones. The beta chain is distinct and the use of highly specific monoclonal antibodies in the FSH ELISA eliminates the interference of TSH and LH in the assay of FSH.

FSH is present in the plasma of normal males and females. The primary physiologic function of FSH in the female is to cause stimulation of primordial follicular growth and steroid (estrogen) production by the developing follicle. In the male it stimulates the Sertoli cells of the testicular spermatogenic tubules to produce inhibin and androgen-binding protein (ABP). ABP transports testosterone from the interstitial cells (Leydig cells) to the spermatogenic tubule for spermatogenesis. Circulating levels of the steroidal hormones exert a negative feedback effect on the hypothalamus to control the secretion of FSH and LH.

In females, FSH values are low before puberty and increase during the reproductive years. In menstruating females, FSH levels are subject to cyclic variations. During the follicular (pre-ovulatory) phase of the menstrual cycle, FSH levels fluctuate and increase slightly. At midcycle, just prior to ovulation, serum FSH levels increase rapidly and peak at approximately the same time as the LH levels. The concentration of FSH decreases to base line levels within two days, marking the luteal (post-ovulatory) phase of the cycle. The elevated estrogen levels in pregnant females, or those taking oral contraceptives, increase negative feedback to the hypothalamus and result in decreased FSH levels. At menopause, when ovarian estradiol secretion decreases, the loss of negative feedback on the hypothalamus results in greatly increased levels of FSH.

In males, FSH values are low before puberty and increase slightly during the reproductive years. Geriatric males may have slightly elevated FSH levels.

In both males and females, primary hypogonadism with decreased production of steroids results in increased secretion of FSH and LH. In secondary or tertiary hypogonadism, the decreased secretion of FSH leads to decreased levels of gonadal steroid secretion.

Principle of the Procedure

The MICRO-ELISA FOLLICLE STIMULATING HORMONE test is based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes two unique antibodies (mouse monoclonal) directed against distinct antigenic determinants on the FSH molecule.

Specifically, plastic wells are coated with anti-FSH (mouse monoclonal). With the addition of a test sample or appropriate controls containing FSH, immune complexes are formed between FSH in the sample and the solid phase anti-FSH. Anti-FSH (mouse monoclonal) enzyme-labeled with horseradish peroxidase is added to each well. During an incubation period (45 minutes at room temperature), the FSH molecule is sandwiched between the solid phase and enzyme-labeled antibodies. The sample is then decanted and the wells are washed to remove unbound-labeled antibody. An enzyme substrate-chromogen (hydrogen peroxide, H_2O_2, and tetramethylbenzidine, TMB) is added to the well and incubated for 15 minutes at room temperature, resulting in the development of a blue color. The addition of 1.0 N H_2SO_4 stops the reaction and converts the color to yellow. The intensity of the yellow color is directly proportional to the concentration of FSH in the sample.

Reagents

Components in Each 96-Test Micro-Elisa FSH Diagnostic Kit

1. 96 wells, FSH ANTIBODY COATED **WELLS**: Coated with anti-FSH (mouse monoclonal); contained in a pack with silica gel desiccant.
2. 1 bottle, 22 ml, FSH ENZYME ANTIBODY **CONJUGATE:** anti-FSH (mouse monoclonal) labeled with horseradish peroxidase in buffered protein solution; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives; contains FD&C red # 40 as coloring agent.
3. 1 bottle, 12 ml, **SUBSTRATE-CHROMOGEN** Buffered hydrogen peroxide and 3,3',5,5'- tetramethylbenzidine (TMB) solution.
4. 1 bottle, 12 ml, **STOP** SOLUTION 1 N H_2SO_4

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5. 1 vial, 4 ml, **0 mIU/ml FSH CALIBRATOR:** Bovine serum; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives.
6. 3 vials, 1 ml, FSH **CALIBRATORS:** FSH in bovine serum; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives; 25, 50 and 100 mIU/ml.
7. 1 bottle, 60 ml, **WASH BUFFER CONCENTRATE (20X):** Buffered detergent solution, contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives. Dilute bottle to 1200 ml with deionized water.

Additional Materials Required

Disposable tip precision pipets - 0.05, 0.1 and 0.2 ml.
microtiter plate reader.
Distilled or deionized water.

Storage and Stability

Store unopened kits at 2°-8°C. The following components may be stored at ambient temperature: WELLS, SUBSTRATE-CHROMOGEN, WASH BUFFER and STOP SOLUTION. Expiration date printed on the kit indicates limits of stability.

The FSH ANTIBODY COATED WELLS are supplied in a resealable bag containing a desiccant and must be stored with the bag sealed to protect from moisture. Wells can be stored at 2°-30°C.

Chemical or Physical Indications of Instability

Alterations in the physical appearance of reagents, or results consistently outside the acceptable limits for control sera, may be due to reagent contamination or deterioration.

Instruments

Performance of the MICRO-ELISA FSH test requires use of a precision microtiter plate reader with a wavelength of 450 ± 20 nm:

Specimen Collection and Preparation

Serum samples are used in the MICRO-ELISA FSH Diagnostic Kit procedure. No special preparation of the patient is necessary; fasting is not required. Repeated freezing and thawing of specimens should be avoided. No additives or preservatives are necessary.

STORAGE: Specimens may be stored in a tightly sealed tube at 2°-8°C for three days. If the serum is not assayed within 3 days, store frozen (-20°C) in a tightly sealed tube for up to 3 weeks. Specimens should be allowed to come to room temperature and should be mixed thoroughly by gentle inversion before assaying.

Do not use grossly lipemic specimens. Moderately lipemic, hemolyzed and icteric specimens should not interfere with the assay.

Micro-Elisa FSH Procedure

Reagent Preparation

Dilute bottle of WASH BUFFER CONCENTRATE (20X) solution to 1200 ml with deionized water. Diluted wash solution will be stable until the expiration date stamped on the kit.

Preliminary Comments and Precautions

1. Patient sample may contain pathogens: treat all samples as potentially infectious.
2. Reagents contain thimerosal; avoid contact with skin.
3. Avoid contact with SUBSTRATE-CHROMOGEN (tetramethyl-benzidine) solution. It is harmful if inhaled or absorbed through skin (may cause irritation).
4. CAUTION: Source material used to prepare Calibrators was derived from human material. The material was tested using FDA-approved methods and found non-reactive for Hepatitis B Surface Antigen (HBsAg) by ELISA and non-reactive for HIV by ELISA. No known test method can offer total assurance that infectious agents are absent. HANDLE THESE REAGENTS AS IF THEY ARE POTENTIALLY INFECTIOUS. Information on handling human serum is provided in the CDC/NIH manual "Bio-safety in Microbiological and Biomedical Laboratories" (1984).

Procedural Notes

1. All test kit components used in an assay must be of the same master lot number. Materials should not be used after the expiration date shown on the package label. Components and test specimens should be at room temperature (18°-30°C) before testing begins.
2. All calibrators, controls, and samples should be tested in duplicate simultaneously. The test samples and controls must be well mixed before use.
3. A separate disposable tip should be used for each sample to avoid cross-contamination. All pipetting steps should be performed with the utmost care and accuracy. Avoid contaminating the reagent pipette tip with the serum sample.
4. The duration of the incubation times must be the same for all wells within a run.
5. Run size should be limited to the number of samples that can be added to antibody coated wells within 5 minutes.
6. Samples should be pipetted to the bottom of the antibody coated wells.
7. If microtiter reader is not capable of reading absorbances greater than 2.0, the color should be read after a shorter incubation time with the SUBSTRATE/CHROMOGEN, i.e., 10 minutes.

Test Procedure

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1. Place sufficient COATED WELLS in a holder to run 0.0 mIU/ml, 25 mIU/ml, 50 mIU/ml and 100 mIU/ml FSH CALIBRATORS, Quality Control Sera and patient samples in duplicate. Limit run size to the number of samples that can be pipetted in 5 minutes.
2. Pipet 50 µl of the CALIBRATORS, Controls or Patient Sample to the corresponding COATED WELL.
3. Pipet or dispense 200 µl of the ENZYME ANTIBODY CONJUGATE solution to all the wells and mix gently.
4. Incubate at room temperature (18°-30°C) for 45 minutes ± 5 minutes.
5. Decant or aspirate and discard liquid contents of all wells. SLAP the inverted wells on a clean piece of absorbent paper. Remove ALL OF THE LIQUID from the wells.
6. Fill each well with diluted WASH BUFFER. Fill the wells to overflowing, you cannot cause any carryover between the wells. Decant or aspirate liquid contents of all wells. SLAP the inverted wells on a fresh clean piece of absorbent paper. Remove ALL OF THE LIQUID from the wells.

WARNING: WASHING THE WELLS IS OF CRITICAL IMPORTANCE. Fill the wells to overflowing, you CANNOT cause any carryover between wells. You CANNOT over wash the wells. Completely decant or aspirate all of the liquid out of the wells. SLAP the inverted wells on a FRESH clean piece of absorbent paper AFTER EACH WASH. YOU CANNOT SLAP TOO HARD, REMOVE ALL OF THE LIQUID FROM THE WELLS.

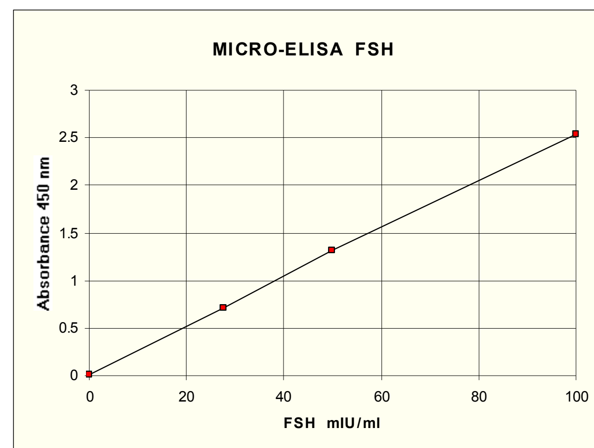
7. Repeat step 6 three more times (for a total of 4 washes).
8. Fill each well with deionized water. Fill the wells to overflowing. Decant or aspirate liquid contents of all wells. SLAP the inverted wells on a fresh clean piece of absorbent paper. Remove ALL OF THE LIQUID from the wells.
9. Pipet or dispense 100 µl (0.1 ml) of SUBSTRATE-CHROMOGEN solution into each well.
10. Mix thoroughly and incubate 15 minutes at room temperature (18°-30°C).
11. Pipet or dispense 100 µl (0.1 ml) of 1 N H_2SO_4 into each well and mix thoroughly.
12. Read the absorbance of each well at 450 ± 20 nm against water.

Calculation of Results

1. Calculate the mean value for each duplicate sample absorbance. Values for duplicate absorbances should be within 10% (or 0.02 absorbance units for absorbances less than 0.2).
2. Construct the standard curve by plotting the mean absorbance obtained for each FSH CALIBRATOR on the vertical (Y) axis versus the corresponding FSH concentration on the horizontal (X) axis, using rectilinear graph paper.
3. Connect the points with straight-line segments.
4. Using the mean absorbance for each sample, read the corresponding FSH concentration in mIU/ml from the curve. Multiply the value by the dilution factor if required.

EXAMPLE DATA
DO NOT USE IN PLACE OF CUREVE
DETERMINED ATHE THE TIME OF ASSAY.

Specimen I.D.	A_{450}	Mean A_{450}	FSH(mIU/ml)
CALIBRATOR	0 mIU/ml	0.015, 0.013	0.014
CALIBRATOR	25 mIU/ml	0.909, 0.866	0.888
CALIBRATOR	50 mIU/ml	1.738, 1.692	1.715
CALIBRATOR	100 mIU/ml	2.894, 2.909	2.902
SAMPLES			
# 1 (UNKNOWN #I)	0.312, 0.335	0.324	9.8
# 2 (UNKNOWN #2)	0.477, 0.451	0.464	14.2
# 3 (UNKNOWN #3)	1.108, 1.125	1.117	33.5



The range of this assay is 0 - 100 mIU/ml. For specimen with FSH concentrations beyond the standard curve (100 mIU/ml), repeat the test by diluting the specimen with the 0 mIU/ml CALIBRATOR. To obtain the final concentration, multiply the concentration of the diluted sample by the dilution factor.

Quality Control

Good laboratory practice requires that quality control specimen be run with each calibration curve to check the assay performance. Controls containing azide can not be used. Two controls with normal and elevated values should be used. Pooled human serum or commercially available control sera without azide are suitable. Any material used should be assayed repeatedly to establish mean values and acceptable ranges to assure proper performance.

Read the absorbance of the test solutions against a water blank. If the absorbance of the 0 mIU/ml CALIBRATOR exceeds 0.100 it is an indication of careless washing and the assay must be repeated.

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Standardization

The MICRO-ELISA FSH CALIBRATORS have been standardized against the World Health Organization International Reference Preparation (2nd IRP 78/549).

Limitations of the Procedure

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.

Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). These samples should not be assayed with the MICRO-ELISA FSH test as erroneous results may be obtained. These conditions should be ruled out prior to clinical evaluation of test results.

The wash procedure (steps 6-8) is critical. Insufficient washing will result in poor precision and falsely elevated absorbances. The use of tap water for washing could result in a higher background absorbance.

FINAL REACTION STABILITY: The spectrophotometric measurement should be made within 30 minutes after the addition of the H_2SO_4 solution.

Samples with elevated levels of FSH (up to 3,700 mIU FSH/ml) will always assay as >100 mIU FSH/ml when tested, and will not result in a "high dose hook effect". When it is necessary to measure levels of FSH greater than the 100 mIU FSH/ml CALIBRATOR, the sample should be diluted with the 0 mIU/ml CALIBRATOR and re-assayed.

Estrogens from either exogenous or endogenous sources cause a decrease in serum levels of FSH. Patients treated with oral contraceptives will have a decreased level of FSH and will not demonstrate a mid-cycle peak in FSH or LH levels. Because the levels of hCG in serum from pregnant females can be greatly elevated, samples from pregnant patients will show false FSH values.

Single observations of FSH levels may give misleading results due to the pulsatile nature of FSH secretion. Samples drawn at different times may vary by as much as the width of the normal range. Elevated levels of FSH should be confirmed by more than one FSH measurement.

Expected Values

FSH values were measured in serum samples from apparently normal individuals and the following results were obtained:

	FSH (mIU/ml)		
	N	Mean	Range
ADULT FEMALE			
premenopausal	65	4.7	0.3 - 25.7
postmenopausal	74	55.3	6.7 – 137
ADULT MALE	66	4.9	1.2 - 17.8
PREPUBERTAL			
MALE & FEMALE	10	1.4	0.5 -3.1

In premenopausal females, the serum level of FSH will change throughout the menstrual cycle.

PHASE	MEAN FSH (mIU/ml)	RANGE (mIU/ml)
Follicular	6 - 10	0 – 20
Mid-cycle Peak	10 - 14	0 – 30
Luteal	5 - 9	0 - 13

These values are consistent with those reported in the literature. It is recommended that each laboratory determine its own normal range.

Performance Characteristics of Test

Assay Specificity

Specificity of this test system was proven by determining a lack of interference of thyroid stimulating hormone (TSH) and luteinizing hormone (LH) when added to a serum sample with a known FSH concentration. The incremental change in FSH concentration was measured and the relative cross reactivity calculated by dividing the apparent change in FSH concentration (mIU/ml) by the cross reactant concentration (in mIU/ml or uIU/ml) times 100. The observed cross reactivity for both TSH and LH was < 1.0%. The observed interference of hCG (3,000 mIU hCG/ml) was an apparent decrease of 0.3% (10 mIU/ml) and at higher levels of hCG (10,000 mIU/ml) there is a 50% inhibition in measured FSH. The use of this test to determine FSH concentrations in serum from pregnant or immediately postpartum females is not recommended.

Assay Sensitivity

The sensitivity of this assay is defined as the smallest single value that can be distinguished from the zero calibrator. This value was calculated from the mean + two standard deviations for twenty-one replicates at the zero concentration. The calculated sensitivity is <1.0 mIU/ml.

Assay Reproducibility

Intra-assay reproducibility was determined by measurement of 20 replicates of three serum pools in a single run.

	Mean FSH (mIU/ml)	SD	%CV
Serum A	9.19	0.52	5.6
Serum B	12.43	0.59	4.7
Serum C	32.33	1.10	3.4

The interassay reproducibility was determined by duplicate measurement of three serum pools in nine separate runs.

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	Mean FSH (mIU/ml)	SD	% CV
Serum A	9.66	0.70	7.3
Serum B	14.08	0.89	6.4
Serum C	33.63	1.64	4.9

Assay Linearity

A study was performed diluting a serum sample containing an elevated level of FSH with the 0 mIU/ml CALIBRATOR to determine the linearity of the MICRO-ELISA FSH test.

FSH mIU/ml

Dilution Factor	Expected Value	Observed Value	% of Expected Value
undiluted	-	100.9	-
3:4	75.7	80.8	107 %
1:2	50.5	52.6	104 %
1:4	25.2	26.7	102 %
1:8	12.6	12.6	100 %
1:16	6.3	6.0	95 %

Assay Recovery

Three aliquots of human sera with a FSH concentrations of 7.0 mIU/ml were spiked with 7.9, 16.5 and 33.0 mIU FSH/ml. The samples were assayed in duplicate.

FSH mIU/ml

Added FSH	Expected Value	Measured Value	% Recovery
0.0	7.0		
7.9	14.9	15.4	103 %
16.5	23.5	22.6	96 %
33.0	40.0	42.0	105 %

Comparison to Other FSH Tests

Correlation studies on a random group of 133 serum samples with a range of values from <1 - 96 mIU/ml, were performed using the quantitative results from the MICRO-ELISA FSH Test and another ELISA FSH test. The correlation coefficient of the test results was 0.976. Correlation studies on a second group of 50 serum samples with a range of values from <1 - 150 mIU/ml, were performed using the quantitative results from the MICRO-ELISA FSH Test and an automated FSH test. The correlation coefficient of the test results was 0.984.

		Slope	Y-Intercept	Correlation Coefficient
ELISA	n= 133	0.982	1.34	0.976
AUTOMATED	n= 50	1.037	3.72	0.984

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Warranty

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