MP Biomedicals
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Follicle Stimulating Hormone (FSH)
ChLIA Kit
Catalog No.: 07M475A, 07M475B

INTENDED USE

The MP Biomedicals Follicle Stimulating Hormone (FSH) ChLIA Kit is intended to be used for the quantitative determination of Follicle Stimulating Hormone Concentration in Human Serum by a Microplate Chemiluminescense Immunoassay (ChLIA). This test is for in vitro diagnostic use only.

SUMMARY AND EXPLANATION OF THE TEST

Follicle Stimulating hormone (FSH) is a glycoprotein consisting of two subunits with an approximate molecular mass of 35,500 daltons. The α-subunit is similar to other pituitary hormones [luteinizing stimulating hormone (LH), thyroid stimulating hormone (TSH) and chorionic gonadotropin (CG)] while the β-subunit is unique. The β-subunit confers the biological activity to the molecule. Stimulation by gonadotropin-releasing hormone (GnRH) causes release of FSH, as well as LH, from the pituitary and is transported by the blood to their sites of action, the testes or ovary.

In men, FSH acts on the Sertoli cells of the testis, stimulating the synthesis of inhibin, which appears to specifically inhibit further FSH secretion, and androgen-binding protein. Thus, it indirectly supports spermatogenesis.

In women, FSH acts on the granulosa cells of the ovary, stimulating steroidogensis. All ovulatory menstrual cycles have a characteristic pattern of FSH, as well as LH, secretion. The menstrual cycle is divided into a follicular phase and a luteal phase by the midcycle surge of the gonadotropins (LH and FSH). As the follicular phase progresses, FSH concentration decreases. Near the time ovulation occur, about midcycle, FSH peaks (lesser in magnitude than LH) to its highest level.

The clinical usefulness of the measurement of Follicle Stimulating hormone (FSH) in ascertaining the homeostasis of fertility regulation via the hypothalamic - pituitary - gonadal axis has been well established (1,2).

In this method, FSH calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of FSH) are added and the reactants mixed. Reaction between the various FSH antibodies and native FSH forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-Follicle Stimulating hormone antibody bound conjugate is separated from the unbound enzyme-follicle stimulating hormone conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce light.

The employment of several serum references of known follicle stimulating hormone levels permits construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with follicle stimulating hormone concentration.

PRINCIPLE

Immunoenzymometric assay (TYPE 3):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-FSH antibody.
Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$Enz_{Ab_{(p)}} + Ag_{FSH} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} Enz_{Ab_{(p)}}\text{-}Ag_{FSH}\text{-}{}^{Btn}Ab_{(m)}$$

${}^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
Ag_{FSH} = Native Antigen (Variable Quantity)
$Enz_{Ab_{(p)}}$ = Enzyme labeled Antibody (Excess Quantity)
$Enz_{Ab_{(p)}}\text{-}Ag_{FSH}\text{-}{}^{Btn}Ab_{(m)}$= Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:

$$Enz_{Ab_{(p)}}\text{-}Ag_{FSH}\text{-}{}^{Btn}Ab_{(m)} + Streptavidin_{C.W.} \Rightarrow \text{Immobilized complex}$$

$Streptavidin_{C.W.}$ = Streptavidin immobolized on well
Immobilized complex = sandwich complex bound to the solid surface

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity, determined by reaction with a substrate that generates light, in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

REAGENTS

Materials Provided:

A. FSH Calibrators – 1 mL/vial
Six (6) vials of references for FSH Antigen at levels of 0(**A**), 5(**B**), 10(**C**), 25(**D**), 50**E**) and 100(**F**) mIU/mL. Store at 2-8°C. A preservative has been added.
Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 2nd IRP (78/549).

B. FSH Tracer Reagent —13 mL/vial
One (1) vial-containing enzyme labeled antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Light Reaction Wells – 96 wells
One 96-well white microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20 mL
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. Signal Reagent A – 7.0 mL/vial
One (1) bottle containing luminol in buffer. Store at 2-8°C.

F. Signal Reagent B – 7.0 mL/vial
One (1) bottle containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Product Instructions

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate

Required but not provided:
1. Pipette capable of delivering 50 µL volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 mL and 0.350 mL volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate luminometer.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100 mL of the specimen is required.

REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of Wash Concentrate to 1000 mL with distilled or deionized water in a suitable storage container. Store diluted buffer at room 2-30°C for up to 60 days.
2. **Working Signal Reagent Solution -** Store at 2 - 30°C.
Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 mL of A and 1 mL of B per two (2) eight well strips (A slight excess of solution is made). **Discard the unused portion if not used within 36 hours after mixing.** If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

Note: Do not use reagents that are contaminated or have bacteria growth.

TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-27°C).
*****Test procedure should be performed by a skilled individual or trained professional*****

1. Format the microplate wells for each serum reference, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C**
2. Pipette 0.050 mL (50 µL) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 mL (100 µL) of FSH-Enzyme Conjugate solution to all wells.
4. Swirl the plate gently for 20-30 seconds to mix and cover.
5. Incubate 45 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 350 µL of wash buffer (see Reagent Preparation Section) decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat four (4) additional times.**
8. Add 0.100 mL (100 µL) of working signal reagent to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells**.

DO NOT SHAKE THE PLATE AFTER SIGNAL ADDITION

9. Incubate at room temperature in the dark for five (5) min.
10. Read the relative light units in each well, for minimum 0.5 – 1.0 seconds, using a microplate luminometer. **The results should be read within thirty (30) minutes of adding the substrate solution.**

QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated ranges for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control records should be maintained and used to monitor batch to batch consistency.

CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of follicle stimulating hormone in unknown specimens.
1. Record the RLU obtained from the printout of the microplate luminometer as outlined in Example 1.
2. Plot the light intensity for each duplicate serum referenceversus the corresponding FSH concentration in mIU/ml on linear graph paper.
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of hFSH for an unknown, locate the average RLU's of the unknown on the vertical axis of the graph, find the intersecting point on the curve,

and read the concentration (in mIU/mL) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average RLU's (51568) of the unknown intersects the calibration curve at (42.3mIU/mL) FSH concentration (See Figure 1).

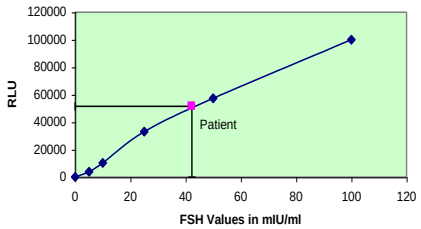
Note: Computer data reduction software designed for chemiluminescence assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	RLU (A)	Mean RLU (B)	Value (mIU/mL)
Cal A	A1	101	99	0
	B1	98		
Cal B	C1	3933	3911	5
	D1	3888		
Cal C	E1	10466	10399	10
	F1	10332		
Cal D	G1	33101	33194	25
	H1	33287		
Cal E	A2	57982	57344	50
	B2	56705		
Cal F	C2	99636	100000	100
	D2	100364		
Ctrl 1	E2	2335	2370	***3.5***
	F2	2405		
Ctrl 2	G2	15106	15789	***13.5***
	H2	16473		
Patient	A3	51569	51568	***42.3***
	B3	51566		

* The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay. In addition, the RLU's of the calibrators have been normalized to 100,000 RLU's for the F calibrator (greatest light output). This conversion minimizes differences caused by efficiency of the various instruments that can be used to measure light output.

Figure 1



LIMITATIONS OF PROCEDURE

Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of signal reagent initiates a kinetic reaction; therefore the signal reagent(s) should be added in the same sequence to eliminate any time-deviation during reaction.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.
8. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from MP Biomedicals' IFU may yield inaccurate results.
9. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
10. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.

Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, MP Biomedicals shall have no liability.
5. If computer-controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. FSH is suppressed by estrogen but in woman taking oral contraceptives the level may be low or normal. Excessive dieting and weight loss may lead to low gonadotropin concentrations.
7. Follicle Stimulating hormones is dependent upon diverse factors other than pituitary homeostasis. Thus, the determination alone is not sufficient to asses clinical status.

EXPECTED RANGES OF VALUES

A study of an apparent normal adult population was undertaken to determine expected values for the FSH ChLIA test. The expected values are shown in Table 1.

TABLE I
Expected Values for the FSH ChLIA
(in mIU/mL (2nd IRP 78/549)

Women		Men
Follicular phase	3.0 -- 12.0	1.0 – 14.0
Midcycle	8.0 -- 22.0	
Luteal phase	2.0 -- 12.0	
Postmenopausal	5.0 -- 151.0	

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

PERFORMANCE CHARACTERISTICS

Precision

The within and between assay precision of the FSH ChLIA test were determined by analyses on three different levels of control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in mIU/mL)

Sample	N	X	σ	C.V.
Level 1	16	4.6	0.21	4.6%
Level 2	16	13.5	0.61	4.5%
Level 3	16	53.2	1.54	2.9%

TABLE 3
Between Assay Precision* (Values in mIU/mL)

Sample	N	X	σ	C.V.
Level 1	10	4.5	0.35	7.7%
Level 2	10	13.3	1.0	7.5%
Level 3	10	52.3	4.5	8.6%

*As measured in ten experiments in duplicate.

Sensitivity

The sensitivity (detection limit) was ascertained by determining the variability of the 0 mIU/mL serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose. It was determined to be 0.084mIU/mL.

Accuracy

This FSH ChLIA test was compared with a reference enzyme immunoassay. Biological specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 106. The least square regression equation and the correlation coefficient were computed for this method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This Method	15.8	y = 0.97(x) – 1.5	0.981
Reference	17.1		

Only slight amounts of bias between this procedure and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

Specificity

The cross-reactivity in this method to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of Follicle Stimulating Hormone needed to produce the same light intensity.

Substance	Cross Reactivity	Concentration
Follitropin (FSH)	1.0000	--
Lutropin Hormone (hLH)	< 0.0001	1000 ng/mL
Chorionic Gonadotropin (hCG)	< 0.0001	1000 ng/mL
Thyrotropin (TSH)	< 0.0001	1000 ng/mL

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