Follicle Stimulating Hormone ELISA Assay Kit

Catalog Number:

FSH31-K01 (1 x 96 wells)

For Research Use Only. Not for use in diagnostic procedures.

v. 12.0 (effective 02Jun23)

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INTENDED USE

The Eagle Biosciences Follicle Stimulating Hormone (hFSH) ELISA kit is for the quantitative measurement of Follicle Stimulating Hormone in human serum. The Follicle Stimulating Hormone ELISA Assay Kit is for research use only and not to be used for diagnostic procedures.

LIMITATIONS RELATED TO INTENDED USE

1. This test is not intended to be used for screening purposes.
2. This test is not intended for home testing or self-testing.
3. The kit is calibrated for the determination of FSH in human serum. The kit is not calibrated for the determination of FSH in other specimens of human or animal origin.
4. The results obtained with this kit shall never be used as the sole basis for a clinical diagnosis and for therapeutic decisions.
5. Although common interfering substances have been evaluated with this test, other substances that have not been evaluated such as drugs and the occurrence of heterophilic antibodies in individuals regularly exposed to animals or animal products that have the potential of causing interferences.
6. Some individuals may have antibodies to mouse protein that can possibly interfere in this assay. Therefore, the results from any patients who have received a preparation of mouse antibodies for diagnosis or therapy should be interpreted with caution.

INTRODUCTION

Human follicle stimulating hormone (FSH) is a glycoprotein hormone produced by the anterior pituitary gland. There are three other glycoprotein hormones, namely Thyroid Stimulating Hormone, Luteinizing Hormone (both produced by anterior pituitary gland) and Human Chorionic Gonadotropin (produced by the placenta) which are structurally similar. Each hormone has an alpha and beta subunit. The α subunits of each hormone are similar while the β subunit is specific to each hormone. The α subunits contain 92 amino acids with the β subunits vary with each hormone. The β subunit of both FSH and LH contain 115 amino acids, TSH 110 amino acids, and hCG 147 amino acids.

The FSH and LH hormones function differently in females and males. It is to be noted that in women the growth and maturation of the ovarian follicle is dependent of FSH, while in men both LH and FSH act on the testes.

PRINCIPLE OF THE ASSAY

The FSH ELISA is a two-step capture or 'sandwich' type immunoassay. The assay makes use of two highly specific monoclonal antibodies: a monoclonal antibody specific for FSH is immobilized onto the microplate and another monoclonal antibody specific or a different region of FSH is conjugated to horse radish peroxidase (HRP). In the first incubation step, FSH present in the specimen samples, calibrators and controls is bound by the antibody immobilized onto the microplate. Excess and unbound materials are removed by a washing step. In the second incubation step, HRP conjugate antibody (HRP conjugate) is added, which binds specifically to any immobilized FSH, thus forming a sandwich complex. Unbound HRP conjugate is removed by a washing step. Next, the TMB substrate (enzyme substrate) is added which reacts with HRP to form a blue colored product that is directly proportional to the amount of FSH present. The enzymatic reaction is terminated by the addition of the stopping solution, converting the color from blue to yellow. The absorbance is measured on a microplate reader at 450 nm. A set of calibrators is used to plot a calibrator curve from which the a mount of FSH in a specimen samples and controls can be directly read.

PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is for use by trained laboratory personnel (professional use only). For laboratory in vitro use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This Includes:
    a. Do not pipette by mouth.
    b. Do not smoke, drink, or eat in areas where specimens or kit reagents are handled
    c. Wear protective clothing and disposable gloves.
    d. Wash hands thoroughly after performing the test.
    e. Avoid contact with eyes; use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
3. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
4. Do not use the kit beyond the expiry date stated on the label.
5. If the kit reagents are visibly damaged, do not use the test kit.
6. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
7. All kit reagents and specimens must be brought to room temperature and mixed geritly but thoroughly before use. Avoid repeated freezing and thawing of specimens.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
10. A calibrator curve must be established for every run.
11. It is recommended to all customers to prepare their own control materials or serum pools which should be included in every run at a high and low level for assessing the reliability of results .
12. The controls (Included in kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper procedural techniques or pipetting, incomplete washing, or Improper reagent storage.
13. When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
14. The TMB Substrate is sensitive to light and should remain colourless if properly stored. Instability or contamination may be Indicated by the development of a blue colour, in which case it should not be used.
15. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
16. Samples or controls containing azide or thlmerosal are not compatible with this kit, they may lead to false results.
17. Samples values above the measuring range of the kit may be reported as >100 IU/L. If further dilution and retesting is required, only calibrator A may be used to dilute serum samples. The use of any other reagent may lead to false results.
18. Avoid microbial contamination of reagents.
19. To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control.
20. To prevent the contamination of reagents, do not pour reagents back into the original containers.

21. Kit reagents must be regarded as hazardous waste and disposed of according lo local and/or national regulations.
22. Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
23. This kit contains 1 M sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
24. The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions
25. Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
26. If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of shaker used can influence the optical densities and test results. If a different type of shaker and/or speed is used, the user is responsible for validating the performance of the kit.
27. Do not reuse the microplate wells, they are for SINGLE USE only.
28. To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
29. When reading the microplate, the presence of bubbles In the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

SAFETY CAUTIONS AND WARNINGS

BIOHAZARDS

The reagents should be considered a potential biohazard and handled with the same precautions applied to blood specimens. All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

The calibrators and controls provided with the kit contain material(s) of human origin that have been tested and found to be negative for the presences of HBsAg, HIV 1 RNA, HCV RNA and antibodies to HIV ½. However, no test method can offer complete assurance that any viable pathogens are absent. Therefore, these components should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen, following good laboratory practices.

CHEMICAL HAZARDS

Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (contains tetramethylbenzidine) and Stopping Solution (contains sulfuric acid). If contacted with any of these reagents, was with plenty of water and refer to SDS for additional information.

SPECIMEN COLLECTION AND STORAGE

Approximately 0.1 mL of serum is required per duplicate determination. Collect 4–5 mL of venous blood into an appropriately labeled tube and allow it to clot. Centrifuge at room temperature and carefully transfer the serum into a new storage tube or container. Serum samples may be stored at 2-8°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT

This assay is a direct system; no specimen pretreatment is necessary.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Calibrated single-channel pipette to dispense 25 µL.
2. Calibrated multiple-channel pipette to dispense 50 µL and 100 µL.
3. Calibrated multiple-channel pipette to dispense 300 µL.
4. Automatic microplate washer (recommended).
5. Microplate shaker:
    a Orbital shaker (3mm diameter) set to 600 rpm or
    b Reciprocating shaker (1.5" stroke length) set to 180 oscillations/minute.
6. Disposable pipette tips.
7. Distilled or deionized water.
8. Calibrated absorbance microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater

REAGENTS PROVIDED

1. **Microplate**

Contents:	One anti-FSH monoclonal antibody-coated microplate 96-well (12x8) in a resealable pouch with desiccant.
Format:	Ready to Use
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

2. **HRP Conjugate Concentrate**

Contents:	One bottle containing anti-FSH monoclonal antibody-Horse Radish Peroxidase (HRP) conjugate in a protein-based buffer with a non-mercury preservative.
Format:	Concentrated; Requites Preparation
Volume:	0.3 mL/bottle
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.
Preparation of HRP Conjugate Working Solution:	Dilute 1:51 in assay buffer before use (e.g. 40 µL of conjugate concentrate in 2 mL of assay buffer). If the whole plate is to be used dilute 240 µL of conjugare concentrate in 12 mL of assay buffer. Discard any that is left over.

3. **Calibrator A – F**

Contents:	Six bottles of calibrator containing specified FSH concentrations. Protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined

quantities of FSH. Calibrated against World Health Organization (WHO) 1st IS 83/575

Listed below are approximate concentrations, please refer to vial labels for exact concentrations.
Concentrations: 0, 5, 10, 20, 50, 100 IU/L

Format: Ready to Use
Volume: Calibrator A: 2.0 mL/bottle
Calibrator B-F: 0.5 mL/bottled
Storage: 2-8°C
Stability: Unopened: Stable until the expiry date printed on the label.
After Opening: Stable for four weeks.

4. **Control 1 – 2**

Contents: Two bottles of control containing different FSH concentrations. Protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of FSH.
Refer to the QC certificated for the target values and acceptable ranges.
Format: Ready to Use
Volume: 0.5 mL/bottle
Storage: 2-8°C
Stability: Unopened: Stable until the expiry date printed on the label.
After Opening: Stable for four weeks.

5. **Assay Buffer**

Contents: One bottle containing a protein-based buffer with a non-mercury preservative.
Format: Ready to Use
Volume: 25 mL/bottle
Storage: 2-8°C
Stability: Unopened: Stable until the expiry date printed on the label.
After Opening: Stable for four weeks.

6. **TMB Substrate**

Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
Format: Ready to Use
Volume: 16 mL/bottle
Storage: 2-8°C
Stability: Unopened: Stable until the expiry date printed on the label.
After Opening: Stable for four weeks.

7. **Stopping Solution**

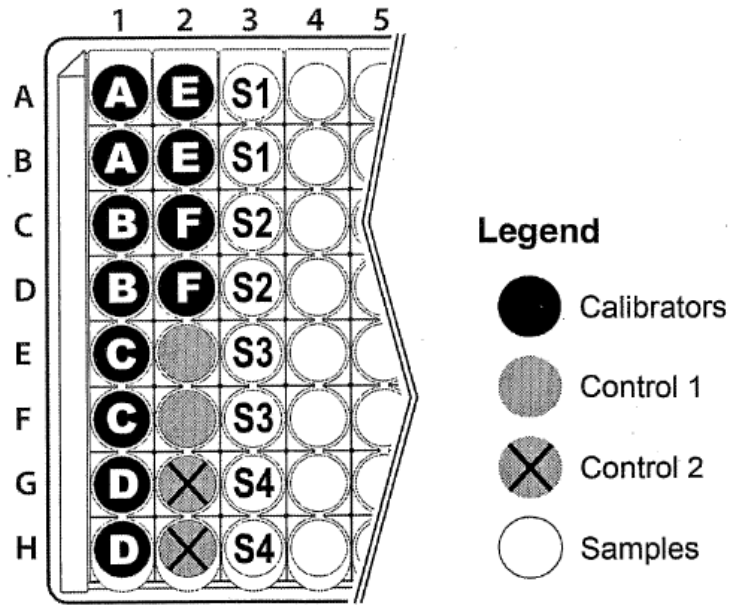
Contents: One bottle containing 1M sulfuric acid.
Format: Ready to Use
Volume: 6 mL/bottle
Storage: 2-8°C

Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.
Safety:	Refer to product SDS

8. **Wash Buffer Concentrate**

Contents:	One bottle containing buffer with non-ionic detergent and a non-mercury preservative.
Format:	Concentrated; Requires Preparation
Volume:	50 mL/bottle
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks. Following Preparation: The wash buffer working solution is stable for 2 weeks following preparation, assuming Good Laboratory Practices are adhered to. To prevent microbial growth, prepare the wash buffer working solution in a clean container and store under refrigerated conditions (2-8oC) when not in use.
Preparation of Wash Buffer Working Solution:	Dilute 1:10 in distilled or deionized water before use. If the whole microplate is to be used dilute 50 mL of the water buffer concentrate in 450 mL of distilled or deionized water.

RECOMMENDED ASSAY LAYOUT



ASSAY PROCEDURE

Specimen Pretreatment: None

All kit components, controls and specimen samples must reach room temperature prior to use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. After all kit components have reached room temperature, mix gently by inversion.
2. Prepare the HRP Conjugate Working Solution and Wash Buffer Working Solution (See *Reagents Provided, HRP Conjugate Concentrate, Wash Buffer Concentrate)*
3. Plan the microplate wells to be used for calibrators, controls, and samples. See *Recommended Assay* Layout. Remove the strips from the microplate frame that will not be used and place them in the bag with desiccant. Reseal the bag with the unused strips and return it to the refrigerator.
4. Pipette 25 uL of each calibrator, control, and specimen sample into assigned wells.
5. Pipette 100 uL of Assay Buffer into each well (the use of a multichannel pipette is recommended.)
6. Incubate on a plate shaker** for 30 minutes at room temperature.
7. Wash the microplate wells with an automatic microplate washer (preferred) or manually as stated below.
    a Automatic: Using an automatic microplate washer, perform a 3-cycle wash using 300 µL/well of Wash Buffer Working Solution (3 x 300 µL). One cycle consists of aspirating all wells then filling each well with 300 µL of Wash Buffer Working Solution. After the final wash cycle, aspirate all wells and then tap the microplate firmly against absorbent paper to remove any residual liquid.
    b Manually: For manual washing, perform a 3-cycle wash using 300 µL/well of Wash Buffer Working Solution (3 x 300 µL). One cycle consists of aspirating all the wells by briskly emptying the contents of the wells over a waste container, then pipetting 300 µL of Wash Buffer Working Solution into each well using a multi-channel pipette. After the final wash cycle, aspirate all wells by briskly emptying the contents over a waste container and then tap the microplate firmly against absorbent paper to remove any residual liquid.
8. Pipette 100 uL of the HRP Conjugate Working Solution into each well (the use of a multi-channel pipette is recommended).
9. Incubate the microplate on a microplate shaker** for 30 minutes at room temperature.
10. Wash the microplate wells again as stated in step 7.
11. Pipette 100 uL of TMB Substrate into each well (the use of a multi-channel pipette is recommended).
12. Incubate the microplate on a microplate shaker** for 15-20 minutes at room.
13. Pipette 50 uL of stopping solution into each well (the use of a multi-channel pipette is recommended) in the same order and speed as was used for addition of the TMB Substrate. Gently tap the microplate frame to mix the contents of the wells.
14. Measure the optical density (absorbance in the microplate wells using an absorbance microplate reader set to 450 nm, within 20 minutes after addition of the Stopping Solution.

**See *Reagents and Equipment Needed But Not Provided* for microplate shaker options.

CALCULATIONS

1. Calculate the mean optical density of each calibrator, controls, and specimen sample duplicate.
2. Use a 4-parameter or 5-parameter curve fit with immunoassay software to generate a calibrator curve.
3. The immunoassay software will calculate the concentrations of the controls and specimen samples using the mean optical density values and the calibrator curve.
4. If a sample reads more than 100 IU/L and needs to be diluted and retested, then dilute it with calibrator A not more than 1:8. The result obtained must be multiplied by the dilution factor.

QUALITY CONTROL

When assessing the validity of the test results, the following criteria should be evaluated:

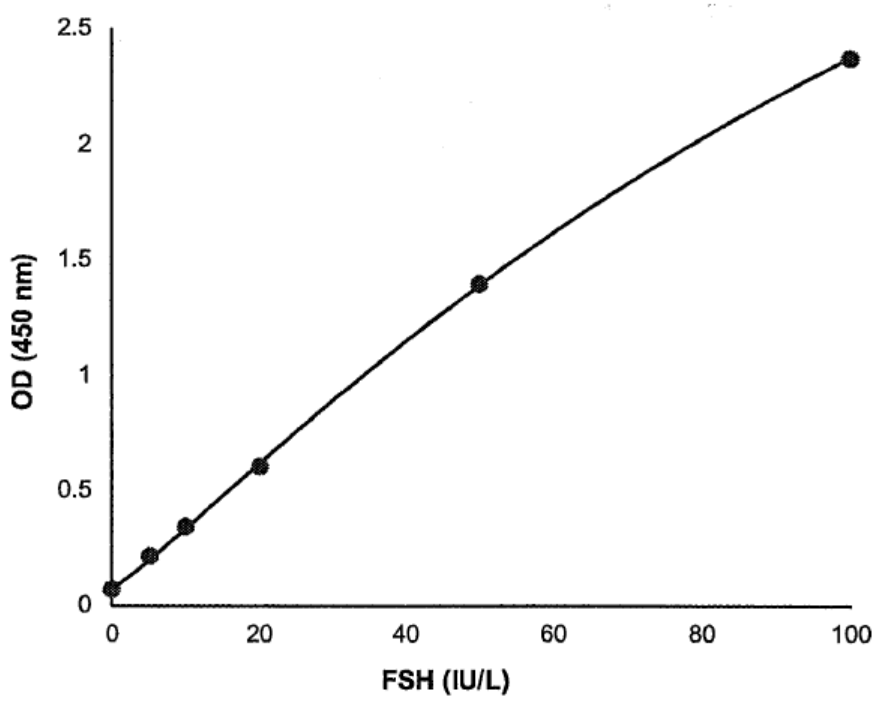
1. The values obtained for the kit controls are within the acceptable ranges as stated in the QC Certificate.
2. The results of any external controls that were used meet the acceptable ranges.

TYPICAL TABULATED DATA

Sample data only. Do not use to calculate results.

Calibrator	Mean OD (450nm)	% Binding	Value (IU/L)
A	0.072	3	0
B	0.214	9	5
C	0.342	14	10
D	0.602	25	20
E	1.397	59	50
F	2.372	100	100
Unknown	0.266	-	7.2

TYPICAL CALIBRATOR CURVE



PERFORMANCE CHARACTERISTICS

SENSITIVITY

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator A (based on 10 replicate analyses) plus 2 SD. Therefore, the sensitivity of the FSH ELISA Assay Kit is **1 IU/L.**

SPECIFICITY (CROSS-REACTIVITY)

The specificity of the FSH ELISA Assay Kit was determined by measuring the apparent FSH value of calibrator A spike with the following compounds.

Substance	Concentration Range	Apparent hFSH Value (IU/L)
hCG Calibrated against WHO 3rd IS 75/537	1000–50,000 IU/L	Not Detected
hLH Calibrated against WHO 2nd IS 80/552	5-250 IU/L	Not Detected
hTSH Calibrated against WHO 2nd IS 80/558	5-250 mIU/L	< 4.0

HIGH-DOSE HOOK EFFECT

The FSH ELISA Assay Kit did not experience any high dose hook effect when tested up to a FSH concentrations of 50,000 IU/L.

INTRA-ASSAY PRECISION

Three serum samples were assayed ten times each on the same calibrator curve. The results (in IU/L) are tabulated below:

Sample	Mean	SD	CV %
1	7.41	0.43	5.8
2	48.57	1.70	3.5
3	138.12	4.70	3.4

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in IU/L) are tabulated below:

Sample	Mean	SD	CV %
1	7.11	0.24	3.4
2	44.31	2.01	4.5
3	120.63	7.74	7.7

RECOVERY

Spiked samples were prepared by adding defined amounts of FSH to three patients serum samples. The results (in IU/L) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1 Unspiked	5.65	-	-
+ 9.7	14.75	15.35	96.1
+ 53.5	56.68	59.15	95.8
+ 107.0	103.33	112.65	91.7
2 Unspiked	17.52	-	-
+ 9.7	26.21	27.22	96.3
+ 53.5	70.07	71.02	98.7
+ 107.0	116.40	124.52	93.5
3 Unspiked	58.47	-	-
+ 9.7	72.71	68.17	106.7
+ 53.5	114.25	111.97	102.0
+ 107.0	171.05	165.47	103.4

LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in IU/L) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1	22.56	-	-
1:2	11.89	11.28	105.4
1:4	6.47	5.64	114.7
1:8	3.27	2.82	116.0
2	123.42	-	-
1:2	66.80	61.71	108.2
1:4	31.78	30.86	103.0
1:8	17.05	15.43	110.5
3	162.67	-	-
1:2	77.93	81.34	95.8
1:4	39.35	40.67	96.8
1:8	21.86	20.33	107.5

REFERENCE VALUES

As for all clincal assays, each laboratory should collect data and establish their own range of expected normal values.

Group	Range (IU/L)
Males	1–18
Females	
Follicular Stage	2–10
Midcycle Peak	7–20
Luteal Stage	1–10
Postmenopausal	18–150

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Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.