ab108641 – Follicle Stimulating Hormone Human ELISA Kit

Instructions for Use

An immunoenzymatic assay for the quantitative measurement of Follicle Stimulating Hormone in Human Serum.

This product is for research use only and is not intended for diagnostic use.
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INTRODUCTION

1. BACKGROUND

Abcam’s Follicle Stimulating Hormone in vitro ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Follicle Stimulating Hormone in Human serum.

A 96-well plate has been precoated with anti-Follicle Stimulating Hormone antibodies. Samples and standards and are added to the wells, where Follicle Stimulating Hormone in the sample and standards binds to the precoated antibody. After incubation and washing, added Anti-Follicle Stimulating Hormone HRP conjugate binds to the antibody-Follicle Stimulating Hormone complex. After incubation, the wells are washed to remove unbound material and TMB substrate is then added which is catalyzed by HRP to produce blue coloration. The reaction is terminated by addition of Stop Solution which stops the color development and produces a color change from blue to yellow. The intensity of signal is directly proportional to the amount of Follicle Stimulating Hormone in the sample and the intensity is measured at 450 nm.

Follicle-Stimulation 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, and HCG, 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 are dependent on the unique beta subunits.

In the female, FSH stimulates the growth and maturation of ovarian follicles by acting directly on the receptors located on the granulosa cells; follicular steroidogenesis is promoted and LH production is
INTRODUCTION

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 in women.

FSH levels are elevated after menopause, castration, and in premature ovarian failure. The levels of FSH may be normalized through the administration of estrogen, which demonstrate a negative feedback mechanism. Abnormal relationships between FSH and LH and between FSH and estrogen have been linked to anorexia nervosa and polycystic ovarian disease. Although there are significant exceptions, ovarian failure is indicated when random FSH concentrations exceed 40 mIU/mL.

The growth of the seminiferous tubules and maintenance of spermatogenesis in men are regulated by FSH. However, androgens, unlike estrogen, do not lower FSH levels, therefore demonstrating a feedback relationship only with serum LH. For reasons not fully understood, azospermic and oligospermic males usually have elevated FSH levels. Tumors of the testes generally depress serum FSH concentrations. High levels of FSH in men may be found in primary testicular failure and Klinefelter syndrome. Elevated concentrations are also present in cases of starvation, renal failure, hyperthyroidism, and cirrhosis.
2. **ASSAY SUMMARY**

**Primary capture antibody**
Prepare all reagents, samples and standards as instructed.

**Sample**
Add samples and standards to wells used. Add Zero Buffer and mix well. Incubate at room temperature.

**HRP conjugated antibody**
After washing, add prepared labeled HRP-Conjugate to each well. Incubate at room temperature.

**Substrate Colored product**
After washing, add TMB substrate solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.
3. **PRECAUTIONS**

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. **STORAGE AND STABILITY**

Store kit at 2-8°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

5. **MATERIALS SUPPLIED**

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Condition (Before Preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Follicle Stimulating Hormone Coated Microplate (12 x 8 wells)</td>
<td>96 Wells</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>11 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Anti-Follicle Stimulating Hormone HRP Conjugate</td>
<td>13 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>TMB Reagent</td>
<td>11 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Follicle Stimulating Hormone Standard 0 – 0 mIU/mL (Lyophilized)</td>
<td>1 vial</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Follicle Stimulating Hormone Standard 1 – 5 mIU/mL (Lyophilized)</td>
<td>1 vial</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Follicle Stimulating Hormone Standard 2 – 15 mIU/mL (Lyophilized)</td>
<td>1 vial</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Follicle Stimulating Hormone Standard 3 – 50 mIU/mL (Lyophilized)</td>
<td>1 vial</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Follicle Stimulating Hormone Standard 4 – 100 mIU/mL (Lyophilized)</td>
<td>1 vial</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Follicle Stimulating Hormone Standard 5 – 200 mIU/mL (Lyophilized)</td>
<td>1 vial</td>
<td>2-8°C</td>
</tr>
</tbody>
</table>
6. **MATERIALS REQUIRED, NOT SUPPLIED**

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm or 620 nm
- Incubator at 37°C
- Multi- and single-channel pipettes to deliver volumes between 10 and 1,000 µL
- Optional: Automatic plate washer for rinsing wells.
- Rotating mixer
- Deionised or (freshly) distilled water.
- Disposable tubes
- Timer
- Absorbent paper or paper towel.
7. **LIMITATIONS**

- ELISA kit intended for research use only. Not for use in diagnostic procedures.
- All components of Human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate, without splashing, accurately to the bottom of wells.
- Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
8. **TECHNICAL HINTS**

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background
- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**
9. **REAGENT PREPARATION**
Equilibrate all reagents, samples and controls to room temperature (18-25°C) prior to use.

9.1 **Follicle Stimulating Hormone Standards**
Reconstitute each lyophilized Follicle Stimulating Hormone Standards with 1.0 mL distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. Reconstituted standards will be stable for up to 30 days when stored sealed at 2-8°C.

- All other solutions are supplied ready to use

10. **SAMPLE COLLECTION AND STORAGE**
- Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.
- The determination of Follicle Stimulating Hormone can be performed in Human serum. Microbiologically contaminated, highly lipemic or haemolysed should not be used in the assay. If the assay is performed on the same day of sample collection, the specimen should be kept at 2-8°C; otherwise it should be aliquoted and stored deep-frozen (-20°C). If samples are stored frozen, mix thawed samples gently for 5 minutes before testing.

_Avoid repeated freezing and thawing_
11. **PLATE PREPARATION**

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For each assay performed, a minimum of 1 well must be used as a blank, omitting sample and conjugate from well addition.
- For statistical reasons, we recommend each standard and sample should be assayed with a minimum of two replicates (duplicates).
ASSAY PROCEDURE

12. ASSAY PROCEDURE

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described.
- If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of washing solution from 300 µL to 350 µL to avoid washing effects.
- Assay all standards, controls and samples in duplicate.

13.1. Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

13.3. Add 50 µL standards, control and samples into their respective wells.

13.4. Add 100 µL of Enzyme Conjugate into each well. Mix gently for 30 seconds.

   Note: Complete mixing is essential for good assay performance

13.5. Cover wells with the foil supplied in the kit and incubate at room temperature for 45 minutes.

13.6. Remove the foil, aspirate the contents of the wells and wash each well five times with 300 µL of deionized or distilled water. Avoid spill over into neighboring wells. The soak time between each wash cycle should be >5 sec. After the last wash, remove the remaining deionized or distilled water by aspiration or decanting. Invert the plate and blot it against clean paper towels to remove excess liquid.

   Note: Complete removal of liquid at each step is essential for good assay performance.
13.7. Add 100 µL TMB Reagent into each well. Gentle mix for 10 seconds.

13.8. Incubate at room temperature for 20 minutes in the dark.

13.9. Stop the reaction by adding 100 µL of Stop Solution to each well. Mix gently for 30 seconds.

   Note: It is important to make sure that all the blue color changes to yellow color completely.

13.10. Measure the absorbance of the sample at 450 nm within 15 minutes of addition of the Stop Solution.
13. **CALCULATIONS**

Calculate the mean background subtracted absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (e. g. Four Parameter Logistic).

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in mU/mL.
14. **TYPICAL DATA**

**TYPICAL STANDARD CURVE** – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

<table>
<thead>
<tr>
<th>Conc. (mIU/mL)</th>
<th>Mean O.D. (-Blank)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.058</td>
</tr>
<tr>
<td>5</td>
<td>0.133</td>
</tr>
<tr>
<td>15</td>
<td>0.265</td>
</tr>
<tr>
<td>50</td>
<td>0.782</td>
</tr>
<tr>
<td>100</td>
<td>1.483</td>
</tr>
<tr>
<td>200</td>
<td>2.885</td>
</tr>
</tbody>
</table>
15. **TYPICAL SAMPLE VALUES**

**REFERENCE VALUES**-
Based on random selected outpatient clinical laboratory samples, the mean FSH values in males (N=100) and females (N=150) are 11 and 12 mIU/mL, respectively. The mean FSH values in post-menopausal (N=60) and pregnant females (N=60) are 94 and 1.0 mIU/mL, respectively.

**SENSITIVITY** –
The minimum detectable concentration of Follicle Stimulating Hormone by this assay is estimated to be 2.5 mIU/mL.

16. **ASSAY SPECIFICITY**
This kit detects Follicle Stimulating Hormone in Human samples. Other species have not yet been tested with this kit.
## 17. TROUBLESHOOTING

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low signal</td>
<td>Incubation time too short</td>
<td>Try overnight incubation at 4 °C</td>
</tr>
<tr>
<td></td>
<td>Precipitate can form in wells upon substrate addition when concentration of target is too high</td>
<td>Increase dilution factor of sample</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
<tr>
<td></td>
<td>Sample prepared incorrectly</td>
<td>Ensure proper sample preparation/dilution</td>
</tr>
<tr>
<td>Large CV</td>
<td>Bubbles in wells</td>
<td>Ensure no bubbles present prior to reading plate</td>
</tr>
<tr>
<td></td>
<td>All wells not washed equally/thoroughly</td>
<td>Check that all ports of plate washer are unobstructed/wash wells as recommended</td>
</tr>
<tr>
<td></td>
<td>Incomplete reagent mixing</td>
<td>Ensure all reagents/master mixes are mixed thoroughly</td>
</tr>
<tr>
<td></td>
<td>Inconsistent pipetting</td>
<td>Use calibrated pipettes &amp; ensure accurate pipetting</td>
</tr>
<tr>
<td></td>
<td>Inconsistent sample preparation or storage</td>
<td>Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)</td>
</tr>
<tr>
<td>Problem</td>
<td>Cause</td>
<td>Solution</td>
</tr>
<tr>
<td>------------------------</td>
<td>--------------------------------------</td>
<td>----------------------------------------------------</td>
</tr>
<tr>
<td>High background</td>
<td>Wells are insufficiently washed</td>
<td>Wash wells as per protocol recommendations</td>
</tr>
<tr>
<td></td>
<td>Contaminated wash buffer</td>
<td>Make fresh wash buffer</td>
</tr>
<tr>
<td></td>
<td>Waiting too long to read plate after</td>
<td>Read plate immediately after adding stop solution</td>
</tr>
<tr>
<td></td>
<td>adding stop solution</td>
<td></td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of ELISA kit</td>
<td>Store all reagents as recommended. Please note all</td>
</tr>
<tr>
<td></td>
<td></td>
<td>reagents may not have identical storage requirements.</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g.</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
<tr>
<td></td>
<td>Serum vs. cell extract)</td>
<td></td>
</tr>
</tbody>
</table>
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