ab108678

Follicle Stimulating Hormone Human ELISA Kit

Instructions for Use

For the quantitative measurement of Human Follicle Stimulating Hormone (FSH) concentration in serum.

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

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1. **Introduction**

ab108678 Follicle Stimulating Hormone Human ELISA Kit is an immunoenzymatic colorimetric method for quantitative determination of Follicle Stimulating Hormone in serum.

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 (hCG)] 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 steroidogenesis. All ovulatory menstrual cycles have a characteristic pattern of FSH, as well as LH, secretion.
2. Assay Summary

In the ab108678 method, calibrators, specimens and/or controls (containing the native FSH antigen) are first added to streptavidin coated wells.

Biotinylated monoclonal and horseradish peroxidase (HRP) labeled antibodies are added and the reactants are mixed. The different types of antibodies used have high affinity and specificity and are directed against distinct and different epitopes of FSH. Reaction between the various FSH antibodies and native FSH occurs in the microwells without competition or steric hindrance, forming a soluble sandwich complex.

Simultaneously, the complex is fixed to the well through the high affinity reaction of streptavidin and biotinylated antibody. After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by aspiration.

The activity of the conjugated HRP is quantified by reaction with TMB substrate to produce blue color. The reaction is terminated by adding stop solution which turns the blue color into yellow. The absorbance is measured on a plate reader.
3. Kit Contents

- Coated Wells: 12 break apart 8-well snap-off strips coated with Streptavidin, in resealable aluminium foil.
- Stop Solution: 1 bottle containing 15 ml sulphuric acid, 0.15 mol/l (avoid any skin contact).
- Anti-FSH conjugate: 1 bottle containing 12 ml of horseradish peroxidase labeled anti-FSH antibodies (polyclonal) and biotinylated monoclonal anti-FSH antibodies.
- TMB Substrate Solution: 1 bottle containing 15 ml 3, 3’, 5, 5’-tetramethylbenzidine (H₂O₂-TMB 0.25g/l) (avoid any skin contact).
- Wash solution 50x conc.: 1 bottle containing 20 ml, 50 mM phosphate buffer (pH 7.4, Tween20 1 g/l).
- FSH Control: 1 bottle containing 1 ml of a lot-specific, ready to use control. The concentration is mentioned on the label.
- FSH Standards: 6 bottles, 1 ml each: The standards are calibrated against the (WHO 2nd IRP 78/549) and have approx. the following concentrations:
  - Standard 0: 0 mIU/ml
  - Standard 1: 5 mIU/ml
  - Standard 2: 10 mIU/ml
  - Standard 3: 25 mIU/ml
  - Standard 4: 50 mIU/ml
  - Standard 5: 100 mIU/ml
- 1 Strip holder
4. Storage and Handling

The closed reagents are stable up to the expiry date stated on the label when stored at 2 - 8 °C in the dark. Opened reagents are stable for 60 days when stored at 2 - 8°C.

5. Additional Materials Required

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450 nm
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 50 μl (precision better than 1.5%)
- Dispenser for repetitive deliveries of 100μl and 300 μl volumes with a precision better than 1.5%
- Vortex tube mixer
- Distilled water
- Quality control material
- Timer
6. Preparation of Reagents

It is very important to bring all reagents, samples and standards to room temperature (22-28°C) before starting the test run.

1. **Coated snap-off Strips:** The ready to use break apart snap-off strips are coated with Streptavidin. Store at 2-8°C. Open the bag only when it is at room temperature. *Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2-8°C. Do not remove the adhesive sheets on the unused strips.*

2. **Anti-FSH Conjugate:** The bottle contains 13 ml of a ready-to-use conjugate mixture of HRP-labeled polyclonal anti-FSH antibodies and biotinylated monoclonal anti-FSH antibodies.

3. **FSH Standards:** Each of the 6 vials contains 1 ml standard solution of the concentration mentioned in Kit Contents. The standards are ready to use.

4. **TMB Substrate Solution:** The bottle contains 12 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2-8°C in the dark. *The solution should be colorless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away. After first use the TMB substrate solution is still stable for another 6 months if stored at 2-8 °C.*
5. **Stop Solution:** The bottle contains 12 ml 0.15 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2-8°C. *After first use stable until expiry date.*

6. **Wash Solution:** Dilute the 50x concentrated wash solution to 1000 ml with distilled or deionised water in a suitable storage container.

**7. Preparation and Collection of Specimen**

Use human serum as samples with this assay. The blood should be collected in a venipuncture tube without additives or anticoagulants. 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 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 repetitive freezing and thawing.

For accurate comparison to established normal values, a fasting morning serum sample should be obtained.

**Precaution:**

- The reagents contain Proclin 300® as preservative
- Do not use heavily haemolysed or highly lipemic samples.
Maximum precision is required for reconstitution and dispensation of the reagents.

This method allows the determination of FSH from 0.6 to 100 mIU/ml.

8. Assay Method

Test Preparation

Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and standards should be carefully established. Select the required number of microtiter strips or wells and insert them into the holder. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than one plate is used, it is recommended to repeat the dose response curve.

Please allocate at least:

- 1 well (e.g. A1) for the substrate blank
- 2 wells (e.g. B1+C1) for standard 0
- 2 wells (e.g. D1+E1) for standard 1
- 2 wells (e.g. F1+G1) for standard 2
- 2 wells (e.g. H1+A2) for standard 3
- 2 wells (e.g. B2+C2) for standard 4
- 2 wells (e.g. D2+E2) for standard 5
- 2 wells (e.g. F2+G2) for control
It is recommended to determine standards and samples in duplicate.

Perform all assay steps in the order given and without any appreciable delays between the steps.

A clean, disposable tip should be used for dispensing each standard and each sample.

Assay Procedure:

1. Dispense 50 μl standards and samples into their respective wells. Add 100 μl conjugate to each well. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour at room temperature (22 – 28 °C).**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 μl diluted wash solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
   
   *Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values*
5. Dispense 100 μl TMB Substrate Solution into all wells.
6. **Incubate for exactly 15 min at room temperature (22 – 28°C) in the dark.**
7. Dispense 100 μl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. 
   Any blue color developed during the incubation turns into yellow.

8. Measure the absorbance of the specimen at 450 nm within 30 min after addition of the Stop Solution.

Measurement:

Adjust the ELISA Microwell Plate Reader to zero using the substrate blank in well A1.

If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at 450 nm and record the absorbance values for each standard and sample.

Where applicable calculate the mean absorbance values of all duplicates.

Quality control:

Each laboratory should assay controls at normal, high and low levels range of FSH for monitoring assay performance. These controls should be treated as unknowns and values determined in every test
procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

9. Data Analysis

A. Validity of the assay

The optical density (OD) of standard 5 should be $\geq 1.3$

B. OD Conversion

The optical densities (O.D.) of some standards and samples may be higher than 2.0, in such a case, they could be out of the measurement range of the microplate reader. It is therefore necessary, for O.D.s higher than 2.0, to perform a reading at 405
nm (=wavelength of peak shoulder) in addition to 450 nm (peak wavelength) and 620 (reference filter for the subtraction of interferences due to the plastic).

For microplate readers unable to read the plate at 3 wavelengths at the same time, it is advisable to proceed as follows:
- Read the microplate at 450 nm and at 620 nm.
- Read again the plate at 405 nm and 620 nm.
- Find out the wells whose ODs at 450 nm are higher than 2.0
- Select the corresponding ODs read at 405 nm and multiply these values at 405 nm by the conversion factor 3.0 (where OD 450/OD 405 = 3.0), that is: OD 450 nm = OD 405 nm x 3.0

Warning: The conversion factor 3.0 is suggested only. For better accuracy, the user is advised to calculate the conversion factor specific for his own reader.

C. Calculation

Automated method

Use the 4 parameter logistic (preferred) or the smoothed cubic spline function as calculation algorithm.

Manual method

A dose response curve is used to ascertain the concentration of follicle stimulating hormone in unknown specimens.
1. Record the OD obtained from the printout of the microplate reader as outlined in Example 1.

2. Plot the OD for each duplicate calibrator versus the corresponding FSH concentration in mIU/ml on linear graph paper (do not average the duplicates of the serum references before plotting).

3. Draw the best-fit curve through the plotted points.

4. To determine the concentration of FSH for an unknown, locate the average OD of the duplicates for each 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).

NOTE: The data presented in Example 1 are for illustration only and should not be used in lieu of a standard curve prepared with each assay. Assigned values for standards are lot specific.
## Example 1

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Well number</th>
<th>OD</th>
<th>Mean OD</th>
<th>Value mIU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 0</td>
<td>A1</td>
<td>0.092</td>
<td>0.072</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>B1</td>
<td>0.053</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard 1</td>
<td>C1</td>
<td>0.375</td>
<td>0.371</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>D1</td>
<td>0.367</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard 2</td>
<td>E1</td>
<td>0.573</td>
<td>0.581</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>F1</td>
<td>0.588</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard 3</td>
<td>G1</td>
<td>1.412</td>
<td>1.301</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>H1</td>
<td>1.190</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard 4</td>
<td>A2</td>
<td>1.923</td>
<td>1.944</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>B2</td>
<td>1.965</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard 5</td>
<td>C2</td>
<td>2.739</td>
<td>2.694</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>D2</td>
<td>2.650</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>E2</td>
<td>0.714</td>
<td>0.697</td>
<td>13.89</td>
</tr>
<tr>
<td></td>
<td>F2</td>
<td>0.6881</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td>G2</td>
<td>1.671</td>
<td>1.627</td>
<td>43.8</td>
</tr>
<tr>
<td></td>
<td>H2</td>
<td>1.496</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
D. Reference Values

Each laboratory must establish its own normal ranges based on population.

The serum or plasma FSH values are comprised in the following intervals:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Range mIU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>1 - 4</td>
</tr>
<tr>
<td>Female:</td>
<td></td>
</tr>
<tr>
<td>Follicular phase</td>
<td>3 - 12</td>
</tr>
<tr>
<td>Midcycle</td>
<td>8 - 22</td>
</tr>
<tr>
<td>Luteal phase</td>
<td>2 – 12</td>
</tr>
<tr>
<td>Menopausal</td>
<td>35 - 151</td>
</tr>
</tbody>
</table>

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 tests 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 analyst using the method
with a population indigenous to the area in which the laboratory is located.

E. Sensitivity

The lowest detectable concentration of FSH by this assay is 0.6 mIU/ml.

F. Reproducibility

Intra-Assay: Within-run precision was determined by replicate determinations of three different control sera in one assay. The within assay variability is shown.

<table>
<thead>
<tr>
<th>Serum Sample</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Replicates</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean FSH (mIU/ml)</td>
<td>5.4</td>
<td>15.6</td>
<td>37.9</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.46</td>
<td>1.32</td>
<td>3.56</td>
</tr>
<tr>
<td>Coefficient of Variation (%)</td>
<td>9.6</td>
<td>8.5</td>
<td>9.4</td>
</tr>
</tbody>
</table>
Inter-Assay: Between-run precision was determined by replicate measurements of three different control sera in several different assays. The between assay variability is shown below.

<table>
<thead>
<tr>
<th>Serum Sample</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Replicates</td>
<td>10</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean FSH (mIU/ml)</td>
<td>5.9</td>
<td>16.9</td>
<td>35.3</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.66</td>
<td>1.59</td>
<td>4.16</td>
</tr>
<tr>
<td>Coefficient of Variation (%)</td>
<td>11.2</td>
<td>9.4</td>
<td>11.8</td>
</tr>
</tbody>
</table>

10. Limitations

- Procedural directions must be followed exactly and careful technique must be used to obtain valid results. Any modification of the procedure is likely to alter the results. FSH is dependent upon diverse factors other than pituitary homeostasis.
- FSH is suppressed by estrogen but in woman taking contraceptives the level may be low or normal. Excessive dieting and weight loss may lead to low gonadotropin concentration.
- Specimens with abnormally high FSH levels can cause a hook effect, that is, paradoxical low absorbance results. If this is
suspected, dilute the specimen 1/100 with standard 0, re-assay (multiply the result by 100). However, values as high as 2000 mIU/ml have been found to absorb greater than the absorbance of the highest calibrator.

- Those receiving preparations of mouse monoclonal antibodies for diagnosis of therapy may contain human anti-mouse antibodies (HAMA) and may show either falsely elevated or depressed values when assayed.
- Samples with an expected FSH concentration of > 100 mIU/ml should be diluted with standard 0 before testing.
11. Specificity

The cross-reactivity of ab108678 Follicle Stimulating Hormone Human ELISA Kit 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 OD.

<table>
<thead>
<tr>
<th>Material Tested</th>
<th>Cross Reactivity</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follitropin (FSH)</td>
<td>1.0000</td>
<td>---</td>
</tr>
<tr>
<td>Lutropin Hormone (hLH)</td>
<td>&lt;0.0001</td>
<td>1000ng/ml</td>
</tr>
<tr>
<td>Chorionic Gonadotropin (hCG)</td>
<td>&lt;0.0001</td>
<td>1000ng/ml</td>
</tr>
<tr>
<td>Tireotropin (TSH)</td>
<td>&lt;0.0001</td>
<td>1000ng/ml</td>
</tr>
</tbody>
</table>
## 12. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>Improper standard dilution</td>
<td>Confirm dilutions made correctly</td>
</tr>
<tr>
<td></td>
<td>Standard improperly reconstituted (if applicable)</td>
<td>Briefly spin vial before opening; thoroughly resuspend powder (if applicable)</td>
</tr>
<tr>
<td></td>
<td>Standard degraded</td>
<td>Store sample as recommended</td>
</tr>
<tr>
<td></td>
<td>Curve doesn't fit scale</td>
<td>Try plotting using different scale</td>
</tr>
<tr>
<td>Low signal</td>
<td>Incubation time too short</td>
<td>Try overnight incubation at 4 °C</td>
</tr>
<tr>
<td></td>
<td>Target present below detection limits of assay</td>
<td>Decrease dilution factor; concentrate samples</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>Issue</td>
<td>Solution</td>
<td></td>
</tr>
<tr>
<td>--------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>All wells not washed equally/thoroughly</td>
<td>Check that all ports of plate washer are unobstructed/wash wells as recommended</td>
<td></td>
</tr>
<tr>
<td>Incomplete reagent mixing</td>
<td>Ensure all reagents/master mixes are mixed thoroughly</td>
<td></td>
</tr>
<tr>
<td>Inconsistent pipetting</td>
<td>Use calibrated pipettes and ensure accurate pipetting</td>
<td></td>
</tr>
<tr>
<td>Inconsistent sample preparation or storage</td>
<td>Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)</td>
<td></td>
</tr>
<tr>
<td>High background</td>
<td>Wells are insufficiently washed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wash wells as per protocol recommendations</td>
<td></td>
</tr>
<tr>
<td>Contaminated wash buffer</td>
<td>Make fresh wash buffer</td>
<td></td>
</tr>
<tr>
<td>Waiting too long to read plate after adding STOP solution</td>
<td>Read plate immediately after adding STOP solution</td>
<td></td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of ELISA kit</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Store all reagents as recommended. Please note all reagents may not have identical storage requirements.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. Serum vs. cell extract)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Detection may be reduced or absent in untested sample types</td>
<td></td>
</tr>
</tbody>
</table>

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select “contact us” on www.abcam.com for the phone number for your region).
ab108678 Follicle Stimulating Hormone Human ELISA Kit
Abcam in the USA
Abcam Inc
1 Kendall Square, Ste B2304
Cambridge,
MA 02139-1517
USA

Toll free: 888-77-ABCAM (22226)
Fax: 866-739-9884

Abcam in Japan
Abcam KK
1-16-8 Nihonbashi
Kakigaracho,
Chuo-ku, Tokyo
103-0014
Japan

Tel: +81-(0)3-6231-094
Fax: +81-(0)3-6231-0941

Abcam in Europe
Abcam plc
330 Cambridge Science Park
Cambridge
CB4 0FL
UK

Tel: +44 (0)1223 696000
Fax: +44 (0)1223 771600

Abcam in Hong Kong
Abcam (Hong Kong) Ltd
Unit 225A & 225B, 2/F
Core Building 2
1 Science Park West Avenue
Hong Kong Science Park
Hong Kong

Tel: (852) 2603-682
Fax: (852) 3016-1888

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