ab108651

Luteinizing Hormone
Human ELISA Kit

Instructions for Use

For the quantitative measurement of Luteinizing Hormone (LH) concentration in Human Serum

This product is for research use only and is not intended for diagnostic use.
# Table of Contents

1. Introduction .................................................. 3

2. Assay Summary ............................................. 4

3. Kit Contents ................................................. 5

4. Storage and Handling ...................................... 5

5. Additional Materials Required ......................... 6

6. Preparation of Reagents .................................. 6

7. Preparation and Collection of Specimen .............. 7

8. Assay Method ................................................ 7

9. Data Analysis ............................................... 9

10. Limitations ................................................ 12

11. Troubleshooting .......................................... 13
1. Introduction

ab108651 Luteinizing Hormone Human ELISA Kit is intended for the quantitative determination of luteinizing hormone (LH) concentration in human serum.

Luteinizing hormone (LH) is produced in both men and women from the anterior pituitary gland in response to luteinizing hormone-releasing hormone (LH-RH or Gn-RH), which is released by the hypothalamus. LH, also called interstitial cell-stimulating hormone (ICSH) in men, is a glycoprotein with a molecular weight of approximately 30,000 daltons. It is composed of two noncovalently associated dissimilar amino acid chains, alpha and beta. The alpha chain is similar to that found in human thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), and human chorionic gonadotropin (hCG). The differences between these hormones lie in the amino acid composition of their beta subunits, which account for their immunological differentiation.

The basal secretion of LH in men is episodic and has the primary function of stimulating the interstitial cells (Leydig cells) to produce testosterone. The variation in LH concentrations in women is subject to the complex ovulatory cycle of healthy menstruating women, and depends on a sequence of hormonal events along the gonado-hypothalamic-pituitary axis.
2. Assay Summary

*ab108651 is based on the principle of a solid phase enzyme-linked immunosorbent assay.*

The assay system utilizes a mouse monoclonal anti-α-LH antibody for solid phase (microtiter wells) immobilization and a mouse monoclonal anti-β-LH antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution.

The test sample is allowed to react simultaneously with the antibodies, resulting in LH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 45 minute incubation at room temperature, the wells are washed with water to remove unbound-labeled antibodies.

A solution of tetramethylbenzidine (TMB) reagent is added and incubated for 20 minutes, resulting in the development of a blue color.

The color development is stopped with the addition of Stop Solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of LH is directly proportional to the color intensity of the test sample.
3. Kit Contents

- Mouse monoclonal anti-α LH antibody coated microtiter plate with 96 wells.
- Enzyme Conjugate Reagent, 13 ml.
- LH reference standards, Lyophilized;
  - LH Reference Standard 0 (0 mIU/ml)
  - LH Reference Standard 1 (5 mIU/ml)
  - LH Reference Standard 2 (15 mIU/ml)
  - LH Reference Standard 3 (50 mIU/ml)
  - LH Reference Standard 4 (100 mIU/ml)
  - LH Reference Standard 5 (200 mIU/ml)
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 11 ml.

4. Storage and Handling

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above.
5. Additional Materials Required

- Distilled or deionized water
- Precision pipettes: 50 μl, 100 μl and 1.0 ml
- Disposable pipette tips
- Microtiter plate reader at 450 nm wavelength, with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater.
- Vortex mixer, or equivalent
- Absorbent paper or paper towel
- Graph paper

6. Preparation of Reagents

1. All reagents should be allowed to reach room temperature (18-25°C) before use.

2. Reconstitute each lyophilized Standard 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. Discard the reconstituted Standards after 8 hours.

7. Preparation and Collection of Specimen

1. Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques.
2. This kit is for use with serum samples without additives only.

8. Assay Method

Assay Procedure:

1. Secure the desired number of coated wells in the holder.
2. Dispense 50 µl of standard, specimens, and controls into appropriate wells.
3. Dispense 100 µl of Enzyme Conjugate Reagent into each well.
4. Gently mix for 30 seconds. It is very important to have complete mixing in this setup.
5. Incubate at room temperature (18-25°C) for 45 minutes.
6. Remove the incubation mixture by flicking plate contents into sink.
7. Rinse and flick the microtiter wells 5 times distilled or deionized water. (Please do not use tap water.)
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.

9. Dispense 100 μl TMB Reagent into each well. Gently mix for 10 seconds.

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

11. Stop the reaction by adding 100 μl of Stop Solution to each well.

12. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.

13. Read the optical density at 450 nm with a microtiter plate reader within 15 minutes.
9. Data Analysis

1. Calculate the mean absorbance value ($A_{450}$) for each set of reference standards, controls and samples.

2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in mIU/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.

3. Using the mean absorbance value for each sample, determine the corresponding concentration of LH in mIU/ml from the standard curve.

A. Typical Data

Results of a typical standard run with absorbency readings at 450nm shown on the Y axis against LH concentrations shown on the X axis.

NOTE: This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each laboratory must provide its own data and standard curve in each experiment.
<table>
<thead>
<tr>
<th>LH (mIU/ml)</th>
<th>Absorbance (450 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.043</td>
</tr>
<tr>
<td>5</td>
<td>0.148</td>
</tr>
<tr>
<td>15</td>
<td>0.328</td>
</tr>
<tr>
<td>50</td>
<td>0.947</td>
</tr>
<tr>
<td>100</td>
<td>1.656</td>
</tr>
<tr>
<td>200</td>
<td>2.704</td>
</tr>
</tbody>
</table>
B. Sensitivity

The minimum detectable concentration of the human luteinizing hormone by this assay is estimated to be 1 mlU/ml.

Each laboratory must establish its own normal ranges based on population. The information provided should be considered only as a guideline.

<table>
<thead>
<tr>
<th>Adult</th>
<th>mlU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>1.24-7.8</td>
</tr>
</tbody>
</table>

Female

<table>
<thead>
<tr>
<th>Phase</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular phase:</td>
<td>1.68-15</td>
</tr>
<tr>
<td>Ovulatory peak:</td>
<td>21.9-56.6</td>
</tr>
<tr>
<td>Luteal phase:</td>
<td>0.61-16.3</td>
</tr>
<tr>
<td>Postmenopausal:</td>
<td>14.2-52.3</td>
</tr>
</tbody>
</table>
10. Limitations

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
- Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
## 11. 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>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 adding STOP solution</td>
<td>Read plate immediately after adding STOP solution</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>--------------------------------------</td>
<td>-------------------------------------------------</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 and 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>Low sensitivity</td>
<td>Improper storage of ELISA kit</td>
<td>Store all reagents as recommended. Please note all reagents may not have identical storage requirements.</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>
</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).
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