LH

Enzyme immunoassay for the quantitative determination of LH in human serum

Only for in-vitro diagnostic use

Product Number: DNOV030 (96 Determinations)
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1. INTRODUCTION

Luteinizing hormone (LH) is a glycoprotein consisting of two subunits with a molecular mass of 30,000 daltons. The \( \alpha \)-subunit is similar to other pituitary hormones [follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH) and chorionic gonadotropin (HCG)] while the \( \beta \)-subunit is unique. The \( \beta \)-subunit confers the biological activity to the molecule. The \( \alpha \)-subunit consists of 89 amino acid residues while the \( \beta \)-subunit contains 129 amino acids. The carbohydrate content is between 15% and 30%. The clinical usefulness of the measurement of luteinizing hormone (LH) in ascertaining the homeostasis of fertility regulation via the hypothalamic-pituitary-gonadal axis has been well established (1,2). In addition, the advent of in vitro fertilization (IVF) technology to overcome infertility-associated problems has provided the impetus for rapid improvement in LH assay methodology from the technically demanding bioassay to the procedurally simple and rapid immunoenzymometric assays.

2. INTENDED USE

Immunoenzymatic colorimetric method (ELISA) for quantitative determination of LH in human serum.

3. PRINCIPLE OF THE ASSAY

In this method, LH standards, patient specimens and/or controls (containing the native antigen) are first added to streptavidin coated wells. Biotinylated monoclonal and enzyme labeled antibodies are added and the reactants mixed: these antibodies have high affinity and specificity and are directed against distinct and different epitopes of LH. Reaction between the various LH antibodies and native LH occurs in the microwells without competition or steric hindrance forming a soluble sandwich complex.

The interaction is illustrated by the following equation:

\[
\text{EnzAb + Ag LH + BtnAb(m)} \leftrightarrow \text{EnzAb - AgLH-BtnAb(m)}
\]

\( \text{ka} \) = Rate Constant of Association

\( \text{k-a} \) = Rate Constant of Dissociation

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

\[
\text{EnzAb -AgLH-BtnAb(m) + StreptavidinC.W.} \rightarrow \text{Immobilized complex}
\]

Streptavidin C.W. = Streptavidin immobilized on well.

Immobilized complex = Antibodies-Antigen sandwich bound.

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. The activity of the enzyme present on the surface of the well quantitated by reaction with a suitable substrate to produce colour. By utilizing several different calibrators of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4. MATERIALS

4.1. Reagents supplied

- **Coated Wells**: 12 breakapart 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), ready to use.
- **Anti-LH-HRP conjugate + Anti LH-Biotin conjugate**: 1 bottle containing 12 ml of horseradish peroxidase labelled anti-LH antibodies and Biotin labelled anit-LH antibodies, ready to use.
- **TMB Substrate Solution**: 1 bottle containing 15 ml 3, 3´, 5, 5´-tetramethylbenzidine (H\(_2\)O\(_2\)-TMB 0.26g/l) (avoid any skin contact), ready to use.
- **Wash solution 50x conc**: one bottle containing 20 ml NaCl 45 g/l, Tween-20 55 g/l)
- **LH control**: one bottle containing 1 ml of a lot-specific control solution. The concentration is mentioned on the label.
LH Standards: 6 bottles, 1 ml each and have approx. the following concentrations:

- Standard 0: 0 mIU/ml
- Standard 1: 5 mIU/ml
- Standard 2: 25 mIU/ml
- Standard 3: 50 mIU/ml
- Standard 4: 100 mIU/ml
- Standard 5: 200 mIU/ml

4.2. Materials supplied

- 1 Strip holder
- 1 Cover foils
- 1 Test protocol
- 1 Distribution and identification plan

4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450, 405 and 620 nm
- Pipettes to deliver 50 µl with a precision better than 1.5%
- Dispenser(s) for repetitive deliveries of 0.100 ml and 0.300 ml volumes with a precision of better than 1.5%.
- Microplate automatic washer or a squeeze bottle (optional).
- Distilled or deionized water and a clean one litre cylinder for the dilution of the wash solution
- Absorbent Paper for blotting the microplate wells.
- Vacuum aspirator (optional) for wash steps.
- Timer
- Quality control materials.

5. STABILITY AND STORAGE

The originally 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.

6. REAGENT PREPARATION

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

6.1. Coated snap-off Strips

The ready to use break apart snap-off strips are coated with Streptavidin. 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.

6.2. LH Control

The bottle contains 1 ml of a lot-specific control solution. The concentration is indicated on the label.

6.3. Standards

Each of the 6 vials contains 1 ml standard solution of the concentration mentioned in 4.1. The standards are ready to use.

6.4. TMB Substrate Solution

The bottle contains 15 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 colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

6.5. Wash Solution

Dilute contents of wash buffer concentrate 50x to 1000 ml with distilled or deionised water in a suitable storage container. For smaller volumes respect the 1:50 ratio. The diluted wash solution is stable for 30 days at 2…8°C.

7. SPECIMEN COLLECTION AND PREPARATION

- The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be followed.
- For accurate comparison to established normal values, a fasting morning serum sample should be obtained.
- The blood should be collected in a 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 5 days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at -20°C for up to 30 days.
- Avoid repetitive freezing and thawing.
- When assayed in duplicate, 40 µl of the specimen is required.
7.1. Precaution

- Maximum precision is required for dispensation of the reagents.
- This method allows the determination of LH from 0.5 mIU/ml to 200 mIU/ml.
- Samples with concentration of LH higher than 200 mIU/ml have to be diluted with standard 0.

8. ASSAY PROCEDURE

8.1. 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 on the result sheet supplied in the kit. 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+E9) for standard 5
2 wells (e.g. E9+F9) for LH control

It is recommended to determine standards and patient 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 patient sample.

1. Dispense 20 µl standards, control and samples into their respective wells. Add 100 µl conjugate to each well. Leave well A1 for substrate blank.
2. **Incubate for 1 hour at room temperature (22…28°C).**
3. When incubation has been completed, aspirate the content of the wells and wash each well three times with 300 µl diluted wash solution. Avoid overflows from the reaction wells. 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.*
4. Dispense 100 µl TMB Substrate Solution into all wells.
5. **Incubate for exactly 15 min at room temperature (22…28°C) in the dark.**
6. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Shake the microplate gently.
   *Any blue colour developed during the incubation turns into yellow.*
7. Measure the absorbance of the specimen at 450 nm.

9. RESULTS

9.1. Validation

The OD of standard 5 should be ≥ 1.3.

9.2. OD Conversion

The optical densities (O.D.s) of some calibrators 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.

9.3. Calculation

**Automated method**

Use the 4 parameters logistic – preferred – or the smoothed cubic spline function as calculation algorithm.

**NOTE:** 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.
Manual method

- A dose response curve is used to ascertain the concentration of LH in unknown specimens.
- Record the OD obtained from the printout of the microplate reader as outlined in Example 1.
- Plot the OD for each duplicate calibrator versus the corresponding LH concentration in ng/ml on linear graph paper (do not average the duplicates of the calibrators before plotting).
- Draw the best-fit curve through the plotted points.
- To determine the concentration of LH for an unknown, locate the average OD of the duplicates for each unknown sample 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).

9.4. Reference values

The serum LH values are comprised in the following intervals:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>0.7 – 7.4 mIU/ml</td>
</tr>
<tr>
<td>Women</td>
<td></td>
</tr>
<tr>
<td>follicular</td>
<td>0.5 – 10.5 mIU/ml</td>
</tr>
<tr>
<td>Ovulation</td>
<td>18.4 – 61.2 mIU/ml</td>
</tr>
<tr>
<td>luteal phase</td>
<td>0.5 – 10.5 mIU/ml</td>
</tr>
<tr>
<td>menopause</td>
<td>8.2 – 40.8 mIU/ml</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 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.

10. QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of LH 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.

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.

11. SPECIFIC PERFORMANCE CHARACTERISTICS

11.1. Precision

Intra Assay Variation
Within-run precision was determined by replicate determinations (20x) of three different control sera in one assay. The within-assay variability is ≤ 9.21%.

Inter Assay Variation
Between-run precision was determined by replicate measurements of three different control sera in 15 different assays. The between-assay variability is ≤ 7.91%.

11.2. Accuracy

The recovery test performed on three different samples, enriched with 5.63 - 11.25 - 22.5 - 45 - 90 mIU/ml of LH, gave a average value (±SD) of 97.17% ± 4.00%.

In the dilution test three different samples were diluted 2, 4, 8 and 16 times with Standard 0; the average value (±SD) obtained is 99.13% ± 7.37%.

11.3. Specificity

The cross-reactivity of the NovaTec LH 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 Luteinizing Hormone needed to produce the same absorbance.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH</td>
<td>100%</td>
</tr>
<tr>
<td>b-HCG</td>
<td>0.007%</td>
</tr>
<tr>
<td>HCG</td>
<td>None detected</td>
</tr>
<tr>
<td>FSH</td>
<td>None detected</td>
</tr>
<tr>
<td>TSH</td>
<td>None detected</td>
</tr>
</tbody>
</table>
11.4. Sensitivity
The minimal detectable concentration of LH by this assay is estimated to be 0.22 mIU/ml.

11.5. Correlation
The NovaTec LH kit was compared to a commercially available LH kit. 36 serum samples were tested. The regression curve is:

\[ \text{NovaTec} = 0.91 \times (\text{commercial kit}) + 0.05 \]
\[ R^2 = 0.971 \]

The new NovaTec LH kit was compared to the old NovaTec LH kit. 36 serum samples were tested. The regression curve is:

\[ (\text{NovaTec new}) = 1.08 \times (\text{NovaTec old}) - 1.22 \]
\[ R^2 = 0.981 \]

12. LIMITATIONS OF THE PROCEDURE
Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values.
LH 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. Luteinizing hormone is dependent upon diverse factors other than pituitary homeostasis. Thus, the determination alone is not sufficient to assess clinical status.

13. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- 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.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- 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.
- Avoid the exposure of TMB substrate to direct sunlight, metal or oxidants.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
- The ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

**WARNING:** Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!

13.1. Disposal Considerations
Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.
14. LITERATURE


15. ORDERING INFORMATION

Prod. No.: DNOV030 LH Determination (96 Determinations)
SCHEME OF THE ASSAY
LH

Test Preparation

Prepare reagents and samples as described.
Establish the distribution and identification plan for all specimens and controls on the result sheet supplied in the kit.
Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

<table>
<thead>
<tr>
<th></th>
<th>Substrate blank</th>
<th>Standard 0 - 5</th>
<th>Control</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 0 - 5</td>
<td>-</td>
<td>20 µl</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>20 µl l</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20 µl</td>
</tr>
<tr>
<td>Conjugate</td>
<td>-</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

**Incubate for 1 h at room temperature (+22 - +28 °C)**
Wash each well three times with 300 µl diluted wash solution

<table>
<thead>
<tr>
<th></th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
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</thead>
<tbody>
<tr>
<td>TMB Substrate</td>
<td></td>
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</table>

**Incubate for exactly 15 min at room temperature in the dark**

<table>
<thead>
<tr>
<th></th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stop Solution</td>
<td></td>
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Shake the microplate gently
Photometric measurement at 450 nm

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