



## LH Elisa

### Enzyme-Linked Immunosorbent Assay for Quantitative Determination of Luteinizing Hormone in Human Serum

#### INTENDED USE

"LH" kit is intended for the quantitative determination of luteinizing hormone (LH) in human serum.

LH is a glycoprotein with a molecular weight of about 30 000 Da that consists of two subunits –  $\alpha$  and  $\beta$ . LH is secreted by frontal lobe of pituitary gland and stimulates testosterone synthesis in testicles. Alongside with other hormones, LH regulates the menstrual cycle in females.

Increased LH levels are observed in patients with different forms of hypogonadism (primary ovary or testicle insufficiency, polycystic ovary, menopause etc.) renal insufficiency and cirrhosis. Decreased LH levels, that may lead to sterility in both sexes, are observed in the case of dysfunctions in hypothalamus or frontal lobe of hypophysis.

LH measurement is useful for the diagnosis of menopause, exact determination of ovulation time and for endocrine therapy monitoring.

#### ASSAY DESCRIPTION AND PRINCIPLES

##### Kit Contents:

- 12 microtitration strips, 12x8 wells, coated with anti-LH monoclonal antibodies, packed in the plastic bag, 1 bag;
- LH calibrators (protein-based buffer containing known LH concentrations). Calibrators have been standardized against the WHO international Reference Preparation 68/40. Exact LH concentrations are indicated on vial labels, 6 vials (0.5 ml each or lyophilized preparations);
- Anti-LH antibodies conjugated with HRP, 1 vial, 14 ml;
- Concentrated wash solution, 1 vial, 20ml;
- Tetramethylbenzidine substrate solution, TMB solution, 1 vial, 14 ml;

- "Stop reagent", 1 vial, 14 ml;
- Control (protein-based buffer containing known LH concentration), labeled "Control", 1 vial (0.5 ml or lyophilized preparation).

LH reagents are sufficient for determination of 40 unknowns, 6 calibrators, 1 control, 1 TMB Substrate control in duplicates, provided that all the strips are used simultaneously.

*NOTE: if used partially, kit should be utilized within a month after opening.*

**Assay principle.** LH is a "sandwich" type of solid phase enzyme immunoassay, based on two monoclonal antibodies that are specific for different epitopes of LH molecule. One of these antibodies (anti-  $\alpha$ -subunit) is conjugated with horse radish peroxidase the other (anti-  $\beta$  subunit) is immobilized on inner surface of microwells. LH molecules from the serum sample bind to both immobilized antibodies and anti-LH-peroxidase conjugate. Then the wells are washed with wash buffer to remove any material not bound to the inner surface of the wells. The quantity of the bound conjugate. Then the wells are washed with wash buffer to remove any material not bound to the inner surface of the wells. The quantity of the bound conjugate is in direct proportion to the LH concentration in tested sample. During the incubation with Substrate solution the coloring appears. The color intensity is in direct proportion to the LH concentration in sample. Optical density of the solution in the wells is measured and the LH concentration in the samples is calculated using calibration curve.

#### PERFORMANCE CHARACTERISTICS

**Specificity.** No cross-interaction of anti- $\beta$ -LH monoclonal antibodies with FSH, TSH and hCG was detected.

**Coefficients of variation (intra-assay precision)** between the results of LH determination in the same sample is less than 8%.

**Linearity (Dilution test).** Dilution of serum sample containing predetermined LH concentration with LH-free serum leads to linear recovery of LH in diluted samples in the concentration range between calibrator N:2 and calibrator N:6.

**Recovery.** To determine this parameter, equal volumes of control and calibrator N:3 were mixed. Then the correspondence between the calculated LH concentration in the obtained sample and the measured concentration was determined. Recovery range is 90-110%.

**Detectability.** Minimal LH concentration detectable by A-LH assay is 0.3 mIU/ml.

**Expected values.** Serum samples collected between 9 and 11 a.m from apparently healthy people, both males

and females, at the age of 19-65 were assayed with LH kit (see table 1 for results).

These limits should be considered as guidelines only.

Group	N	Mean (mIU/ml)	Range (mIU/ml)
Females			
Normally menstruating (19-35 years old)	120		
Follicular phase		4.2	1.1-8.7
Midcycle peak		32.0	13.2-72
Luteal phase		5.3	<0.9-14.4
Postmenopausal (49-65 years old)	15	19.3	18.6-72
Males (21-39 years old)	40	3.9	0.8-8.4

It is highly recommended for each laboratory to determine its own reference range of LH concentrations.

#### WARNINGS AND PRECAUTIONS

1. All the components are non-toxic.
2. Stop reagent is 1N HCL solution. Avoid contacts with skin and mucosa. In case of contact rinse affected region thoroughly with plenty of water.
3. It is highly recommended to handle kit components in accordance with established good laboratory practice. The operator should wear disposable latex or plastic gloves and handle patients' samples as if capable of transmitting infectious agents.

#### MATERIALS REQUIRED BUT NOT SUPPLIES WITH THE KIT:

- Digital variable pipettes that cover volume range from 0.005 to 0.05 ml; form 0.04 to 0.2 ml to 1 ml and from 1 to 5 ml, with appropriate disposable tips;
- 8-channel digital variable pipette that covers volume range up to 0.3 ml, with appropriate disposable tips;
- Microplate shaker-thermostat, able to maintain temperature +37°C and shaking speed 500 to 800 rpm;
- Automatic microplate reader;
- 200ml volumetric cylinder;
- 300 ml volumetric beaker;
- Distilled water.

#### REAGENT PREPARATION FOR ASSAY

1. LH calibrators and control. Add 0.5ml of distilled water to vials with lyophilized calibrators and control, leave for 20 min at room temperature (+18...+25°C), then stir gently, avoiding foaming. Store at +2...+8°C for no more than 1 month. Liquid calibrators and control ready to use. Once opened, store at +2...+8°C for not more than 1 month.
2. Microtitration strips. Before opening keep the bag at room temperature (+18...+25°C) for 30 minutes open the bag and

place required number of strips on strip holder. Put remaining strips back in plastic bag and close tightly. Keep at +2... +8oC until expiry date stated on the label.

3. Wash buffer. Prepare the necessary volume of Wash buffer by dilution of Buffer 10-fold with distilled water. For example 5 ml of buffer+ 45 ml of distilled water. Mix thoroughly. Avoiding foaming .Keep firmly closed.  
Store at room temperature (+18...25°C) for no more than 5 days.The rest of the Buffer P should be stored firmly closed at +2...8°C until expiry date.
4. Conjugate is ready for use. Once opened, store at +2...8°C for no more than 1 month.
5. TMB Substrate solution is ready to use. One opened store at +2...8°C for more than 1 month.
6. Stop reagent is ready to use. One opened, store at +2...8°C

#### ASSAY PROECEDURE

1. All kit components and serum samples should be brought to room temperature and stirred thoroughly before the assay. Assay scheme is given on the last page.
2. Mark the wells as follows:

A1, A2—No 1 for TMB substrate control;	E1, E2—No 5 for calibrator No 4;
B1, B2—No 2 for calibrator No 1	F1, F2—No 6 for calibrator No 5;
C1, C2—No 3 for calibrator No 2	G1, G2—No 7 for calibrator No 6;
D1, D2—No 4 for calibrator No 3	H1, H2—No 8 for calibrator

3. Perform each assay in duplicate for both calibrators and unknowns.
4. Pipette 100µl of conjugate into each well **except A1 and A2**.
5. Pipette 20µl of the LH calibrators and serum samples into the corresponding wells.
6. Incubate strips for 1 hour on shaker-thermostat at +37oC (500-800 rpm).
7. After incubation aspirate the liquid from the wells and wash the stips five times with wash buffer. Each time add 300 µl of wash buffer per well and shake for 5-10 sec, then aspirate the buffer. After the last washing cycle, if necessary, invert the plate and firmly tap on a clean paper towel to remove remaining wash buffer.
8. Add 100 µl of TMB reagent to each well .Incubate at room temperature (+18-25°C) in the dark for 15-30 minutes depending on the color intensity.
9. Add 100 µl of Stop reagent to all wells and shake well for 1-2 minutes.
10. Read the optical density of the solution in the microwells at a wavelength of 450 nm.
11. Calculate the mean absorbency for each duplicate. Subtract the mean absorbency value of A1-A2 wells from the mean absorbency values of calibrators; control and unknown

samples. Draw the calibration curve in linear coordinates by plotting absorbency values for calibrators against corresponding LH concentrations. Determine the LH concentrations in the unknown samples and control.

12. An appropriate computer software may be used for calculations. Use the mean absorbency value of A1-A2 wells as "blank".

#### PROCEDURAL NOTES

"GonadotropinEIA-LH" kit should be stored at +2...8°C until expiry date stated on the label. If used for separate experiments kit contents should be stored as follows:  
- put remaining strips in plastic bag and close tightly. Keep at +2...8oC until expiry date;  
- store TMB substrate solution and Conjugate E at +2..8oC for no more than 1 month after opening;  
- once opened, store Buffer P at +2...8oC until expiry date;  
- store prepared wash buffer firmly closed at room temperature for no more than 5 days.  
- store reconstituted calibrators and control at +2...8oC for no more than 1 moth after reconstitution;  
-store stop reagent at +2...8oC until expiry date.

Do not use plasma, hemolysed or lipimic serum or samples with sodium azide as a preservative.

When open and reconstitute the lyophilized reagents, make sure that no dry matter was left on the caps and walls of the vials.

Please take into consideration that calibrators should be measured in each separate assay. It is also recommended to measure LH concentration in the control each time.

do not use Stop reagent fromother manufacturers.

The operator should follow all points of the manual to obtain the reliable result.

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