

Instruction Manual No. M-0100

Human Luteinizing Hormone (LH)

**For Quantitative Determination of LH
In Human Serum**

For In Vitro Research Use Only

Human Luteinizing Hormone (LH) ELISA KIT

Cat. No. 0100

For Quantitative Determination of Human LH In Serum

Kit Contents: (reagents for 96 tests)

Components	96 tests
Anti-human LH coated microwell strip plate (96 wells),	1 Plate
LH Sample Diluent , 0 mIU/ml	11 ml,
LH Calibrator, 10 mIU/ml,	0.75 ml
LH Calibrator, 20 mIU/ml,	0.75 ml
LH Calibrator, 40 mIU/ml	0.75 ml
LH Calibrator, 100 mIU/ml	0.75 ml
LH Calibrator, 200 mIU/ml,	0.75 ml
Anti-hLH-Enzyme Conjugate, 11 ml,	1 bottle
Solution A, 11 ml, Cat# 100SA	1 bottle
Solution B, 11 ml, Cat# 100SB	1 bottle
Stop solution, 10 ml, Cat. # T-10	1 bottle
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Note: Standards are calibrated to WHO 2nd IS 80/552.

Introduction

The Analysis of hLH is an important tool in the diagnosis and treatment of infertility in the female. Detection of the hLH surge can aid in predicting the time of ovulation. The onset of the serum hLH surge precedes ovulation by 34 to 35 hours with peak hLH levels occurring several hours later in urine than in serum. The analysis of urinary hLH has been used successfully to time oocyte retrieval for in vitro fertilization and would similarly assist timing of artificial insemination.

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

Based on sixteen replicates determinations of the zero standard, the minimum concentration of human LH detected using this assay is ~ 2 mIU/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

2. PRECISION

Intra-assay precision:

Three serum samples (mean LH concentrations 11.9, 19.5, 46.9 mIU/ml) were run in an assay. The samples showed good intra-assay precision with % CV of 5-620.

Inter-assay precision:

Three serum samples were run in duplicate in sixteen ten assays. The samples showed good inter-assay precision (4-7% CV). The actual values were: mean 10.30 mIU/ml, SD 1.16 mIU/ml, %CV 7.59; mean 18.40 mIU/ml, SD 1.07 IU/l, %CV 5.84; mean 47.80 mIU/ml, SD 4.16 mIU/ml, %CV 1.9, respectively.

3. LINEARITY

A patient samples (with original LH concentrations 200 mIU/ml) was diluted (1:2, 1:5, and 1:10) with the zero standard and their final LH values determined. The samples showed excellent mean recoveries of about 102% (range 100-105%).

4. HIGH DOSE HOOK EFFECT

LH concentrations of up to 2000 mIU/ml did not show any hook effect.

5. SPECIFICITY

The specificity of LH ELISA kit was determined by measuring interference from high concentrations of hFSH (up to 200 mIU/ml), hTSH (up to 50 uIU/ml), and hCG (25 mIU/ml). These hormones produced color intensity equal to 5, 2.5, and 16 mIU/ml, respectively.

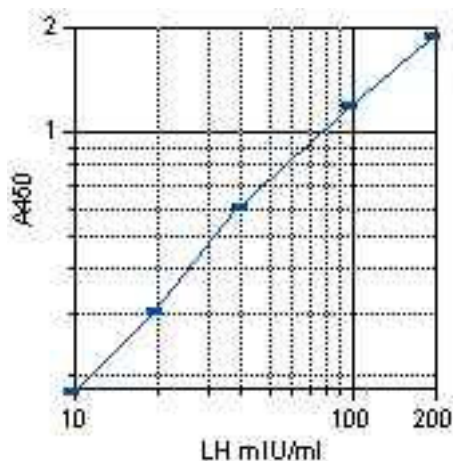
5. INTERFERENCE

The addition of 200 ug/ml each of the following compounds: hemoglobin, atropine, genisic acid, ascorbic acid, acetyl-salicylic acid, in 4 different pool of hLH samples (0, 40, 80, 200 5. SPECIFICITY

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A _{450nm}	Calculated Conc. (IU/l)
A1, A2	(0 mIU/ml)	0.007	
B1, B2	(10.0 mIU/ml)	0.183	
C1, C2	(20.0 mIU/ml)	0.310	
D1, D2	(40 mIU/ml)	0.450	
E1, E2	(100 mIU/ml)	1.210	
F1, F2	(200 mIU/ml /l)	1.920	
G1, G2	Sample 1	0.348	19.9

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical std. assay curve (do not use this for calculating sample values)

PRINCIPLE OF THE TEST

LH ELISA kit is based on simultaneous binding of human LH from samples to two antibodies, one immobilized on microtiter well plates, and other conjugated to the enzyme horseradish peroxidase. After a washing step, chromogenic substrate is added and color developed. The enzymatic reaction (color) is directly proportional to the amount of LH present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm and the concentration of LH in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (25-100 µl) and Multichannel pipet with disposable plastic tips. Reagent troughs, Plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The LH ELISA test is intended for *in vitro research* use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions. The Control Serum has been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site. TMB (substrate), H₂SO₄ (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates). All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera cannot be immediately assayed, these could be stored at -20°C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

EXPECTED VALUES

LH concn of less than or equal to 20 mIU/ml (baseline levels) are normally found during the major portions of follicular and luteal phase of the menstrual cycle. Levels of LH equal to or greater than 40 mIU/ml (40-200) are usually found at the time of hLH surge. Comparison studies between serum and urinary LH concn. throughout the cycle indicate a high degree of correlation between the urinary and serum LH levels starting on day 11 from the initiation of the last menses, which continued through day 16. Peak levels of LH in both the urine and serum were detected on the same day (13-15) following the start of the last menses. The studies indicate that evaluation of urinary LH is just as efficient as serum LH for detecting ovulation in normally cycling females.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping. Standards are stable for two month at 2-8°C. The unused portions of the standards can be frozen in suitable aliquots for long-term use. Repeated freezing and thawing is not recommended.

TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. Dispense 200-300 μ l of wash buffer to all wells. Mix for 5 seconds and discard or aspirate the solution. The step should be done just before adding the samples, do not allow the wells to dry at any time during the assay.

1. Label or mark the microtiter well strips to be used on the plate.
2. Pipet **25 μ l of standards**, control, and serum samples into appropriate wells in *duplicate*.
3. Add **100 μ l of enzyme conjugate** into each well. Mix gently.
4. Cover the plate and incubate for **30 minutes** at room temperature.
5. Aspirate and **wash the wells 5 times** with 300 μ l of running tap water or distilled water. We recommend using an automated ELISA plate Washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
6. Add **200 μ l of HRP-substrate mix** at timed intervals into each well (prepared by mixing equal volume of Soln. A and B. Example, for 96 wells, mix 10 mls of Soln A and 10 ml of Soln B in separate clean tube or dispensing tray. Prepare substrate mix as needed. Substrate mix must not be stored). Mix gently.
7. Cover the plate and incubate for **10 minutes** at room temperature.
8. Stop the reaction by adding **50 μ l of stop solution** to all wells at the same timed intervals as in step 6. Mix gently.
9. Measure the **absorbance at 450 nm** using an ELISA reader within 30 min.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4°C.

Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

DILUTION OF SAMPLES

Serum samples do not usually require dilution. However, if dilution is desired, the zero standard (Sample Diluent) must be used and the results obtained should be multiplied by the appropriate dilution factor.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standard from the mean absorbance values of standards, control, and samples. Draw the standard curve on log-log graph paper by plotting net absorbance values of standards against appropriate LH concentrations. Read off the LH concentrations of the control and patient samples.