Human LH ELISA Kit

For the quantitative determination of human luteinizing hormone (LH) concentrations in serum.

Catalogue Number: EL10011

96 tests

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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S7.5 (03) LH

TABLE OF CONTENTS

	Page
INTENDED USE	2
INTRODUCTION	2
PRINCIPLE OF THE ASSAY	3
REAGENTS PROVIDED	4
MATERIALS REQUIRED BUT NOT SUPPLIED	5
PRECAUTIONS	5
SAMPLE PREPARATION	6
.....Collection, Handling, and Storage	6
PREPARATION OF REAGENTS	6
ASSAY PROCEDURE	7
CALCULATION OF RESULTS	8
TYPICAL DATA	8
.....Example	8
PERFORMANCE CHARACTERISTICS	9
.....Sensitivity	9
.....Specificity	9
.....Calibration	9
.....Hook effect	9
.....Expected Normal Values	10
CITATIONS	10

INTENDED USE

This Human LH ELISA Kit is to be used for the *in vitro* quantitative determination of human luteinizing hormone (LH) concentrations in serum. This kit is intended FOR LABORATORY RESEARCH USE ONLY and is not for use in diagnostic or therapeutic procedures.

INTRODUCTION

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. It is composed of two non-covalently 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 upon a sequence of hormonal events along the gonado-hypothalamic-pituitary axis. The decrease in progesterone and estradiol levels from the preceding ovulation initiates each menstrual cycle. As a result of the decrease in hormone levels, the hypothalamus increases the secretion of gonadotropin-releasing hormone (GnRH), which in turn stimulates the pituitary to increase FSH production and secretion. The rising FSH levels stimulate several follicles during the follicular phase, one of these will mature to contain the egg. As the follicle develops, estradiol is secreted, slowly at first, but by day 12 or 13 of a normal cycle increasing rapidly. LH is released as a result of this rapid estradiol rise because of direct stimulation of the pituitary and increasing GnRH and FSH levels. These events constitute the pre-ovulatory phase.

Ovulation occurs approximately 12 to 18 hours after the LH reaches a maximum, level. After the egg is released, the corpus luteum is formed which secretes progesterone and estrogen, feedback regulators of LH. The luteal phase rapidly follows this ovulatory phase and is characterized by high progesterone levels, a second estradiol increase, and low LH and FSH levels. Low LH and FSH levels are the result of the negative feedback effects of estradiol and progesterone on the hypothalamic-pituitary axis. After conception, the developing embryo produces HCG, which causes the corpus luteum to continue producing progesterone and estradiol. The corpus luteum regresses if pregnancy does not occur, and the corresponding drop in progesterone and estradiol levels results in menstruation. The hypothalamus initiates the menstrual cycle again as a result of these low hormone levels.

Patients suffering from hypogonadism show increased concentrations of serum LH. A decrease in steroid hormone production in females may be a result of immature ovaries, primary ovarian failure, polycysticovary disease, or menopause. In these cases, LH secretion is not regulated. A similar loss of regulatory hormones occurs in males when the testes develop abnormally or anorchia exists. High concentrations of LH may also be found in primary testicular failure and Klinefelter syndrome, although LH levels will not necessarily be elevated if the secretion of androgens continues. Increased concentrations of LH are also present during renal failure, cirrhosis, hyperthyroidism, and severe starvation.

A lack of secretion by the anterior pituitary may cause lower LH levels. As may be expected, low levels may result in infertility in both males and females. Low levels of LH may also be due to the decreased secretion of GnRH by the hypothalamus, although the same effect may be seen by a failure of the anterior pituitary to respond to GnRH stimulation. Low LH values may therefore indicate some dysfunction of the pituitary or hypothalamus, but the actual source of the problem must be confirmed by other tests. In the differential diagnosis of hypothalamic, pituitary, or gonadal dysfunction, assays of LH concentration are routinely performed in conjunction with FSH assays since their roles are closely interrelated. Furthermore, the hormone levels are used to determine menopause, pinpoint ovulation, and monitor endocrine therapy.

PRINCIPLE OF THE ASSAY

This LH enzyme linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for LH. Standards or samples are then added to the microtiter plate wells and LH if present, will bind to the antibody pre-coated on the wells. In order to quantitate the amount of LH present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated monoclonal antibody, specific for LH are added to each well to “sandwich” the LH immobilized on the plate. The microtiter plate undergoes incubation, then the wells are thoroughly washed to remove all unbound components. Next, a TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain LH and enzyme-conjugated antibody will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm.

In order to measure the concentration of LH in the sample, this Human LH ELISA Kit includes a set of calibration standards (6 standards). The calibration standards are assayed at the same time as the samples and allow the operator to produce a standard curve of Optical Density (O.D.) versus LH concentration (mIU/mL). The concentration of LH in the samples is then determined by comparing the O.D. of the samples to the standard curve.

REAGENTS PROVIDED

All reagents provided are stored at 2-8° C. Refer to the expiration date on the label.

	96 tests

1. **MICROTITER PLATE** (Part EL11-1) ***96 wells***
   Pre-coated with anti-human LH monoclonal antibody.
2. **CONJUGATE** (Part EL11-2) ***12 mL***
   Anti-human LH monoclonal antibody conjugated to horseradish peroxidase (HRP) with preservative. *Ready-to-use.*
3. **STANDARD - 240 mIU/mL** (Part EL11-3) ***1 vial***
   Lyophilized human LH in a buffered protein base with preservative that will contain 240 mIU/mL after reconstitution.
4. **STANDARD - 120 mIU/mL** (Part EL11-4) ***1 vial***
   Lyophilized human LH in a buffered protein base with preservative that will contain 120 mIU/mL after reconstitution.
5. **STANDARD - 60 mIU/mL** (Part EL11-5) ***1 vial***
   Lyophilized human LH in a buffered protein base with preservative that will contain 60 mIU/mL after reconstitution.
6. **STANDARD - 30 mIU/mL** (Part EL11-6) ***1 vial***
   Lyophilized human LH in a buffered protein base with preservative that will contain 30 mIU/mL after reconstitution.
7. **STANDARD - 7.5 mIU/mL** (Part EL11-7) ***1 vial***
   Lyophilized human LH in a buffered protein base with preservative that will contain 7.5 mIU/mL after reconstitution.
8. **STANDARD - 0 mIU/mL** (Part EL11-8) ***1 vial***
   Lyophilized buffered protein base with preservative that will contain 0 mIU/mL after reconstitution.
9. **SUBSTRATE A** (Part EL11-9) ***10 mL***
   Buffered solution with H_20_2.
10. **SUBSTRATE B** (Part 30007) ***10 mL***
    Buffered solution with TMB.
11. **STOP SOLUTION** (Part 30008) ***14 mL***
    2N Sulphuric Acid (H_2SO_4). Caution: Caustic Material!

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100 μL and 50-200 μL for running the assay.
2. Pipettes: 1 mL, 5 mL, and 10 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Incubator (37±2°C)
7. Microtiter plate reader (450 nm±2 nm)
8. Automatic microtiter plate washer or squirt bottle
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Human serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate human viruses.
   Solid Waste: Autoclave 60 min. at 121°C.
   Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate B contains 20% acetone, keep this reagent away from sources of heat or flame.

SAMPLE PREPARATION

COLLECTION, HANDLING AND STORAGE

Serum: Blood should be drawn using standard venipuncture techniques and serum separated from blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) and serum extracted. This kit is for use with serum samples without additives only.

- Avoid grossly hemolytic, lipidic or turbid samples.
- Serum samples to be used within 24-48 hours may be stored at 2-8°C, otherwise samples must be stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- When performing the assay slowly bring samples to room temperature.
- It is recommended that all samples be assayed in duplicate.
- DO NOT USE HEAT-TREATED SPECIMENS.

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

1. **LH Standards:** Reconstitute each LH Standard vial with **0.6 mL** of distilled or deionized water. Allow each solution to sit for at least 15 minutes with gentle agitation. The LH standard stock solutions are stable at 4°C for 3 months. Avoid freeze-thaw cycles

2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table below for correct amounts of Substrate Solution to prepare.

Wells Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
16 wells	1.5	1.5	3.0
32 wells	3.0	3.0	6.0
48 wells	4.0	4.0	8.0
64 wells	5.0	5.0	10.0
80-wells	6.0	6.0	12.0
96 wells	7.0	7.0	14.0

ASSAY PROCEDURE

1. Prepare all LH Standards before starting assay procedure (see Preparation Reagents). *It is recommended that all Standards and Samples be added in duplicate to the Microtiter Plate.*

2. First, secure the desired number of coated wells in the holder, then add 50 µL of Standards or samples to the appropriate well of the antibody pre-coated Microtiter Plate.

3. Add 100 µL of Conjugate to each well. COMPLETE MIXING IN THIS STEP IS IMPORTANT. Cover and incubate for **1 hour at 37°C.**

4. Prepare Substrate Solution no more than 15 minutes before end of incubation (see Preparation of Reagents).

5. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with distilled or de-ionized water, then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

   Automated Washing: Aspirate all wells, then wash plate **FIVE times** using distilled or de-ionized water. Always adjust your washer to apirate as much liquid as possible and set fill volume at 350 µL/well/wash (range: 350-400 µL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.*

6. Add 100 µL Substrate Solution to each well. Cover and incubate for 15 minutes at 37°C.

7. Add 100 µL of Stop Solution to each well. Mix well.

8. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 30 minutes.

CALCULATION OF RESULTS

This standard curve is used to determine the amount of luteinizing hormone (LH) in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the six standard concentrations on the vertical (Y) axis versus the corresponding LH concentration (mIU/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D. value for each standard and sample. All O.D. values are subtracted by the mean value of the zero-standard (0 mIU/mL) before result interpretation. Construct the standard curve using graph paper or statistical software.

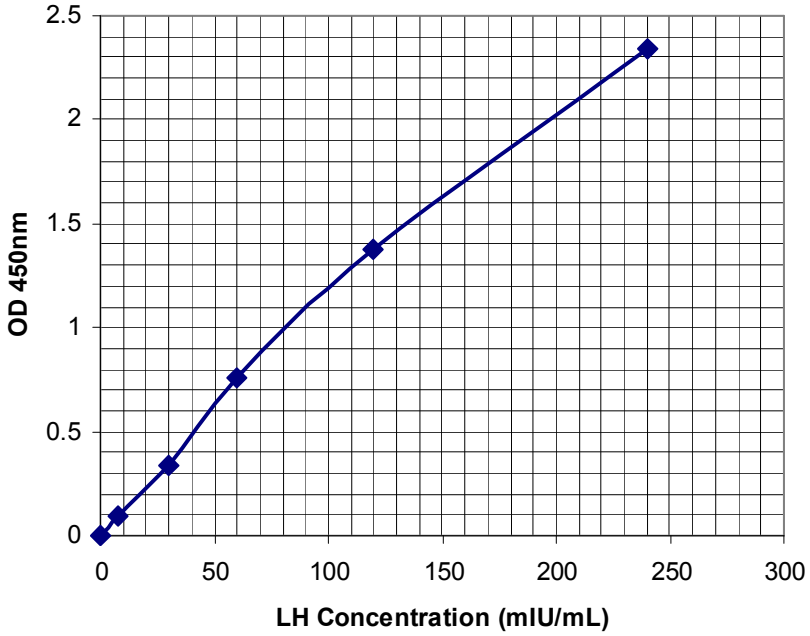
2. To determine the amount of LH in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding LH concentration.

TYPICAL DATA

Results of a typical standard run of LH ELISA are shown. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain their own standard curve.

EXAMPLE
Results of a typical standard run are shown below:

Standard (mIU/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted
0	0.013, 0.014	0.014	0
7.5	0.108, 0.110	0.109	0.095
30	0.344, 0.358	0.351	0.337
60	0.773, 0.781	0.777	0.763
120	1.412, 1.360	1.386	1.372
240	2.408, 2.299	2.354	2.340



PERFORMANCE CHARACTERISTICS

1. **SENSITIVITY**
   The minimal detectable concentration of LH by this assay is estimated to be 3 mIU/ml.

2. **SPECIFICITY**
   This kit exhibits no detectable cross reaction with human; FSH, TSH, Prolactin, or GH. But there is cross reaction with hCG in this assay. After pregnancy hCG levels are elevated, therefore the use of LH ELISA Kit is not recommended during pregnancy or immediately post-parturition.

3. **CALIBRATION**
   This immunoassay is calibrated against W.H.O. 2nd IS, 80/552.

4. **HOOK EFFECT**
   In this assay, no hook effect is observed up to 2500 mIU/ml.

5. **EXPECTED NORMAL VALUES**

Each laboratory must establish its own normal ranges based on patient population. The results provided below are based on randomly selected out-patient clinical laboratory samples.

	Age	(N)	Mean LH (mIU/mL)	Range (mIU/mL)
Male (pre-pubsecent)	<10	25	1.3	0 to 2.5
Male (normal adult)	15-60	56	4.8	1.0 to 15.0
Female(pre-pubsecent)	<10	25	1.1	0 to 2.0
Female (normal adult)	20-35	60	15.0	1.0 to 90.0
Female (post-menopausal)	46-60	40	38.0	8.0 to 120.0

CITATIONS

1. S. S. Dharap, Y. Wang et al. Tumor-specific targeting of an anticancer drug delivery system by LHRH peptide. Proc. Natl. Acad. Sci. U.S.A. Sep 6, 2005; 102(36): 12962-12967.

2. Q Xu, X Yuan et al. Isolation of tumour stem-like cells from benign tumours, Br J Cancer, Jul 21, 2009; 101(2):303-311.