



### AccuDiag™ LH ELISA Kit

REF 4225-16

IVD See External Label 2°C 8°C 96 Tests

LUTEINIZING HORMONE (LH) ELISA	
Method	Enzyme Linked Immunosorbent Assay
Principle	Sandwich Complex
Detection Minimum	2 mIU/ml
Sample	50 µl
Incubation Time	80 minutes
Shelf Life	12-14 Months from the manufacturing date

### PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

### INTENDED USE

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

### SIGNIFICANCE AND SUMMARY

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 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 on 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 factors (GnRF), 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, increases rapidly. LH is released as a result of this rapid estradiol rise because of direct stimulation of the pituitary and increasing GnRF 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 is a result of immature ovaries, primary ovarian failure, polycystic ovary 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.

### ASSAY PRINCIPLE

The LH Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-LH antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-LH antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in the LH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 60-minute incubation at room temperature, the wells are washed to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 2N HCl, 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.

### SPECIMEN COLLECTION AND PREPARATION

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

### REAGENTS

#### Materials provided with the test kit

1. Antibody-coated microtiter plate with 96 wells.
2. Reference standard set, contains 0, 5, 20, 50, 100, and 200 mIU/ml. (WHO, 1st IRP, 68/40), lyophilized.
3. Enzyme Conjugate Reagent, 12 ml.
4. TMB Substrate, 12 ml.
5. Stop Solution, 12 ml.
6. Wash Buffer Concentrate (50X), 15 ml
7. Control Set (Optional)

#### Materials required but not provided

1. Precision pipettes: 40µl ~ 200µl and 1.0ml.
2. Disposable pipette tips.
3. Distilled water.
4. Vortex mixer or equivalent.
5. Absorbent paper or paper towel.
6. Graph paper.
7. Microtiter plate reader.

### REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-22°C) before use.
2. Reconstitute each lyophilized standard with 0.5 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be stored and sealed at 2-8°C.
3. Dilute 1 volume of Wash Buffer Concentrate (50x) with 49 volumes of distilled water. For example, dilute 15 ml of Wash Buffer Concentrate (50x) into distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.

### ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Make data sheet with sample identification.
2. Dispense 50µl of standard, specimens, and controls into appropriate wells.
3. Dispense 100µl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to have complete mixing in this step.
5. Incubate at room temperature (18-22°C) for 60 minutes.
6. Remove the incubation mixture by flicking plate content into sink.
7. Rinse and flick the microtiter wells 5 times with washing buffer (1X).
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100µl of TMB solution into each well. Gently mix for 5 seconds.
10. Incubate at room temperature for 20 minutes.
11. Stop the reaction by adding 100µl 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 optical density at 450nm with a microtiter well reader within 15 minutes.

#### Important Note:

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

### RESULTS

Calculate the mean absorbance value (A<sub>450</sub>) for each set of reference standards, specimens, controls and patient samples. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in mIU/ml on graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of LH in mIU/ml from the standard curve.

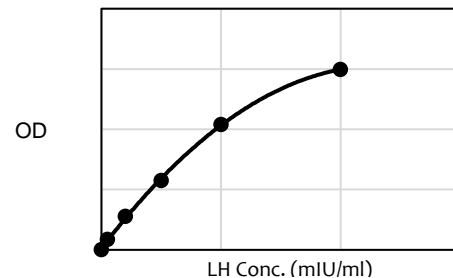
### EXAMPLE OF STANDARD CURVE

Results of typical standard run with optical density reading at 450nm shown in the Y- axis against LH concentrations shown in the X-axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

LH (mIU/ml)	Absorbance (450nm)
0	0.000
5	0.169
20	0.552
50	1.150
100	2.079
200	2.990

$$y = -6E-05x^2 + 0.026x + 0.0241$$

$$R^2 = 0.9995$$



### EXPECTED VALUES AND SENSITIVITY

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

	LH (mIU/ml)
Adult male	0~25 mIU/ml
Female Follicular	0~40 mIU/ml
Mid-cycle	40~150 mIU/ml
Luteal	0~30 mIU/ml



Post Menopausal	20~200 mIU/ml
Pre pubertal, female	0~9 mIU/ml
Pre pubertal, male	0~17 mIU/ml

The minimal detectable concentration of human luteinizing hormone by this assay is estimated to be 2 mIU/ml.

### STORAGE

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 expiring date shown, provided it is stored as prescribed above. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2.5 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

### LIMITATIONS



There are some limitations of the assay:

- As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
- Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot guarantee it will eliminate all the effects of that.

### REFERENCES

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### MANUFACTURER AND BRAND DETAILS

<b>ISO 13485:2016</b>	
	
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<b>Brand Name</b>	<b>AccuDiag™</b>
<div style="border: 1px solid black; padding: 2px; display: inline-block;">EC</div> <div style="border: 1px solid black; padding: 2px; display: inline-block; margin-left: 10px;">REP</div>	CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands. <a href="http://www.cepartner4u.eu">www.cepartner4u.eu</a>
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