
LUTEINIZING HORMONE (LH) CHEMILUMINESCENCE

IMMUNOASSAY KIT

Catalog No. CL1101-2

INTENDED USE

The Autobio luteinizing hormone (LH) chemiluminescence immunoassay (CLIA) kit is intended for the quantitative determination of LH concentration in human serum.

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 daltons. It is composed of two noncovalently 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.

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.

PRINCIPLE OF THE TEST

The LH CLIA test is a solid phase two-site immunoassay. One monoclonal antibody is coated on the surface of the microtiter wells and another monoclonal antibody labeled with horseradish peroxidase is used as the tracer. The LH molecules present in the standard solution or serum are "sandwiched" between the two antibodies. Following the formation of the coated antibody-antigen-antibody-enzyme complex, the unbound antibody-enzyme labels are removed by washing. The horseradish peroxidase activity bound in the wells is then assayed by chemiluminescence reactions. The related light unit (RLU) of the reaction is proportional to the concentration of LH present in the sample.

MATERIALS PROVIDED

1. Antibody Coated Microtiter Plate: Microplate with anti-LH monoclonal antibody (MAb) coated wells (1 plate, 48wells/96 wells)
2. Enzyme Conjugate Reagent: Horseradish peroxidase (HRP) labeled anti-LH (MAb) in Stabilizing Buffer (1 vial, 3.0 ml/6.0ml)
3. Reference Standards: 1.0, 2.5, 10, 40, 160mIU/m. (5 vials, Lyophilized)
4. Substrate A: (1 vial, 3.5ml/6.0ml)
5. Substrate B: (1 vial, 3.5ml/6.0ml)
6. PBS-T powder: PBS-Tween (1bag, 5g)

MATERIALS NOT PROVIDED

The following materials are required but not provided in the kit.

1. Precision pipettes and tips: 0.05ml, 0.1ml, 1.0ml.
2. Distilled water.
3. Vortex mixer
4. Absorbent paper or paper towel
5. Graph paper
6. Luminometer

STORAGE OF TEST KIT AND INSTRUMENTATION

1. 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. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiration date shown, provided it is stored as prescribed above.

SPECIMEN COLLECTION AND PREPARATION

1. Blood should be drawn using standard venipuncture techniques and the serum should be separated from the red blood cells as soon as practical. Avoid grossly hemolytic, lipemic or turbid samples.
2. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with test procedures and should be avoided.
3. Specimens should be capped and may be stored up to 48 hours at 2~8°C, prior to assaying. Specimens held for a longer time can be frozen at -20°C. Thawed samples must be mixed prior to testing.

PRECAUTIONS AND WARNINGS

1. For *in vitro* diagnostic use only.
2. Handling of reagents, serum specimens should be in accordance with local safety procedures.
3. The reference standards contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody. All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, the reference standards and components containing animal substances should be treated as potentially infectious.
4. Avoid any skin contact with all reagents.
5. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18~25°C) prior to use. All reagents should be mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. Reconstitute each lyophilized standard with 0.5 ml distilled water. Allow the reconstituted material to stand for at least 10 minutes. Reconstituted standards should be stored sealed at 2 ~ 8°C. Reconstituted standards should be used within 14 days and be frozen at -20°C for long term storage.
3. Prepare Wash Solution: add 1 bag of PBS-T powder to 500ml of distilled water, and mix well with magnetic stirrer. The Wash Solution is stable at room temperature for 2 months.

IMPORTANT NOTES

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated RLU values.
2. It is recommended that no more than 32 wells be used for each assay run, if manual pipetting is used, since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of all standards and specimens, although not required, is recommended.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense 50µl of LH standards, specimens, and controls into appropriate wells.
2. Dispense 50µl of Enzyme Conjugate Reagent to each well. Mix gently for 30 seconds.

3. Incubate for 60 minutes at 37°C.
4. At the end of the incubation, remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with washing buffer. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets. The volume of the well is about 300µl.
5. Dispense 50µl substrate A and 50µl substrate B reagent into each well. Gently mix for 10 seconds.
6. Incubate at room temperature in the dark for 5 minutes without shaking and read the RLU values with a Luminometer.

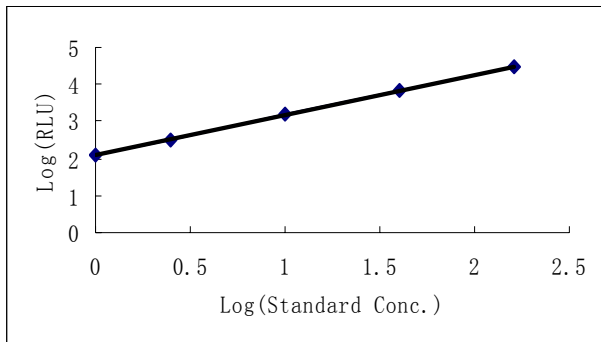
CALCULATION OF RESULTS

1. Calculate the mean value from any duplicate reagents. Where appropriate, the mean values should be used for plotting.
2. Plot the log₁₀RLU for each reference standard against the common logarithm of the corresponding concentration of LH in mIU/ml on logarithmic graph paper, with RLU values on the Y-axis and concentration on the X- axis.
3. Read the concentration for each control and sample by interpolating on the calibration curve.
4. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a linear regression logistic function curve fitting is recommended.

EXAMPLE OF STANDARD CURVE

A typical standard curve shown below is for the purpose of illustration only, and should never be used instead of the real time calibration curve.

LH (mIU/ml)	RLU
1	128.464
2.5	313.808
10	1480.23
40	7205.73
160	28300.9



EXPECTED 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:

		Normal Range (mIU/ml)
Male		1.0 ~ 12.5
Female	Follicular Phase	1.2 ~ 12.7
	Ovulation Phase	15.5 ~ 90.0
	Luteal Phase	0.5 ~ 14.6
	Menopause	15.6 ~ 72.0

PERFORMANCE

A. Sensitivity

The lower detection limit is calculated from the standard curve by identifying the concentration corresponding to the mean RLU of standard diluent (based on 10 replicate analyses) plus 2 SD. Therefore, the sensitivity of the Autobio LH CLIA kit is estimated to be not higher than 0.3mIU/ml.

B. Specificity

No interference was detected with the performance of Autobio LH CLIA upon addition of massive amounts of the following substances to a human serum pool.

Interferents	Concentration
FSH	500mIU/ml
TSH	520 μ IU/ml
hCG	22800mIU/ml

C. Precision

a. Intra-assay Precision

Intra-assay Precision was determined by assaying 20 replicates of each control sera.

Serum	Number	Mean	SD	CV (%)
Low titer	20	4.53	0.32	7.07
High titer	20	18.93	1.31	6.92

b. Inter-assay Precision

Inter-assay Precision was determined by assaying duplicates of each control sera in 10 separate runs.

Serum	Number	Mean	SD	CV (%)
Low titer	10	4.51	0.23	5.08
High titer	10	24.83	1.33	5.35

D. High Dose Hook Effect

In this LH assay, patient samples spiked to LH levels up to 20,000mIU/ml do not demonstrate a paradoxical decrease in the RLUs (high dose hook effect).

E. Accuracy

For samples in the range of 2.5mIU/ml to 160mIU/ml, the relationship between the Autobio LH CLIA kit and Beckman access 2® LH assay, is described by the equation:

Reference	Number of Specimens	Least Square Regression Analysis	Correlation Coefficient
Beckman access 2®	180	$y = 0.9526x + 2.0239$	0.9760




LIMITATIONS



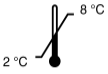


1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert and with adherence to good laboratory practice.
2. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference thus anomalous values may be observed. Additional information may be required for diagnosis.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

QUALITY CONTROL

Good laboratory practice requires that quality control specimens be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges. Controls containing sodium azide should not be used.

SYMBOLS


	BATCH CODE
	USE BY
	MANUFACTURER

	CONTAINS SUFFICIENT FOR <n> TESTS
	<i>IN VITRO</i> DIAGNOSTIC MEDICAL DEVICE
	TEMPERATURE LIMITATION
	CATALOGUE NUMBER
	CONSULT INSTRUCTIONS FOR USE

REFERENCES

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3. Shome, B. and Parlow, A.F. *J. Clin. Endocrinol. Metabl.* 39:199-202; 1974
4. Shome, B. and Parlow, A.F. *J. Clin. Endocrinol. Metabl.* 39:203-205; 1974
5. Uotila, M.; Ruoslahti, E. and Engvall, E. *J. Immunol. Methods.* 42: 11-15; 1981

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