



Human Luteinizing Hormone (LH) MICRO-ELISA Test Kit

Prod. No.: T110
Pkg. Size: 96 Tests

Description

The **MICRO-ELISA LUTEINIZING HORMONE** Test Kit is a solid phase sandwich-type enzyme immunoassay (ELISA) for the *in vitro* quantitative determination of luteinizing hormone concentration in human serum or urine.

Summary and Explanation of the Test

Human luteinizing hormone is a product of the pituitary and has a molecular weight of approximately 30,000 daltons. Control of synthesis and secretion is provided by the hypothalamic product, gonadotrophin releasing hormone (GnRH). GnRH acts to stimulate the production and release of both LH and follicle stimulating hormone (FSH) by the pituitary. LH, like the other pituitary glycoproteins, thyroid stimulating hormone (TSH), follicle stimulating hormone (FSH) and human chorionic gonadotropin (HCG), is composed of two subunits designated alpha and beta. The alpha chain is common to all of these hormones. The beta chain is distinct and the use of highly specific monoclonal antibodies in the **MICRO-ELISA LUTEINIZING HORMONE** eliminates the interference of TSH and FSH in the assay of LH.

LH is present in the serum and urine of normal males and females. The primary physiologic function of luteinizing hormone in the female is to cause ovulation and steroid (estrogen and progesterone) production by the corpus luteum. In the male it stimulates interstitial cells (Leydig cells) to produce androgens and estrogens. Circulating levels of these steroidal hormones exert a negative feedback effect on the hypothalamus to control the secretion of LH.

In females, LH values are low before puberty and increase during the reproductive years. In menstruating females, LH levels are subject to cyclic variations. During the follicular (pre-ovulatory) phase of the menstrual cycle, LH levels fluctuate but remain at less than 10 mIU/ml. Approximately 35 hours prior to ovulation, serum LH levels increase rapidly and peak at values greater than 25 mIU/ml. The concentration of LH decreases to base line levels within two days marking the luteal (post-ovulatory) phase of the cycle. The elevated estrogen levels in pregnant females, or those taking oral contraceptives, increase negative feedback to the hypothalamus and result in decreased LH levels. At menopause, when ovarian estradiol secretion decreases, the loss of negative feedback on the hypothalamus results in greatly increased levels of LH.

In males, LH values are low before puberty and increase slightly during the reproductive years.

In both males and females, primary hypogonadism with decreased production of steroids results in increased secretion of LH. In secondary or tertiary hypogonadism, the decreased secretion of LH leads to decreased levels of gonadal steroid secretion.

Principle of the Procedure

The **MICRO-ELISA LUTEINIZING HORMONE** test is based on the principle of a solid phase enzyme-linked immunosorbent assay (**ELISA**). The assay system utilizes two unique antibodies (mouse monoclonal) directed against distinct antigenic determinants on the LH molecule.

Specifically, plastic wells are coated with anti-LH (mouse monoclonal). With the addition of a test sample or appropriate controls containing LH, immune complexes are formed between LH in the sample and the solid phase anti-LH. Anti-LH (goat polyclonal) enzyme-labeled with horseradish peroxidase is added to each well. During an incubation period (45 minutes at room temperature), the LH molecule is sandwiched between the solid phase and enzyme-labeled antibodies. The sample is then decanted and the wells are washed to remove unbound labeled antibody. An enzyme substrate chromogen (hydrogen peroxide, H_2O_2 , and tetramethylbenzidine, **TMB**) is added to the well and incubated for 15 minutes at room temperature, resulting in the development of a blue color. The addition of 1.0 N H_2SO_4 stops the reaction and converts the color to yellow. The intensity of the yellow color is directly proportional to the concentration of LH in the sample.

Reagents

Components in Each 96-Test

Micro-Elisa LH Test Kit

- 96 wells, **LH ANTIBODY COATED WELLS**: Coated with anti-LH (mouse monoclonal); contained in a pack with silica gel desiccant.
- 1 bottle, 22 ml, **LH ENZYME ANTIBODY CONJUGATE**: anti-LH (goat polyclonal) labeled with horseradish peroxidase in buffered protein solution; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives; contains FD&C red # 40 as a coloring agent.
- 1 bottle, 12 ml, **SUBSTRATE-CHROMOGEN**: Buffered hydrogen peroxide and 3,3',5,5'- tetramethylbenzidine (TMB) solution.
- 1 bottle, 12 ml, **Stop Solution** - 1 N H_2SO_4
- 1 vial, 3 ml, 0 mIU/ml LH **CALIBRATOR**: Bovine serum: contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives.
- 3 vials, 1 ml, LH **CALIBRATOR**: LH in bovine serum; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives; **30,60 and 120 mIU/ml**.
- 1 bottle, 60 ml, **WASH BUFFER CONCENTRATE (20X)**: Buffered detergent solution, contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives. Dilute to 1200 ml with deionized water.



Additional Materials Required

Disposable tip precision pipets - 0.025 ml, 0.05 ml and 0.2 ml.
microtiter well holder.
Microtiter plate reader.
1 N H₂SO₄
Distilled or deionized water.

Storage and Stability

Store all components at 2°-8°C when not in use. Expiration date printed on the kit indicates limits of stability.

The LH ANTIBODY COATED WELLS are supplied in a resealable bag containing a desiccant and must be stored with the bag sealed to protect from moisture. Wells can be stored at 2° - 8°C.

Chemical or Physical Indications of Instability

Alterations in the physical appearance of reagents, or results consistently outside the acceptable limits for control sera, may be due to reagent instability or deterioration.

Instruments

Performance of the MICRO-ELISA LH test requires use of a precision microtiter plate reader with the following specifications at a wavelength of 450 ± 20 nm:

Bandwidth:	10 nm ± 2 nm
Absorbance Range:	0 to 2.0 A ₄₅₀
Repeatability:	better than 0.005 A ₄₅₀ or 1%, whichever is greater.
Linearity:	better than 0.005 A ₄₅₀ or 2%, whichever is greater.
Accuracy:	± 0.005 A ₄₅₀ at 1.0 A ₄₅₀
Drift:	less than 0.005 A ₄₅₀ per hour.

Specimen Collection and Preparation

Serum samples: No special preparation of the patient is necessary; fasting is not required. Repeated freezing and thawing of specimens should be avoided. No additives or preservatives are necessary.

Urine samples: No special preparation of the patient is necessary; fasting is not required. Collect a timed urine specimen (3 - 24 hours), keeping the specimen refrigerated during collection. Centrifugation of the specimen is required if it is cloudy and only the clear supernatant should be used for testing. Repeated freezing and thawing of specimens should be avoided. No additives or preservatives are necessary.

STORAGE: Specimens may be stored in a tightly stoppered tube at 2°-8°C for three days. If the sample is not assayed within 24 hours, store frozen (-20°C) in a tightly stoppered tube for up to 2 weeks. Specimens should be allowed to come to room temperature and should be mixed thoroughly by gentle inversion before assaying.

Micro-Elisa LH Procedure

Reagent Preparation

Dilute bottle of **WASH BUFFER CONCENTRATE (20X)** solution to 1200 ml with deionized water. Diluted wash solution will be stable until the expiration date stamped on the kit.

Preliminary Comments and Precautions

Patient sample may contain pathogens: treat all samples as potentially infectious.

Reagents contain thimerosal; avoid contact with skin.

Avoid contact with **SUBSTRATE-CHROMOGEN** (tetramethylbenzidine) solution. It is harmful if inhaled or absorbed through skin (may cause irritation).

CAUTION: Source material used to prepare Calibrators was derived from human material. The material was tested using FDA-approved methods and found non-reactive for Hepatitis B Surface Antigen (HBsAg) by ELISA and non-reactive for HIV by ELISA. No known test method can offer total assurance that infectious agents are absent. **HANDLE THESE REAGENTS AS IF THEY ARE POTENTIALLY INFECTIOUS.** Information on handling human serum is provided in the CDC/NIH manual "Bio-safety in Microbiological and Biomedical Laboratories" (1984).

Procedural Notes

- All test kit components used in an assay must be of the same master lot number. Materials should not be used after the expiration date shown on the package label. Components and test specimens should be at room temperature (18°-30°C) before testing begins.
- All calibrators, controls, and samples should be tested in duplicate simultaneously. The test samples and controls must be well-mixed before use.
- A separate disposable tip should be used for each sample to avoid cross-contamination. All pipetting steps should be performed with the utmost care and accuracy. Avoid contamination of the reagent pipette tip with the serum sample.
- The duration of the incubation times must be the same for all wells within a run.
- Good laboratory practice requires that quality control specimens be run with each assay to check the assay performance. Interassay reproducibility should be assessed from a pool of LH-containing serum obtained from commercial sources or prepared by the user. The acceptable limits for control sera must be established according to the requirements of the individual laboratory.
- Run size should be limited to the number of samples that can be added to antibody coated wells within 10 minutes.
- Samples should be pipetted to the bottom of the antibody coated wells.
- If microtiter reader is not capable of reading absorbances greater than 2.0, the color should be read after a shorter incubation time with the SUBSTRATE/CHROMOGEN, i.e., 10 minutes.**

Test Procedure

- Place sufficient coated wells in a holder to run 0.0 mIU LH/ml, 25 mIU LH/ml, 50 mIU LH/ml and 100 mIU LH/ml CALIBRATORS, Quality Control Sera and patient samples in duplicate. Record the position of samples on a work sheet.
- URINE:** Pipet **100 ml** of the Patient **URINE** Sample to a correspondingly labeled test tube. Pipet or dispense **100 ml** of the **0 mIU/ml CALIBRATOR** to all of the tubes, mix thoroughly. **THIS IS A 2 FOLD DILUTION OF THE URINE SAMPLE. SERUM:** Serum sample is assayed without dilution.



- Pipet **50 ml** of the **CALIBRATORS**, Controls or Patient Sample (**serum** or **diluted urine**) to the corresponding coated well.
- Pipet or dispense **200 ml** of the ENZYME ANTIBODY **CONJUGATE** solution to all the wells and mix gently.
- Incubate at room temperature (18°-30°C) for **45 minutes** ± 5 minutes.
- Decant** or aspirate and discard liquid contents of all wells.
- Fill each well with **DILUTED WASH BUFFER**. Decant or aspirate liquid contents of all wells. Do not use tap water.
- Repeat step 6 three more times (for a **total of 4 washes**). Tap wells free of any liquid or aspirate thoroughly.
- Fill each well with deionized water. Decant or aspirate liquid contents of all wells. **Tap wells free of any liquid or aspirate thoroughly**. Do not use tap water.
- Pipet or dispense **100 ml** (0.1 ml) of **SUBSTRATE-CHROMOGEN** solution into each well.
- Mix thoroughly and incubate **15 minutes** at room temperature (18-30°C).
- Pipet or dispense **100 ml** (0.1 ml) of 1 N H₂SO₄ **Stop Solution** into each well and mix thoroughly.
- Read the absorbance of each well at **450 ± 20 nm** against water.

Pooled human serum or commercially available control sera without azide are suitable. Any material used should be assayed repeatedly to establish mean values and acceptable ranges to assure proper performance.

Read the absorbance of the test solutions against a water blank. If the absorbance of the 0 mIU/ml CALIBRATOR exceeds 0.200 it is an indication of careless washing and the assay must be repeated.

Standardization

The **MICRO-ELISA LH CALIBRATORS** have been standardized against the World Health Organization 1st International Reference Preparation (1st IRP #68/40).

Limitations of the Procedure

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. These conditions should be ruled out prior to clinical evaluation of test results.

The wash procedure (steps 6-8) is critical. **Insufficient washing will result in poor precision and falsely elevated absorbances**. The use of tap water for washing could result in a higher background absorbance.

FINAL REACTION STABILITY: The spectrophotometric measurement should be made within 2 hours minutes after the addition of the H₂SO₄ solution.

Samples with elevated levels of LH (up to 11,600 mIU LH/ml) will always assay as >100 mIU LH/ml when tested, and will **not** result in a "high dose hook effect". When it is necessary to measure levels of LH greater than the 100 mIU LH/ml CALIBRATOR, the sample should be diluted with the 0 mIU/ml CALIBRATOR and reassayed.

Calculation of Results

- Calculate the mean value for each duplicate sample absorbance. Values for duplicate absorbances should be within 10% (or 0.02 absorbance units for absorbances less than 0.2).
- Construct the standard curve by plotting the mean absorbance obtained for each LH CALIBRATOR on the vertical (Y) axis versus the corresponding LH concentration on the horizontal (X) axis, using **rectilinear graph paper**.
- Connect the points with straight line segments.**
- Using the mean absorbance for each sample, read the corresponding LH concentration in mIU/ml from the curve. Multiply the value by the dilution factor if required. **Multiply the value of URINE samples by 2.**

EXAMPLE DATA

Specimen I.D.	A ₄₅₀	Mean A ₄₅₀	LH(mIU/ml)
CALIBRATOR	0 mIU/ml	0.135, 0.120	0.127
CALIBRATOR	25 mIU/ml	0.543, 0.485	0.514
CALIBRATOR	50 mIU/ml	0.954, 0.921	0.937
CALIBRATOR	100 mIU/ml	1.439, 1.476	1.457

	Mean Abs.	Net Abs.	mIU/ml
Unknown Sample	>2.100	>2.100	>100
Diluted 1:10	>2.100	>2.100	>100
Diluted 1:100	1.713	1.695	116 11,600

SAMPLES

# 1 (Unknown #1)	0.138, 0.144	0.141	0.9
# 2 (Unknown #2)	0.387, 0.376	0.381	16.4
# 3 (Unknown #3)	1.043, 1.050	1.046	60.5

The range of this assay is 0 - 100 mIU/ml. For specimen with LH concentrations beyond the standard curve (100 mIU/ml), repeat the test by diluting the specimen with the 0 mIU/ml CALIBRATOR. To obtain the final concentration, multiply the concentration of the diluted sample by the dilution factor.

Estrogens from either exogenous or endogenous sources cause a decrease in serum levels of LH. Patients treated with oral contraceptives will have a decreased level of LH and will not demonstrate a mid-cycle surge in LH levels.

Single observations of LH levels may give misleading results due to the pulsatile nature of LH secretion. Samples drawn at different times may vary by as much as the width of the normal range. Elevated levels of LH should be confirmed by more than one LH measurement.

Quality Control

Good laboratory practice requires that quality control specimen be run with each calibration curve to check the assay performance. Controls containing azide can not be used. Two controls with normal and elevated values should be used.

Expected Values

Luteinizing hormone values were measured in serum samples from apparently normal individuals and the following results were obtained:



	SERUM LH (mIU/ml)		Range
	N	Mean	
ADULT FEMALE			
premenopausal	65	4.2	< 2.0 - 25.7
postmenopausal	74	55.3	6.7 – 137
ADULT MALE	66	3.2	< 2.0 - 10.8
PREPUBERTAL			
MALE & FEMALE	10	0.7	< 2.0 - 2.1

In premenopausal females, the serum level of LH will change throughout the menstrual cycle.

PHASE	MEAN LH (mIU/ml)	RANGE (mIU/ml)
Follicular	3 - 5	0 - 10
Mid-cycle Peak	20 - 30	0 - 60
Luteal	2 - 4	0 - 8

The normal levels of LH reported in urine are dependent on sex and age.

	URINE LH (mIU/HOUR)	
	Range	
ADULT FEMALE		
premenopausal	200 – 3500	
postmenopausal	>900	
ADULT MALE	500 – 2500	
PREPUBERTAL MALE & FEMALE	<100	

These values are consistent with those reported in the literature, however, it is recommended that each laboratory determine its own normal range.

Performance Characteristics

Assay Specificity

Specificity of this test system was proven by determining a lack of interference of thyroid stimulating hormone (TSH) and follicle stimulating hormone (FSH) when added to a serum sample with a known LH concentration. The incremental change in LH concentration was measured and the relative cross reactivity calculated by dividing the apparent change in LH concentration (mIU/ml) by the cross reactant concentration (in mIU/ml or uIU/ml) times 100. The observed cross reactivity for both TSH and FSH was < 1.0%. The observed cross reactivity for hCG was < 1.0% (at hCG = 4,000 mIU/ml). The use of this test to determine LH concentrations in serum from pregnant or immediately postpartum females is not recommended.

The following substances were tested in urine samples and found to have no effect on the results up to and including the stated concentration.

URINE:			
Acetaminophen	20 mg/dl	Ketones	
	10 gm/dl		
Acetylsalicylic Acid	20 mg/dl	Bilirubin	2
Ascorbic acid	20 mg/dl	Hemoglobin	130 mg/ml
Atropine	20 mg/dl	pH	5 to 9.5
Caffeine	20 mg/dl	Peptide hormones	
Gentisic Acid	20 mg/dl		
Phenothiazine	20 mg/dl	hFSH	500 mIU/ml
Protein	2 gm/dl	hTSH	1000 uIU/ml
Glucose	4 gm/dl		

Assay Sensitivity

The sensitivity of this assay is defined as the smallest single value which can be distinguished from the zero calibrator. This value was calculated from the mean + two standard deviations for twenty-one replicates at the zero concentration. The calculated sensitivity is < 2.0 mIU/ml.

Assay Reproducibility

Intra-assay reproducibility was determined by measurement of 13 replicates of three serum pools in a single run.

	Mean LH (mIU/ml)	SD	%CV
Serum A	9.81	1.28	13.1
Serum B	18.56	1.93	10.4
Serum C	63.36	4.37	6.9

The **interassay** reproducibility was determined by duplicate measurement of three serum pools in nineteen separate runs.

	Mean LH (mIU/ml)	SD	%CV
Serum A	10.60	1.32	12.5
Serum B	17.92	1.24	6.9
Serum C	65.29	4.70	7.2

Assay Linearity

SERUM: A study was performed diluting a serum sample containing an elevated level of LH with the 0 mIU/ml CALIBRATOR to determine the linearity of the **MICRO-ELISA LUTEINIZING HORMONE** test.

Dilution Factor	Expected Value (mIU/ml)	Observed Value (mIU/ml)	% of Expected Value
Undiluted	-	67.2	-
3:4	50.4	51.4	102 %
1:2	33.6	36.0	107 %
1:4	16.8	17.0	101 %

URINE: A study was performed diluting a urine sample containing an elevated level of LH with the 0 mIU/ml CALIBRATOR to determine the linearity of the test.

Dilution Factor	Expected Value (mIU/ml)	Observed Value (mIU/ml)	% of Expected Value
Undiluted	-	42.2	-
3:4	31.7	29.6	93 %
1:2	21.1	23.0	109 %
1:4	10.6	9.1	86 %

Assay Recovery

SERUM: Three aliquots of human sera with LH concentrations of 9.1 and 21.7 mIU/ml were spiked with 10.0, 25.0 and 50.0 mIU LH/ml. The samples were assayed in duplicate.

Added LH	LH mIU/ml		% Recovery
	Expected Value	Measured Value	
0.0	9.1	-	-
10.0	19.1	18.7	98 %
25.0	34.1	34.7	102 %
50.0	59.1	61.3	104 %
0.0	21.7	-	-
10.0	31.7	29.4	93 %
25.0	46.7	46.6	100 %
50.0	71.7	69.0	96 %



URINE: Three aliquots of human urine with an LH concentration of 12.7 mIU/ml were spiked with 10.0, 25.0 and 50.0 mIU LH/ml. The samples were assayed in duplicate.

<u>Added LH</u>	<u>LH mIU/ml</u>		<u>% Recovery</u>
	<u>Expected Value</u>	<u>Measured Value</u>	
0.0	12.7	-	-
10.0	22.7	21.6	95 %
25.0	37.7	34.2	91 %
50.0	62.7	65.3	104 %

Comparison to Other LH Tests

Correlation studies on a random group of 110 serum samples with a range of values from <1.0 - 97.5 mIU/ml, were performed using the results from the MICRO-ELISA LUTEINIZING HORMONE Test and a LH IRMA test. The correlation of test results was 0.977.

<u>n=</u>	<u>Slope</u>	<u>Y-Intercept</u>	<u>Correlation Coefficient</u>
110	0.992	2.00	0.977

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Warranty

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